

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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INARI AGRICULTURE, INC.  
Petitioner,

v.

CORTEVA AGRISCIENCE LLC.  
Patent Owner.

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Case PGR2023-00022  
Patent No. 11,371,055

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**DECLARATION OF PAUL CHRISTOU, Ph.D.**

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## **I. INTRODUCTION**

1. I, Paul Christou, Ph.D., have been retained by Finnegan, Henderson, Farabow, Garrett & Dunner, LLP on behalf of Corteva Agriscience LLC as an independent expert in the field of plant biochemistry and biotechnology, including genetic transformation of plants. In this declaration, I address the aspects of the '055 patent directed to transgenic plants and plant cells, including the patent's disclosures relating to plant transformation. I understand that another witness will be addressing the aspects of the '055 patent directed to AAD-12 enzymes and variants.

2. I am being compensated at my usual hourly consulting rate for the time I spend on this matter. My compensation is not dependent upon my opinions or the outcome of this proceeding.

## **II. QUALIFICATIONS**

3. I am a plant biochemist and an endowed ICREA [Catalan Institution for Research and Advanced Studies] Professor in the Applied Plant Biotechnology research group at the University of Lleida, Spain. I studied the biosynthesis of secondary products in plant cell culture in graduate school and received my Ph.D. in Plant Biochemistry from The University of London, University College, UK in 1980.

4. From 1982 to 1994, I worked at Agracetus Inc. on molecular and cellular plant biology and crop transformation, initially as a project leader and later as Senior Scientist. While working there, I generated the first stably transformed

soybean line in the world by particle bombardment (also referred to as “biolistics”) and published my findings in Nature Biotechnology in 1988. Using biolistics, I also led our Agracetus research group in generating the first commercialized and genetically transformed soybean crop, Roundup Ready soybean. Roundup Ready soybean was transformed with the EPSPS gene to confer tolerance to the herbicide glyphosate.

5. I joined the John Innes Centre, Norwich, UK in 1994 as an associate professor and the head of the Laboratory of Transgenic Technology & Metabolic Pathway Engineering. I became a full professor in 1998. From 2001 to 2004, I worked as a full professor at Fraunhofer Institute of Molecular Biotechnology and Applied Ecology, Schmallenberg-Aachen, Germany. From 1997 to 2004, I also served as an adjunct professor at the Mediterranean Agronomic Institute of Chania, Crete in Greece. In 2004, I established a research group in Applied Plant Biotechnology at the University of Lleida, Spain as an ICREA Professor and continue to work there today.

6. I am highly familiar with, and have expertise in, the science of generating transgenic plants and plant transformation. My academic research, for example, has focused on genetically engineered crops, production of pharmaceutical macromolecules in crop plants, and engineering of novel agronomic traits in crop plants, including herbicide tolerance traits. For my research, I have received grants

from the Bill and Melinda Gates Foundation and the European Research Council (ERC). In 2020, I was awarded the Narcís Monturioll Medal for excellence in scientific research.

7. Over the last three decades, I have taught numerous Masters-level courses in plant molecular biology and transformation, including Applied Plant Molecular Biology-Green Biotechnology, Plant Biotechnology and Applications, Applied Plant Biotechnology and Genome Editing, and Plant Transformation. I have also given approximately 220 invited lectures on plant biotechnology and transgene expression in engineered crops to a variety of universities, academic institutions, and professional organizations. I also served as an organizing committee member, a panel chairman, or a panel review member for numerous international workshops and meetings on plant biotechnology and crop genetic engineering. I have directed the research activities of numerous graduate students and post-doctoral researchers, and I have made over 200 presentations based on our research on transgene expression in crop plants at national and international conferences relating to transgenic plants and plant transformation technologies.

8. I have held leadership roles in professional organizations in the field of plant biotechnology. For instance, since 2019, I have served as a member of the International Scientific Advisory Board of The Max Planck Institute of Molecular Plant Physiology, Golm, Germany. I was also appointed to the Scientific Advisory

Board of the Center for Research and Technology Hellas (CERTH), Ministry of Development, General Secretariat of Research and Technology, Greece in 2000.

9. I have served on the editorial advisory boards for scientific journals since 1990. I joined the Editorial Board of Molecular Breeding in 1998 and served as the Chief Editor on the board from 2002 to 2020. I also became a member of the Editorial Board of Transgenic Research in 1999 and served as the Chief Editor of the journal from 1999 to 2022.

10. I have authored or co-authored 286 peer-reviewed publications on plant biotechnology and transgenic crops. I have also authored approximately 50 chapters in books and conference proceedings relating to plant biotechnology, including chapters in widely recognized plant biotechnology handbooks and protocols such as the Springer Laboratory Handbook: Gene Transfer to Plants (1995), the Plant Molecular Biology Manual (1994), and the Handbook of Plant Biotechnology (2004). In addition, I authored or served as an editor of five books relating to genetic engineering of plants. A copy of my curriculum vitae is attached as **Appendix A**.

### **III. MATERIALS CONSIDERED**

11. My opinions are based on my education, training, background and experience, the documents I reviewed, and information available to me to date. I have reviewed and considered the documents and materials cited in this declaration, as well as those listed in **Appendix B**. To the extent I am provided with additional

documents or information in this proceeding, I reserve the right to modify or expand upon my opinions based on such new information, and in response to any additional reports and testimony.

#### **IV. SUMMARY OF OPINIONS**

12. I disagree with Dr. Silverstone's opinion that the '055 patent lacks written description and enablement. The '055 patent provides extensive, detailed descriptions of how to make and use the transgenic plants and plant cells of the claims, including numerous examples in a variety of monocot and dicot species. It also describes suitable expression vectors, transformation techniques, and transformation protocols. In view of the advanced state of the art in 2005-2006 (as well as the 2017 date Dr. Silverstone relies upon), a skilled artisan would also have had significant background knowledge and experience in plant transformation. Accordingly, in my opinion, the '055 patent's disclosure is more than sufficient.

13. The '055 patent provides detailed descriptions of making transgenic plants and plant cells of the claims. It discloses examples of generating seven different monocot or dicot transgenic plant species (*Arabidopsis*, soybean, cotton, tobacco, canola, rice, and corn) that express AAD-12(v1) (an AAD-12 protein variant). These examples provide detailed descriptions of (1) codon-optimizing an AAD-12 transgene; (2) useful expression vectors comprising known monocot or dicot promoters; (3) successfully transforming plant cells or tissues using a variety

of known techniques (e.g., *Agrobacterium*-mediated, WHISKERS-mediated, and biolistics-mediated transformation), and confirming successful transformation through the use of the AAD-12 gene as a selectable marker. The '055 patent demonstrates that transgenic plants were stably transformed and displayed high levels of tolerance to 2,4-D, triclopyr, and fluroxypyr.

14. Regarding enablement, once a skilled artisan had AAD-12 genes in hand, the time required to produce the transgenic plants and plant cells of the claims was mainly a function of plant biology (i.e., letting tissues grow and regenerate into plants after transformation). The '055 patent discloses several exemplary expression vectors that a skilled artisan would have been able to use in carrying out known transformation methods. Carrying out the transformation techniques themselves (e.g., biolistics) and plant regeneration were routine in 2005-2006. Accordingly, it would not have required undue experimentation to produce the transgenic plants and plant cells of the '055 patent claims.

15. Regarding written description, the '055 patent provides representative examples of successfully transformed monocot and dicot plants: *Arabidopsis*, soybean, cotton, tobacco, canola, rice, and corn. These plants are among the most widely used plants in agriculture and agricultural research. The field of plant transformation was also advanced in 2005-2006, with skilled artisans predictably able to produce transgenic crops using known and established protocols and

techniques. A skilled artisan in 2005-2006 therefore would have understood that the '055 patent's inventors possessed the claimed plants and plant cells.

## **V. LEGAL STANDARD**

16. I am not an attorney but have been informed of certain patent law principles by counsel, including the written description and enablement requirements.

17. I understand that patents are written for a person skilled in the relevant field at the time of the invention, who is presumed to be aware of the relevant prior art. In this case, I believe that the relevant time period is in 2005-2006, when Corteva filed its initial patent applications that led to the '055 patent. Ex. 2017 (WO 2007/053482); Ex. 2013 (Provisional 60/731,044). In this declaration, I also provide my opinions from the perspective of a skilled artisan as of the March 24, 2017, filing date relied upon by Dr. Silverstone. I provide my opinions on who would qualify to be a skilled artisan for the '055 patent in Section VII, below.

18. Regarding the enablement requirement, I understand that a patent must provide sufficient description and guidance to enable a skilled artisan to make and use the full scope of the claimed invention without undue experimentation. I understand that enablement is not measured against the cumulative time and effort required to make every embodiment within the scope of a patent claim. Rather, I understand that whether a patent claim is enabled depends on several factors,

including the breath of the claim, the state of the art at the time of the invention, the level of skill in the art, the predictability of the technical field, the guidance provided in the patent, the amount of experimentation required to make and use the invention, and the existence of working examples in the patent.

19. I understand that a patent need not describe what is well known in the art. I also understand that the fact that some experimentation is required does not mean that it necessarily takes undue experimentation to practice a claimed invention, particularly where such experimentation would have been routine or reasonable to a person skilled in the art.

20. Regarding the written description requirement, I understand that a patent's disclosure (its specification, figures, and claims) must convey with reasonable clarity that the inventors possessed what is claimed. I further understand that analyzing the written description requirement takes into account the perspective and knowledge of a skilled artisan. A number of factors are considered for evaluating the adequacy of the disclosure, including the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, and the predictability of the technology at issue.

21. I understand that, when a patent claims a genus, the patent specification must provide an adequate written description of the claimed genus. I understand that the written description of a genus can be analyzed in two ways: (1) whether the

specification discloses a representative number of species or (2) whether the specification discloses structural features common to the members of the genus, correlated to the claimed function.

## **VI. BACKGROUND AND STATE OF THE ART**

### **A. By 2005-2006, Transgenic Plants Had Been Routinely Produced and Achieved Commercial Success**

22. Plants can be genetically modified by introducing one or more genes foreign to the native plants (i.e., transgenes) into the plant genome to express recombinant proteins that are encoded by the transgenes. Ex. 2057 (Twyman 2002), 107. Before 2005, plants had been routinely genetically engineered to create transgenic plants with improved traits such as resistance or tolerance to pests, pathogens, or herbicides. Ex. 2057 (Twyman 2002), 107. For instance, in the 1980s, transgenic plants were generated to produce insecticidal proteins that protected the plants from insect attack. Ex. 2058 (Vaeck 1987), 33. Since the 1980s, researchers have generated transgenic plants with herbicide-tolerance genes that expressed enzymes conferring tolerance to a variety of herbicides. Ex. 2059 (Block 1987), 2513; Ex. 2060 (Owen 2000), 766-768 (glyphosate-resistant and glufosinate-tolerant transgenic plants). The creation of herbicide-resistant transgenic crops was an important advance in the agricultural field, allowing the selective use of herbicides to minimize the growth of soil-depleting weeds and improving crop yields. Ex. 2060 (Owen 2000), 767.

23. Transgenic plants were also generated before 2005 to express transgenes encoding recombinant proteins useful in the pharmaceutical and healthcare industries, for example in the production of antibodies, vaccines, human blood products, hormones, and growth regulators. Ex. 2061 (Twyman 2003), 570; Ex. 2057 (Twyman 2002), 107. By 2005, transgenic plants had reached commercial status and achieved commercial success. Ex. 2061 (Twyman 2003), 570; Ex. 2057 (Twyman 2002), 107.

24. Plants generally fall into two categories: monocotyledonous and dicotyledonous plants (also referred to as monocots and dicots). Monocot plants have a single embryonic leaf (a cotyledon) that emerges from a seed. Dicot plants typically have two cotyledons. The most common and representative monocot crops in agriculture include corn (i.e., maize) and other grass-like crops such as wheat and rice. Dicot crops in agriculture include tobacco, soybean, and cotton, among others. Long before 2005, there had been a great interest and substantial efforts in the field to genetically modify crops to express recombinant proteins and improve crop traits.

25. For example, it was reported that “plant-based expression systems [had] emerged as a serious competitive force in the large-scale production of recombinant proteins.” Ex. 2061 (Twyman 2003), 570.

**B. By 2005-2006, Transgene Preparation and Plant Transformation Were Routinely Carried Out to Generate Transgenic Plants**

**1. Transgene Preparation and Codon Optimization**

26. A transgene is a nucleotide sequence that can be transferred to the host organism and encode a protein foreign to a host organism (i.e., a recombinant protein). By 2005, it was routine to prepare transgenic DNA sequences using standard molecular biology techniques that were described in detail in basic molecular biology handbooks. *E.g.*, Ex. 2063 (Handbook of Plant Biotechnology, Vol. 1, Chapter 5).

27. Within a transgene, each codon comprises three successive nucleotides that code for a specific amino acid. Different codons can code for the same amino acid, which are referred to as synonymous codons. Codon usage bias refers to an organism's preferential use of synonymous codons, which is a ubiquitous natural phenomenon observed in bacteria, plants, and animals. It was well known before 2005 that plants prefer G and C rich codons compared to bacteria and mammals. Thus, the codons in a foreign gene can be modified (without changing the encoded amino acids) to adapt to the codon usage bias in plant species and optimize the expression of a foreign gene in plant cells. This is referred to as codon optimization. As an approach to optimize transgene expression, codon optimization is not always

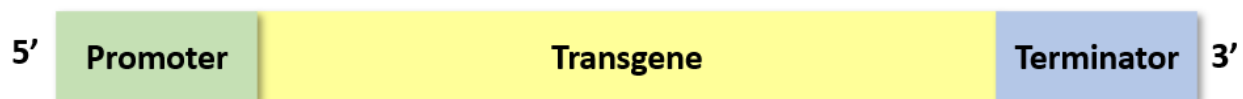
necessary. However, this well-known approach was conveniently used and generally led to improved transgene expression and performance.

28. Indeed, codon optimization had become a standard molecular biology strategy well before 2005 to optimize transgene expression in plants. For example, in the 1990s, researchers modified codons in transgenes from bacteria that encoded insect control proteins to increase the overall G+C content in the transgenes and increase their expression levels in plants. *See e.g.*, Ex. 2064 (Perlak 1991), 3325. Suitable codons for many plant species were well known before 2005, and tools for codon optimization design were readily available to persons skilled in the art, further simplifying codon design. Ex. 2065 (Murray 1989), Table 3 (compilation of plant codon usage from six monocot plant species and 36 dicot plant species). For example, before 2000, researchers had tabulated the codon usage of multiple maize genes and provided detailed methods of optimizing codons for transgene expression in maize and other monocot plants. *E.g.*, Ex. 2081 (WO1997/013402), Table 2; Ex. 2065 (Murray 1989), 485 (“the maize codon usage pattern resembles that of monocots in general”). Following codon optimization, those DNA sequences were rapidly synthesized using standard protocols.

29. Thus, as of 2005, codon optimization had been carried out without difficulty and routinely achieved success in a variety of transgenic plants, although it was optional and not always required for transgene expression.

## **2. Transgenic Expression Cassettes Had Been Developed and Used in a Variety of Plants to Achieve Transgene Expression**

30. To deliver a transgene into host plant cells, skilled artisans in 2005-2006 routinely built expression cassettes having regulatory elements flanking the transgene and then inserted those expression cassettes into expression vectors. Components of such expression cassettes included a promoter upstream of the transgene and other regulatory elements either further upstream of the promoter or downstream of the transgene. In other words, as illustrated below, an expression cassette can be pictured as a sandwich with the transgene sequence located between standard regulatory elements. These regulatory elements, which I discuss separately below, were known to facilitate high expression levels of transgenes in plants. All of these components of expression cassettes had been extensively studied since the 1980s and were well understood and readily available to skilled artisans by 2005. *See e.g., Ex. 2057 (Twyman 2002).*



### **Main Components of an Expression Cassette**

31. A promoter facilitates the transcription of the transgene DNA and drives high-level expression of the desired protein. Over the years, many promoters have been isolated from a wide variety of organisms and used in the production of

transgenic plants. Ex. 2062 (Potenza 2004), 1-2. By 2005, promoters that drive high, constitutive expression of transgenes in plants had been well characterized and were routinely available to skilled artisans. *Id.* at 2.

32. The most common promoters used for robust transgene expression are derived from plant virus sources, such as the cauliflower mosaic virus promoter (CaMV 35S), which has been widely used in plant transformation since the 1990s. Ex. 2062 (Potenza 2004), 3. The CaMV 35S promoter was known to drive high levels of transgene expression in dicots. *Id.* The success of the CaMV 35S promoter motivated researchers to develop other viral promoters for transgene expression in plants, and, before 2005, many such viral-derived promoters were reported to perform as well as or better than the CaMV 35S promoter and to drive high levels of transgene expression in both dicots and monocots. *Id.*

33. For example, the cassava vein mosaic virus (CsVMV promoter) was reported before 2005 to also achieve gene expression in transgenic plants. *See e.g.*, Ex. 2066 (Verdaguer 1998), 1055. Researchers also developed other viral promoters for transgene expression in plants, and, as of 2005, several such viral-derived promoters (e.g., Australian banana streak virus (BSV) promoter, figwort mosaic virus (FMV) promoter, etc.) had been reported to perform similarly or better than the CsVMV and CaMV 35S promoters, driving high levels of transgene expression in dicots and monocots. Ex. 2062 (Potenza 2004), 3.

34. Endogenous or native plant promoters had also been regularly used before 2005 to achieve high levels of transgene expression in plants. *Id.* at 4. Those promoters included promoters derived from plant actin genes (*Act*) or ubiquitin genes (*Ubi*) that are conserved and expressed in almost all plant cells. *Id.*; Ex. 2069 (McElroy 1991), 150; Ex. 2070 (Christensen and Quail 1996), 213.

35. To further improve transgene expression in plants, persons skilled in the art also had successfully developed synthetic or artificial promoters by coupling a basal or core promoter with other regulatory elements. Ex. 2062 (Potenza 2004) at 15.

36. A transgenic expression cassette may also contain other regulatory elements flanking the transgene. These regulatory elements include DNA sequences that do not encode a portion of the protein but regulate the expression of the protein and are usually located upstream (i.e., 5'-) or downstream (i.e., 3'-) of the regulated gene. The regions flanking a gene and containing the regulatory elements are also called the 5'-untranslated region (5'-UTR) and the 3'-untranslated region (3'-UTR).

37. In the context of transgenic plants, it had been reported before 2005 that certain introns (non-coding segments) from some genes, when placed in the 5'-UTR upstream of unrelated transgenes, can increase the expression of these transgenes in different plants. Ex. 2071 (Mascarenhas 1990), 913, 915; Ex. 2069 (McElroy 1991), 150-51.

38. A terminator is a common regulatory element located at the end of a gene (or downstream of the gene), which causes gene transcription to stop at the correct position. Before 2005, researchers routinely reported successful transgene expression in a variety of plants using expression cassettes containing a terminator downstream of the transgene in the 3'-UTR. *E.g.*, Ex. 2067 (De Bolle 2003), 171, 177.

39. As of 2005-2006, using these standard regulatory elements and promoters, persons skilled in the art could routinely build expression cassettes to deliver various transgenes into plants and achieve successful protein expression in the tested transgenic plants.

40. Mitsuhashi et al., for example, reported using expression cassettes comprising a core CaMV 35S promoter for expression of transgenes in tobacco. Ex. 2072 (Mitsuhashi 1996), 51. The CaMV 35S core promoter, three different 5'-upstream sequences of the 35S promoter, the first intron of a gene for phaseolin, and a 5'-untranslated sequence (X2 sequence) of tobacco mosaic virus were used in various combinations to build expression cassettes. *Id.*

41. The authors found that the expression cassettes “caused extremely high-level expression” in tobacco and selected one of them (E\2Q) as “representative of promoters of highest-level expression in dicotyledonous plants.” Ex. 2072 (Mitsuhashi 1996) at 53. Based on the successful expression results in tobacco,

Mitsuhara reported that the tested promoters were “potentially useful for the production of at least 10-fold higher amounts of foreign gene products in transgenic plants both for basic studies of problems in plant molecular biology and for practical purposes.” Ex. 2072 (Mitsuhara 1996), 58.

42. Before 2005, persons skilled in the art had also routinely achieved successful delivery and expression of transgenes in monocot plants. McElroy et al., for example, constructed *Act1*-based expression vectors for expression of transgenes in rice. Ex. 2069 (McElroy 1991), 150. Based on the positive results in rice, the authors concluded that the tested expression vectors “should promote the expression of foreign genes *in most, if not all, transformed monocot cells* to levels that have not previously been attainable with alternative expression vectors.” *Id.* (emphasis added).

43. The strength of the maize ubiquitin (*Ubi-1*) promoter had also been documented before 2005 “in transient and/or stable transformation experiments in a number of monocot systems including rice.” Ex. 2070 (Christensen and Quail 1996), 217. Using expression cassettes containing the regulatory elements from the *Ubi-1* gene to successfully express a Ubi-gus fusion protein in rice, the researchers (Christensen and Quail) reported that their data were “consistent with a potential for targeting a wide spectrum of cells” by the tested expression cassettes. *Id.*

44. There had also been reported examples of using the same expression cassette to deliver homologous genes (i.e., genes carrying out the same function in

different species) from different plant species to a plant and achieving successful expression of all of the tested homologous genes. Dr. Silverstone, for example, reported in 2005 using *Agrobacterium* containing the same expression cassette comprising a maize *Ubi-1* promoter to drive expression of the *psy* transgenes from different plant species (rice, maize, pepper, tomato, and daffodil) in rice. Ex. 2010 (Paine 2005), 483. In this paper, Dr. Silverstone and his co-authors reported that the carotenoid levels, which reflect transgene efficacy, were increased, with the transgenic rice expressing PSY from the monocot plant species rice and maize achieving the most substantial increase of carotenoid. Ex. 2010 (Paine 2005), 483, Figure 2C.

45. Thus, as of 2005, transgene expression cassettes had been routinely developed, were readily available, and had been shown to be efficient in driving high-level expression of transgenes across a variety of monocot and dicot plants.

### **3. Standard Plant Transformation Techniques Had Been Routinely Used to Generate Transgenic Plants**

46. As of 2005, methods of transforming plants (i.e., plant transformation) had become widely used in both basic and applied studies in plant biology. Ex. 2058 (Vaeck 1987), 33; Ex. 2074 (Bhat 2002), 673. As I explained in the book “Genetic Engineering – Principles and Methods,” published in 2002, gene transfer to a plant was by then “a routine procedure.” Ex. 2057 (Twyman 2002), 107. There are two basic strategies for plant transformation, both of which were well known and

routinely available to skilled artisans before 2005. *Id.* One strategy involves using the soil bacterium *Agrobacterium tumefaciens* to infect a plant cell and transfer a small segment of DNA from a resident plasmid in the bacteria into the host plant cell. *Id.* at 107-08. Specifically, *Agrobacterium tumefaciens* carries a naturally occurring resident tumor-inducing plasmid (Ti plasmid) that enables the bacteria to enter and colonize wounded plant cells and transfer a DNA segment from the Ti plasmid to the plant nuclear genome. *Id.* at 108. Because Ti plasmids contain oncogenic genes, the plasmids were subsequently modified to remove these oncogenic genes to create binary vectors to use for plant transformation. *Id.* By 2005, researchers had inserted transgenes into the modified *Agrobacterium* vectors and delivered the transgenes into plant cells such that the transgenes could be incorporated into the plant genome to express the encoded recombinant proteins.

47. Another plant transformation strategy involves directly transferring the foreign DNA into the plant cell by physical force. Ex. 2057 (Twyman 2002), at 107. Routine techniques of directly transferring foreign genes into host cells, including particle bombardment, had been used by researchers well before 2005. *Id.*

48. Particle bombardment (biolistics) was (and still is) a commonly used approach for direct gene transfer to plants. It involves shooting small DNA-coated metal particles (e.g., gold) into plant tissue with sufficient force to break through the cell wall and membrane and release the foreign DNA. Ex. 2057 (Twyman 2002) at

115. As I reported in one of my publications in 2005, “particle bombardment is a versatile and effective transformation method, not limited by cell type, species or genotype. There are no intrinsic vector requirements so transgenes of any size and arrangement can be introduced, and multiple gene cotransformation is straightforward.” Ex. 2075 (Altpeter 2005), 305-06. Because this transformation method was simple and highly efficient in delivering a large number of transgene expression vectors into plant cells, skilled artisans routinely used this standard method before 2005 and achieved successful transformation of a variety of plants. *E.g.*, Ex. 2075 (Altpeter 2005).

49. There are also quick and simple methods of transiently expressing genes in plant cells by delivering a transgene into the cell without incorporating it into the genome. For instance, in my lab, we routinely simultaneously shoot plant tissues with metal-coated particles to deliver the expression vectors into the plant cells and measure recombinant protein expression by *in vitro* assays shortly after transformation. This method, known as transient expression, can provide a strong prediction for stable-integrated *in planta* expression performance within about two days. It can also be used with large numbers of tissue samples for parallel testing.

#### **4. Plant Regeneration Was Routine by 2005-2006**

50. By 2005-2006, protocols of transforming various types of plant cells/tissues were readily available, including those for transforming embryonic cell

suspensions, tissues (such as leaves), segments (such as cotyledon segments, hypocotyl segments, segments of stalk), explants, roots, etc. Regenerating treated plant cells into whole plants for production of seeds and progeny plants was known and routine by 2005-2006.

51. The regeneration process often comprises incubating the treated plant cells in callus induction medium that contains micronutrients and hormones to induce callus formation, transferring the callus tissues onto media to induce shoot and root formation to form plantlets, and planting the plantlets into soil (in a greenhouse or field). Ex. 2076 (Schroeder 1993) at 752. As such, the plant regeneration process as of 2005-2006 was simple and straightforward, only involving using known media to let the treated plant cells grow into plantlets before planting them and, if desired, growing them to seed to produce progeny.

## **5. Transgene Expression Detection and Transgenic Plant Characterization Were Standard and Efficient**

52. By 2005-2006, standard laboratory and field-testing procedures were available to detect the expression of transgenes and identify transgenic plants with improved agronomic properties.

53. For example, standard *in vitro* assays such as western blot and ELISA identify encoded recombinant proteins using antibodies recognizing and specifically binding to the recombinant proteins and detecting signals attached to or elicited from the antibodies bound to the recombinant proteins. These assays can quickly detect

transgene expression in transformed plant cells. Ex. 2077 (Engvall 1972) (ELISA); Ex. 2078 (Burnette 1981) (western blot).

54. Selectable markers could also be used as a simpler technique for identifying successfully transformed plants. A selectable marker refers to a transgene in an expression cassette that, when expressed, produces an effect allowing skilled artisans to identify the plant as having been transformed. Herbicide resistance genes, for example, can be used as selectable markers. Successfully transformed plants exhibit the ability to degrade an applied herbicide (e.g., via spray, leaf-painting, or incubation) while the untransformed plants exhibit injury or death from the herbicide, permitting a skilled artisan to visually select the transformed plants.

55. Long before 2005, multiple herbicide resistance genes were readily available and widely used as selectable markers. For example, the *bar* gene encodes phosphinothricin acetyltransferase (PAT), which can detoxify glufosinate (the active ingredient in the herbicide Liberty®) and bialaphos (the active ingredient in the herbicide Hebiace®) and had been widely used as a plant transformation selectable marker. Ex. 2079 (Zhang 1999); 37. As another example, the *crs1* gene encodes acetohydroxy acid synthase (AHAS) that confers tolerance to herbicides such as imazethapyr and also served as a well-known selectable marker. *See* Ex. 2080 (Brasileiro 2001), 114-15. Co-transforming plants with the transgenes of interest and

these selectable markers enabled skilled artisans to quickly apply the corresponding herbicides to the plants/plant tissues after transformation and identify successful transformants. As I also discuss in Section VIII.B below, the '055 patent established that AAD-12 genes function as effective selectable markers, permitting selection of transformants based on their ability to degrade herbicides such as 2,4-D, triclopyr, and fluroxypyr.

56. Identifying transgenic plants with improved traits resulting from the successful expression of transgenes was therefore straightforward in 2005-2006. It involved incubating the plant cells that underwent transformation with an herbicide of interest or growing the plant cells into plants and then painting, spraying, or incubating them with the herbicide at a concentration sufficient to damage untransformed plants and identify transformants with lesser or no damage.

57. This identification process can also be carried out in later generations. After growing the primary transformants (referred to as T<sub>0</sub> plants), their seeds can be harvested, planted, and grown, and the herbicide may be applied (sprayed) on the progeny (T<sub>1</sub> or later generation plants).

## **VII. LEVEL OF SKILL IN THE ART**

58. Regarding aspects of the '055 patent relating to plant transformation, a person skilled in the art would have generally possessed a Ph.D. in molecular biology, biotechnology, or plant biochemistry, and 3-5 years of experience in plant

transformation. This technical background would include familiarity with building transgene expression cassettes, transforming plant cells, and plant regeneration.

59. With the advanced stage of plant transformation technology in 2005-2006, skilled artisans at that time delegated many of the physical tasks involved in plant transformation to laboratory technicians. For example, plant transformation protocols were well established in 2005-2006, and the task of preparing plant tissues for transformation often required manipulating small tissues (e.g., extracting embryos on the order of one millimeter or trimming small seedlings into explants), requiring a high degree of dexterity but not scientific training or knowledge. For instance, I have typically worked with about five technicians since 2005, and my colleagues in the field follow a similar practice. Generally, the technicians I work with have a high school or bachelor's degree, and there is no requirement for them to have any scientific background.

## **VIII. THE '055 PATENT**

### **A. The '055 Patent Claims**

60. The '055 patent claims are directed to transgenic plants cells and plants comprising a recombinant polynucleotide encoding AAD-12 proteins having at least 85% sequence identity to SEQ ID NO: 2 and a common motif and exhibiting aryloxyalkanoate dioxygenase enzymatic activity that degrades the recited herbicides.

61. Independent claim 1 recites:

A transgenic plant cell comprising a recombinant polynucleotide that encodes an AAD-12 protein that exhibits aryloxyalkanoate dioxygenase activity wherein said activity enzymatically degrades a phenoxy auxin herbicide and a pyridyloxy auxin herbicide, further wherein said AAD-12 protein comprises:

i) an amino acid sequence having at least 85% sequence identity with SEQ ID NO: 2; and

ii) an AAD-12 motif having the general formula of:

$HX_{109}D(X)_{111-134}T(X)_{136-261}H(X)_{263-272}R$ , wherein

$X_{109}$  represents a single amino acid at position 109, relative to the sequence of SEQ ID NO: 2;

$(X)_{111-134}$  represents a sequence of 24 amino acids;

$(X)_{136-261}$  represents a sequence of 126 amino acids; and

$(X)_{263-272}$  represents a sequence of 10 amino acids.

Ex. 1001 at 125:2-18.

62. Independent claim 32, which is similar to claim 1, requires that the AAD-12 motif of the encoded protein share 90% sequence identity with corresponding amino acids of position 108 to 273 of SEQ ID NO: 2. Ex. 1001 at 126:52-127:4. Independent claim 33 is directed to a method of controlling weeds in

a field by applying a pyridyloxy auxin herbicide to the field, wherein the field has been planted with seeds expressing a recombinant polynucleotide with the same features as stated in claim 1. Ex. 1001 at 127:5-26.

63. Claims 2-31 all ultimately depend from claim 1. I summarize the additional elements in those claims as below:

- The AAD-12 protein of claim 1 with an amino acid sequence having at least 90% sequence identity with SEQ ID NO: 2 (claim 2);
- The plant cell of claim 1 is a dicot plant cell selected from cotton, tobacco, canola, soybean, and *Arabidopsis* cells (claim 3);
- A transgenic plant comprising a plurality of the plant cells of claim 1, wherein expression of the polynucleotide renders the plant tolerant to an aryloxyalkanoate herbicide (claim 4);
- The aryloxyalkanoate herbicide in claim 1 being particular herbicide classes or herbicides such as 2,4-D, triclopyr, or fluroxypyr (claims 5-9);
- A transgenic plant of claim 1 having a second herbicide resistance gene (claims 10 and 11);
- Methods of controlling at least one weed in a field, wherein the field contains at least one plant of claim 4 and the method comprises applying a first herbicide selected from the group of a phenoxy auxin herbicide and a pyridyloxy herbicide (or more specific herbicides), and

a second herbicide (or more specific herbicides) and sequence of applying the herbicides (claims 12-27 and 29);

- A seed comprising a plurality of the plant cell of claim 1 (claim 28);
- A plant grown from the seed of claim 28, wherein the plant comprises the polynucleotide (claim 30); and
- A part, progeny, or asexual propagate of the plant of claim 30 comprising the polynucleotide of claim 1 (claim 31).

#### **B. The '055 Patent's Specification**

64. As I explained in Section VI.B, transgenic plant production (or plant transformation) was a well-established process in 2005-2006. It generally involved three steps: (1) building an expression cassette comprising a transgene (which can be optionally codon-optimized); (2) transforming host plant cells with the expression cassette; and (3) regenerating the transformed plant cells into whole plants. The '055 patent provides detailed descriptions of and guidance on all three steps and further describes methods of confirming successful transformation.

65. The '055 patent describes methods of optimizing codons in transgenes to improve their expression in monocot and dicot plants. Ex. 1001 at 22:57-23:2, Example 2. In particular, Example 2 describes generating a transgene encoding an AAD-12 variant (AAD-12(v1)) (SEQ ID NO:3) by optimizing the codons based on calculated maize codon usage bias. Ex. 1001 at Example 2, 35:1-29, Table 5. This

maize codon-optimized AAD-12(v1) gene was used in the transformation examples for both monocot and dicot plants.

66. For expression cassettes, the '055 patent provides examples of known promoters (such as the CaMV 35S promoter from the cauliflower mosaic virus, actin promoters, and ubiquitin promoters). Ex. 1001 at 27:35-28:53, 29:43-30:9. As the '055 patent acknowledges, “[n]umerous plant-specific gene transfer vectors are known in the art.” Ex. 1001 at 28:18-19. Example 3 discloses the construction of a series of binary expression vectors comprising known promoters including the CsVMV promoter, the AtUbi10 promoter, the ZmUbi1 promoter, the AtUbi3 promoter, or the AtAct2 promoter and well-known 3'- and/or 5'-UTRs for the expression of AAD-12 proteins. Ex. 1001 at 42:1-36, Table 8. The '055 patent summarizes the elements of exemplary expression cassettes in Table 8, reproduced below:

TABLE 8

| Binary constructs used in transformation of various plant species. |        |                       |                        |          |           |            |       |           |                          |                            |                      |          |               |
|--------------------------------------------------------------------|--------|-----------------------|------------------------|----------|-----------|------------|-------|-----------|--------------------------|----------------------------|----------------------|----------|---------------|
| pDAB #                                                             | pDAS # | Species* Trans-formed | Gene of interest (GOI) | Promoter | Feature 1 | Feature 2  | GOI 2 | Pro-moter | Bacterial Selection gene | Bacterial Selection gene 2 | Plant Selection gene | Promoter | Trxn Method   |
| 724                                                                | —      | A, Ct, S              | AAD12 v1               | AtUbi10  | NtOsm     | —          | —     | —         | Erythro-mycin            | —                          | pat                  | CsVMV    | Agro binary   |
| 3274                                                               | —      | A                     | AAD12 v1               | AtUbi10  | NtOsm     | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | —                    | —        | Agro binary   |
| 3278                                                               | 1580   | T                     | AAD12 v1               | CsVMV    | NtOsm     | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | AtUbi10  | Argo binary   |
| 3285                                                               | —      | A                     | AAD12 v1               | CsVMV    | NtOsm     | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | AtUbi10  | Argo binary   |
| 3759                                                               | —      | A, Ca, S              | AAD12 v1               | CsVMV    | NtOsm     | RB7 Mar v2 | EPSPS | AtUbi10   | Spectino-mycin           | —                          | pat                  | AtUbi10  | Argo binary   |
| 4101                                                               | 1863   | Cn, R                 | AAD12 v1               | ZmUbi1   | —         | RB7 Mar v2 | —     | —         | Ampicillin               | —                          | AHAS v3              | OsAct1   | Whiskers/ Gun |
| 4464                                                               | —      | S                     | AAD12 v1               | CsVMV    | —         | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | CsVMV    | Argo binary   |
| 4468                                                               | —      | S                     | AAD12 v1               | AtUbi10  | —         | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | CsVMV    | Argo binary   |
| 4472                                                               | —      | S                     | AAD12 v1               | AtUbi3   | —         | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | CsVMV    | Argo binary   |
| 4476                                                               | —      | S                     | AAD12 v1               | ZmUbi1   | —         | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | CsVMV    | Argo binary   |

TABLE 8-continued

| Binary constructs used in transformation of various plant species. |        |                       |                        |          |           |            |       |           |                          |                            |                      |          |             |
|--------------------------------------------------------------------|--------|-----------------------|------------------------|----------|-----------|------------|-------|-----------|--------------------------|----------------------------|----------------------|----------|-------------|
| pDAB #                                                             | pDAS # | Species* Trans-formed | Gene of interest (GOI) | Promoter | Feature 1 | Feature 2  | GOI 2 | Pro-moter | Bacterial Selection gene | Bacterial Selection gene 2 | Plant Selection gene | Promoter | Trxn Method |
| 4480                                                               | —      | S                     | AAD12 v1               | AtAct2   | —         | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | CsVMV    | Argo binary |

\*A = *Arabidopsis*

CsVMV = Cassava Vein Mosaic Virus Promoter

ZmUbi1 = *Zea mays* Ubiquitin 1 Promoter AtUbi10 = *Arabidopsis thaliana* Ubiquitin 10 Promoter

T = Tobacco

HptII = hygromycin phosphotransferase

Atubi3 = *Arabidopsis thaliana* Ubiquitin 3 Promoter

S = Soybean

AtAct2 = *Arabidopsis thaliana* Actin 2 Promoter

Ct = Cotton

RB7 Mar v2 = *Nicotiana tabacum* matrix associated region (MAR)

R = Rice

Nt Osm = *Nicotiana tabacum* Osmotin 5'

Cn = Corn

Untranslated Region and the *Nicotiana tabacum*

Ca = Canola

Osmotin 3' Untranslated Region

67. For plant transformation, the patent provides detailed descriptions of methods that were known in the art such as *Agrobacterium*-mediated transformation, biolistics-mediated transformation (microparticle bombardment), and whisker-

mediated transformation. Ex. 1001 at 24:25-62 (“A large number of techniques are available for inserting DNA into a plant host cell.”). Examples 7, 8, 10-12, 14, 21, and 22 describe use of the expression cassettes from Table 8 and confirm successful transformation of seven different monocot or dicot plant species (*Arabidopsis*, soybean, cotton, tobacco, canola, rice, and corn) with AAV-12(v1). Ex. 1001 at Examples 7, 8, 10-12, 14, 21, and 22.

68. The '055 patent discloses that plant cells, tissues, or parts (such as leaves, segments of stalk, root, etc.) that underwent the AAD-12 transformation processes can be regenerated into whole plants by well-known methods. Ex. 1001 at 23:24-35, 24:54-58, 26:44-52 (describing known methods of inducing shoot and root formation after plant cell transformation). The examples further describe detailed protocols for plant regeneration after transformation. *E.g.*, Ex. 1001 at 58:60-59:42, 80:31-67.

69. The '055 patent also describes methods of confirming successful transformation.<sup>1</sup> For example, the '055 patent describes the known technique of using selectable markers to quickly identify transformed plants shortly after transformation. *Id.* at 25:10-31. The examples further demonstrate successful use of

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<sup>1</sup> The '055 patent provides detailed descriptions of known *in vitro* assays, including western blot, ELISA, and colorimetric assays. Exs. 1001, 2013-2017 at Examples 5, 6, and 9. However, those assays are not required for the confirmation of successful transformation, because using a selectable marker is an easy, effective alternative.

AAD-12(v1) as a new selectable marker for identifying transformed plants by applying herbicides including 2,4-D, triclopyr, and dichlorprop. Ex. 1001 at 53:18-39, Examples 7, 8, 10, 14, 21, 22, 24.

70. The '055 patent also discloses several examples that provide detailed descriptions of the processes of transforming monocot and dicot plant species. I briefly summarize those examples below.

Transformation in *Arabidopsis* (Examples 7 and 14)

71. Example 7 describes *Agrobacterium*-mediated transformation of *Arabidopsis*, a dicot plant. Ex. 1001 at 48:65-50:33. As Example 7 states, the transformation involved dipping one-month old *Arabidopsis* plants for 15 seconds into media containing the *Agrobacterium* cells containing the binary vector (either pDAB724 or pDAB3759) comprising an expression cassette as described in Table 8 that includes the AtUbi10 promoter, the AAD-12(v1)-encoding gene, and the *pat* selectable marker gene. Ex. 1001 at 50:26-28, 54:63-55:9. Example 7 further discloses that the treated plants were then grown for 4 weeks, and the T<sub>1</sub> seeds were harvested. Ex. 1001 at 50:32-33.

72. Example 7 discloses using an herbicide to confirm the expression of the conventional *pat* selectable marker gene and proper performance of the expression cassette. Seven days and eleven days after the T<sub>1</sub> seeds were planted, the T<sub>1</sub> plants were sprayed with the glufosinate (Liberty<sup>®</sup>) herbicide, and survivors were identified

4-7 days after the final spray. Ex. 1001 at 50:52-61. Example 7 also describes the novel testing of AAD-12 as a new selectable marker by applying 2,4-D to T<sub>4</sub> generation *Arabidopsis* plants, reporting that the 2,4-D treatment “was equally effective in selection frequency, and resulted in less herbicidal injury to the transformed individuals than the Liberty selection scheme.” Ex. 1001 at 53:17-38. The ’055 patent concludes that “[t]hese results indicate AAD-12(v1) can be effectively used as an alternative selectable marker.” Ex. 1001 at 53:38-40.

73. Example 7 further describes applying auxin herbicides including 2,4-D to T<sub>1</sub> plants at different doses and presents response in terms of percent visual injury, with individuals exhibiting little or no injury (< 20%), moderate injury (20-40%), or severe injury (> 40%). Ex. 1001 at 51:2-60, Table 11. Based on the results, Example 7 concludes that the AAD-12(v1) gene “imparted herbicide resistance” to the transformed *Arabidopsis* plants compared to the wild type plants. Ex. 1001 at 51:59-52:11. By applying 2,4-D to T<sub>2</sub> *Arabidopsis* plants and measuring the level of injury, Example 7 further shows that AAD-12(v1) was stably integrated to the transformed *Arabidopsis* plants. Ex. 1001 at 53:40-56.

74. Example 7 further describes spraying transgenic *Arabidopsis* plants with pyridyloxyacetate herbicides including triclopyr and fluroxypyr and evaluating the plants three and fourteen days after treatment. Ex. 1001 at 54:1-10. Based on the results, the ’055 patent concludes that “AAD-12-transformed plants were also

clearly protected from the triclopyr and fluroxypyr herbicide injury” that was seen in the non-resistant controls and that “[t]hese results confirm that AAD-12(v1) in *Arabidopsis* provides resistance to the pyridyloxyacetic auxins tested.” Ex. 1001 at 54:13-18, Table 13.

75. Example 14 further demonstrates the superiority of *Arabidopsis* plants transformed with AAD-12(v1) over *Arabidopsis* plants transformed with AAD-2(v1). Example 14 describes the process of cloning AAD-2(v1) into a binary vector that also contains the AtUbi10 promoter and transforming *Arabidopsis* plants by the same method described in Example 7. Ex. 1001 at 83:25-84:48. The '055 patent reports that “AAD-2 (v1) and AAD-2(v2) transformants were far less resistant to 2,4-D than the AAD-12 (v1) gene....” Ex. 1001 at 84:60-85:9.

#### Transformation in corn (Example 8)

76. Example 8 describes preparing maize tissue for AAD-12(v1) transformation by harvesting approximately 1 mm sized maize embryos 9-10 days after pollination and growing callus tissue, which is a mass of dedifferentiated cells. The maize cells were placed into suspension and agitated for 10 seconds with silicon carbide whiskers coated with an AAD-12(v1) vector pDAB4101 from Table 8 comprising the ZmUbil monocot promoter (see Table 8). Ex. 1001 at 56:43-60, 58:24-32. Example 8 further discloses the process of plant regeneration, which involves incubating the callus tissues grown from treated plant cells in medium

(under selection) containing a plant growth factor for two weeks, transferring them into regeneration medium, letting them grow into plantlets with a sufficient root and shoot system, and planting them to soil in a greenhouse. Ex. 1001 at 59:1-16.

77. Example 8 further discloses that T<sub>1</sub> AAD-12(v1) corn plants were grown and two of the three tested events “were robustly tolerant to 2,4-D and 2,4-D + imazethapyr or 2,4-D + glyphosate.” Ex. 1001 at 62:36-55, Table 16. Example 8 also shows testing AAD-12(v1) heritability in T<sub>1</sub> corn and concludes that “AAD-12 is heritable as a robust glyphosate aryloxyalkanoate auxin resistance gene in multiple species....” Ex. 1001 at 63:15-29. The patent further describes field level tolerance trials conducted on two AAD-12(v1) events, during which the transgenic corn’s responses to 2,4-D, triclopyr, and fluroxypyr were tested at day 14 after spraying one of those herbicides on those plants. Ex. 1001 at 64:28-67. Based on the field tolerance data, the ’055 patent concludes that “AAD-12 (v1) conveys high level tolerance in corn to 2,4-D, triclopyr and fluroxypyr at rates far exceeding those commercially used.” Ex. 1001 at 65:1-20, Tables 18-19.

#### Transformation in tobacco (Example 10)

78. Example 10 describes preparing tobacco leaves from 6–8-week-old plants for transformation and using *Agrobacterium* cells comprising a binary vector from Table 8 (pDAB3278) comprising the CsVMV promoter, the AAD-12(v1)-encoding gene, and the *pat* selectable marker gene. Ex. 1001 at 66:46-67:16, Table 8.

79. Example 10 discloses that after tobacco leaves were grown into T<sub>0</sub> plants that were 3-4 inches tall, they were sprayed with a range of 2,4-D, triclopyr, or fluroxypyr concentrations. Ex. 1001 at 68:11-34. The '055 patent discloses that “[e]very event tested was more tolerant to 2,4-D than the untransformed control line.” Ex. 1001 at 68:11-34. Table 21 further shows that the T<sub>0</sub> AAD-12(v1) tobacco plants gained tolerance to all three tested herbicides. Ex. 1001 at Table 21. The '055 patent further describes verification of high 2,4-D tolerance in T<sub>1</sub> tobacco plants and concludes that the results “show resistance can be imparted to the pyridyloxyacetic acid herbicides, triclopyr and fluroxypyr.” Ex. 1001 at 69:13-52, Table 21. Example 10 also describes AAD-12(v1) heritability in tobacco and reports field herbicide tolerance testing, concluding that the results “indicate that AAD-12(v1) transformed event lines displayed a high level of tolerance to 2,4-D, triclopyr and fluroxypyr at multiples of commercial use rates....” Ex. 1001 at 70:15-71:9, Table 22.

#### Transformation in soybean (Example 11)<sup>2</sup>

80. Example 11 describes generating a series of binary constructs comprising a dicot promoter such as the CsVMV promoter and the AtUbi10 promoter, the AAD-12(v1)-encoding gene, and the *pat* selection marker gene,

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<sup>2</sup> Soybeans are also often referred to as soya. For consistency with the '055 patent's terminology, I have used “soybeans” in this declaration.

preparing *Agrobacterium* that contains the binary vectors, conducting *Agrobacterium*-mediated transformation of soybean explants derived from soybean seeds growing on medium for 5 days, and painting the soybean leaves with glufosinate during plant regeneration to screen for injury. Ex. 1001 at 73:20-75:33.

81. The '055 patent further describes that when 2,4-D was painted on the leaves of a subset of the initial T<sub>0</sub> soybean transformants or sprayed on another subset of T<sub>0</sub> transformants as described earlier in the other examples, “[a]ny plant confirmed as having the AAD-12 (v1) coding region, regardless of the promoter driving the gene did not respond to the 2,4-D leaf painting whereas wild type Maverick soybeans did,” confirming that “[a]ll AAD-12 (v1)-containing plants were clearly resistant to the herbicide application versus the wild type Maverick soybeans.” Ex. 1001 at 76:32-54, Table 25.

#### Transformation in cotton (Example 12)

82. Example 12 describes preparing cotyledon segments from 7-10 day old cotton seedlings for transformation, preparing *Agrobacterium* that contains the same pDAB724 binary vector used in Example 7 to transform *Arabidopsis* (i.e., comprising the AtUbi10 promoter, the AAD-12(v1)-encoding transgene, and the *par* selection marker gene), *Agrobacterium*-mediated transformation, generating callus, and selecting the cotton callus on glufosinate ammonium. Ex. 1001 at 79:65-81:57.

Example 15 discloses western blot data confirming expression of AAD-12(v1) in the transformants. Ex. 1001 at 81:38-57.

#### Transformation in rice (Example 21)

83. Example 21 describes preparing embryogenic rice tissues, transforming those tissues by microprojectile bombardment with the same pDAB4101 vector used in Example 8 to transform corn (comprising the ZmUbil promoter and the AAD-12(v1)-encoding gene), and regenerating plants from the transformed rice explants. Ex. 1001 at 91:40-93:8. The '055 patent confirms the transformation and heritability of AAD-12(v1) in T<sub>1</sub> rice, verifying high 2,4-D and triclopyr tolerance. Ex. 1001 at 93:9-94:67, Tables 27-28. The patent concludes that “[t]hese results indicate that the AAD-12(v1) transformed rice displayed a high level of resistance to 2,4-D and triclopyr....” Ex. 1001 at 94:60-67.

#### Transformation in canola (Example 22)

84. Example 22 describes preparing canola hypocotyl segments for transformation, transforming the segments with *Agrobacterium* containing the same binary vector pDAB3759 used to transform *Arabidopsis* in Example 7, comprising the CsVMV promoter and the AAD-12(v1)-encoding transgene. Ex. 1001 at 95:20-41. It also describes regenerating the callused hypocotyl segments into T<sub>0</sub> plants and confirming their tolerance to 2,4-D, confirming AAD-12(v1) heritability in canola, and demonstrating transgenic canola plants’ field tolerance to 2,4-D,

triclopyr and fluroxypyr herbicides. Ex. 1001 at 95:42-99:20. The patent concludes that “[t]hese results indicate that AAD-12(v1) transformed events displayed a high level of resistance to 2,4-D, triclopyr and fluroxypyr ....” Ex. 1001 at 98:62-65.

85. The ’055 patent further discloses that in light of the detailed examples, additional crops may be transformed to express an AAD-12 protein variant of the invention using known methods such as biolistics or *Agrobacterium*-mediated transformation. Ex. 1001 at Example 13. Example 13 discloses other exemplary crops that would benefit from AAD-12(v1) transformation and subsequent increased application of auxinic herbicides. Ex. 1001 at Example 13. Example 15 describes pre-plant burndown applications to kill weeds by herbicides that the transgenic plants of the invention can tolerate. Ex. 1001 at 85:25-86:21. Examples 16 and 17 provide guidance on in-crop use of phenoxy auxin herbicides in soybeans, cotton, corn, rice, and other dicot or monocot plants that are stably transformed with AAD-12. Ex. 1001 at Examples 16 and 17. Examples 18-20 describe stacking AAD-12 with the glyphosate tolerate trait (Example 18), the glufosinate tolerance trait (Example 19), and the imidazolinone herbicide tolerance (AHAS) trait (Example 20). Ex. 1001 at 86:23-91:28. Example 23 describes stacking AAD-12 with insect resistance or other input traits. Ex. 1001 at 99:21-100:5. Example 24 discloses using the AAD-12(v1) gene as an *in vitro* selectable marker in transformed dicot crops. Ex. 1001 at 100:6-102:49.

86. In sum, the specification of the '055 patent provides extensive and detailed descriptions and examples of how to make and use the claimed AAD-12 transgenic plants.

**IX. THE '055 PATENT SPECIFICATION WOULD HAVE ENABLED A SKILLED ARTISAN TO PRACTICE THE CLAIMED INVENTION WITHOUT UNDUE EXPERIMENTATION**

87. As I discussed above in Section V, I was informed that whether a patent enables skilled artisans in the field to make and use the claimed invention without undue experimentation can be determined by multiple factors, including (1) the nature of the invention; (2) the state of the art; (3) the level of skill required to practice the claimed invention; (4) the predictability of the field; (5) breadth of the claim; (6) the guidance and examples provided in the patent specification; and (7) the quantity of the required experimentation. After considering these factors, I conclude that the '055 patent would have enabled skilled artisans in the field in 2005-2006 to practice the claimed invention without undue experimentation.

**A. Level of Skill in the Art**

88. As I explained in Section VII, persons skilled in the art in 2005-2006 would have had a Ph.D. degree in molecular biology, biotechnology, or plant biochemistry and a few years of experience in transformation of a variety of plant species. Such skilled artisans were familiar with the biology and characteristics of different monocot and dicot plant species, methods of preparing and codon

optimizing transgenes and expression vectors suitable for plants, methods of transforming plant cells and regenerating plants, and plant transformant identification. Skilled artisans were also capable of tailoring and optimizing known methods to improve transformation efficiency for different plant species based on their knowledge and experience.

**B. Nature of the Invention and Scope of the Claims**

89. The '055 patent claims are generally directed to transgenic plant cells and plants that produce AAD-12 proteins sharing a common motif and at least 85% sequence identity to SEQ ID NO: 2 and having dual herbicide-degrading activity (i.e., degrading phenoxy and pyridyloxy auxin herbicides), as well as methods of controlling weeds in a field including such transgenic plants. Ex. 1001 at claims 1-32.

90. With AAD-12-encoding genes and the disclosure of the '055 patent in hand, a skilled artisan would have been able to routinely make and use the claimed AAD-12 transgenic plants without undue experimentation. Indeed, the scope of the claimed transgenic plants is reasonable. A skilled artisan would understand that the transgenic plants covered by the patent claims generally fall into two categories: monocot and dicot plants. As I explained in Section VI.B, as of 2005-2006, the field had advanced to the point that transformation protocols were well established, expression cassettes suitable for transforming both monocots and dicots were readily available, and transgenic monocot and dicot plant species were routinely produced

with successful expression. *E.g.*, Ex. 2069 (McElroy 1991), 150; Ex. 2070 (Christensen and Quail 1996), 217; Ex. 2072 (Mitsuhara 1996), 51; Ex. 2062 (Potenza 2004), 3. When successful transformation, expression, and activity had been confirmed across multiple transformed monocots, as in the '055 patent, a skilled artisan would have expected similar performance generally across monocots. The same holds true for dicots. *E.g.*, Ex. 2072 (Potenza 2004), 3 (acknowledging that many promoters “drive high expression” across dicots or monocots); Ex. 2075 (Altpeter 2005), 305-306 (describing “versatile and effective transformation ... not limited by cell type, species or genotype”).

**C. The Specification’s Direction, Guidance, and Working Examples in View of the State of the Art**

91. Once a skilled artisan had AAD-12-encoding genes in hand, optionally codon-optimizing them and using them to make AAD-12 transgenic plants across the scope of the claims would have involved only routine, standard techniques in 2005-2006.

92. Plant transformation generally involves steps of (1) building an expression cassette to drive transgene expression, (2) transforming plant cells, and (3) regenerating (under selection in most cases) the post-transformation plant cells into whole plants. The transgene may optionally be codon optimized. By 2005-2006, the techniques required for each of these steps were readily available and routinely used by skilled artisans to successfully generate transgenic monocot and dicot plants.

*Supra* § VI.B.2. Validation of successful transformation using a selectable marker was also straightforward and well established. *Supra* §§ VI.B.3-5.

93. The '055 patent provides detailed descriptions of each step and includes working examples demonstrating successful AAD-12 transformation in multiple monocot and dicot plants. In my opinion, the '055 patent provides more than sufficient guidance and examples for skilled artisans to make the claimed transgenic plant cells and plants without undue experimentation. Indeed, the time required to make the AAD-12 transgenic plants would have mainly been a function of plant biology (e.g., letting tissues grow and regenerate into plants after transformation), not the routine tasks performed by skilled artisans to transform plants, which generally do not require extensive time.

#### **1. Codon Optimization (optional)**

94. As explained in Section VI.B.1, it was basic knowledge that plant codon usage is biased towards higher G+C content. To further improve transgene expression in plants, skilled artisans in 2005-2006 could optionally optimize the codons of a transgene to increase the G+C content and adapt to the codon bias in plants. As of 2005, the codon usage bias of various plant species had been published, and skilled artisans had improved transgene expression in multiple plant species by codon optimization. *See e.g.*, Ex. 2064 (Perlak 1991), 3325; Ex. 2081 (WO1997/13402); Ex. 2065 (Murray 1989).

95. The '055 patent explains in detail the rationale and strategy of codon optimization for transgene expression in plants and refers to known codon usage calculation tools and codon optimization methods. Ex. 1001 at 32:27-34:67; Ex. 2013 at [00125]-[00132]; Exs. 2014, 2017 at [00152]-[00159]; Ex. 2015 at [00155]-[00162]; Ex. 2016 at [00159]-[00166]. Example 2 of the '055 patent describes generating a transgene encoding an AAD-12 variant (AAD-12(v1)) (SEQ ID NO:3) by optimizing the codons for expression in monocot and dicot plants based on calculated maize codon usage bias. Ex. 1001 at 35:1-29, Table 5; Ex. 2013 at [00134], Table 5; Exs. 2014, 2017 at [00161], Table 5; Ex. 2015 at [00164], Table 5; Ex. 2016 at [00167], Table 5. The patent shows that when the codon-optimized AAD-12(v1) gene was used to transform different monocot and dicot plants, it achieved success in each example. Exs. 1001, 2013-2017 at Examples 7, 8, 10-12, 14, 21, 22.

96. Dr. Silverstone states that “the scientist needs to know ... how to codon optimize the coding portion of the gene for a given plant species.” Ex. 1003 at ¶ 72. As detailed above, however, codon optimization was routine and well within the abilities of a skilled artisan in 2005-2006. The '055 patent specification's detailed descriptions would have enabled a skilled artisan to similarly optimize codons of other AAD-12 proteins for enhanced expression in plants. Moreover, by 2005-2006, computer programs existed that automated codon optimization, minimizing the effort required by a skilled artisan. Ex. 1001, 39:27-40:25 (describing use of the

OptGene™ program); Ex. 2013 at [00141]-[00142]; Exs. 2014, 2017 at [00168]-[00169]; Ex. 2015 at [00171]-[00172]; Ex. 2016 at [00174]-[00175].

## **2. Expression Vectors**

97. As explained above in Section VI.B.2, in 2005-2006, researchers routinely built plant expression cassettes that achieved successful expression of transgenes in different plant species.

98. For example, expression cassettes comprising promoters known to be suitable for expression in monocot plants, such as those derived from monocot plant actin genes (*Act*) or ubiquitin genes (*Ubi*), were commonly used. Ex. 2069 (McElroy 1991), 150; Ex. 2070 (Christensen and Quail 1996), 213. By 2005-2006, skilled artisans also routinely built expression cassettes comprising dicot promoters, such as CaMV 35S, CsVMV, dicot *Act* promoters, and dicot *Ubi* promoters, for dicot plant transformation. Ex. 2062 (Potenza 2004), 3-4. It was also known that the same expression cassette can be used to successfully deliver different transgenes. Ex. 2010 (Paine 2005), 483. As the '055 patent acknowledges, a variety of known promoters and other regulatory components could also be used. Ex. 1001 at 27:36-28:19; Ex. 2013 at [00117]-[00118]; Exs. 2014, 2017 at [00127]-[00128]; Ex. 2015 at [00130]-[00131]; Ex. 2016 at [00133]-[00134].

99. The '055 patent provides detailed guidance on generating expression cassettes to deliver AAD-12-encoding transgenes. As shown in Table 8, which is

copied here, the '055 patent describes a series of expression cassettes comprising monocot or dicot promoters and other regulatory elements, which were tested for AAD-12(v1) expression in seven monocot or dicot plant species including *Arabidopsis*, cotton, soybean, tobacco, canola, rice, and corn.<sup>3</sup> Ex. 1001 and Exs. 2014-2017 at Table 8; Ex. 2013 at Table 7.

TABLE 8

| Binary constructs used in transformation of various plant species. |        |                       |                        |          |           |            |       |           |                          |                            |                      |          |               |
|--------------------------------------------------------------------|--------|-----------------------|------------------------|----------|-----------|------------|-------|-----------|--------------------------|----------------------------|----------------------|----------|---------------|
| pDAB #                                                             | pDAS # | Species* Trans-formed | Gene of interest (GOI) | Promoter | Feature 1 | Feature 2  | GOI 2 | Pro-moter | Bacterial Selection gene | Bacterial Selection gene 2 | Plant Selection gene | Promoter | Trxn Method   |
| 724                                                                | —      | A, Ct, S              | AAD12 v1               | AtUbi10  | NtOsm     | —          | —     | —         | Erythro-mycin            | —                          | pat                  | CsVMV    | Agro binary   |
| 3274                                                               | —      | A                     | AAD12 v1               | AtUbi10  | NtOsm     | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | —                    | —        | Agro binary   |
| 3278                                                               | 1580   | T                     | AAD12 v1               | CsVMV    | NtOsm     | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | AtUbi10  | Argo binary   |
| 3285                                                               | —      | A                     | AAD12 v1               | CsVMV    | NtOsm     | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | AtUbi10  | Argo binary   |
| 3759                                                               | —      | A, Ca, S              | AAD12 v1               | CsVMV    | NtOsm     | RB7 Mar v2 | EPSPS | AtUbi10   | Spectino-mycin           | —                          | pat                  | AtUbi10  | Argo binary   |
| 4101                                                               | 1863   | Cn, R                 | AAD12 v1               | ZmUbi1   | —         | RB7 Mar v2 | —     | —         | Ampicillin               | —                          | AHAS v3              | OsAct1   | Whiskers/ Gun |
| 4464                                                               | —      | S                     | AAD12 v1               | CsVMV    | —         | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | CsVMV    | Argo binary   |
| 4468                                                               | —      | S                     | AAD12 v1               | AtUbi10  | —         | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | CsVMV    | Argo binary   |
| 4472                                                               | —      | S                     | AAD12 v1               | AtUbi3   | —         | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | CsVMV    | Argo binary   |
| 4476                                                               | —      | S                     | AAD12 v1               | ZmUbi1   | —         | RB7 Mar v2 | —     | —         | Spectino-mycin           | —                          | pat                  | CsVMV    | Argo binary   |

<sup>3</sup> Dr. Silverstone states that the '055 patent only includes five examples of plant transformation (cotton, tobacco, canola, soybean and *Arabidopsis*). Ex. 1003 at ¶¶ 78, 92. I disagree and believe he fails to appreciate that Examples 21 and 22 of the '055 patent describe successful transformation of two more plant species, i.e., rice and canola, by AAD-12(v1). Exs. 1001, 2013-2017 at Examples 21 and 22.

TABLE 8-continued

| Binary constructs used in transformation of various plant species.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |        |                       |                        |          |           |            |       |           |                          |                            |                      |                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-----------------------|------------------------|----------|-----------|------------|-------|-----------|--------------------------|----------------------------|----------------------|-------------------|
| pDAB #                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | pDAS # | Species* Trans-formed | Gene of interest (GOI) | Promoter | Feature 1 | Feature 2  | GOI 2 | Pro-moter | Bacterial Selection gene | Bacterial Selection gene 2 | Plant Selection gene | Trxn Method       |
| 4480                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | —      | S                     | AAD12 v1               | AtAct2   | —         | RB7 Mar v2 | —     | —         | Spectinomycin            | —                          | pat                  | CsVMV Argo binary |
| <p>*A = <i>Arabidopsis</i> CsVMV = Cassava Vein Mosaic Virus Promoter<br/> ZmUbi1 = <i>Zea mays</i> Ubiquitin 1 Promoter AtUbi10 = <i>Arabidopsis thaliana</i> Ubiquitin 10 Promoter<br/> T = Tobacco<br/> HptII = hygromycin phosphotransferase Atubi3 = <i>Arabidopsis thaliana</i> Ubiquitin 3 Promoter<br/> S = Soybean AtAct2 = <i>Arabidopsis thaliana</i> Actin 2 Promoter<br/> Ct = Cotton RB7 Mar v2 = <i>Nicotiana tabacum</i> matrix associated region (MAR)<br/> R = Rice Nt Osm = <i>Nicotiana tabacum</i> Osmotin 5'<br/> Cn = Corn Untranslated Region and the <i>Nicotiana tabacum</i> Osmotin 3' Untranslated Region<br/> Ca = Canola</p> |        |                       |                        |          |           |            |       |           |                          |                            |                      |                   |

Exs. 1001 and 2014-2017, Table 8; Ex. 2013 at Table 7.

100. The '055 patent examples describe in detail building these expression cassettes comprising the AAD-12(v1)-encoding transgene into expression vectors and detecting successful expression of AAD-12(v1) in all tested monocot and dicot plants. Ex. 1001 at 42:1-29, 54:46-60, 56:43-60, 65:16-20, 66:57-59, 71:34-49, 73:20-74:16, 79:20-22, 81:23-55, 92:50-52, 94:5-17, 95:20-27, 96:47-55; Exs. 2013-2017 at Examples 3, 7, 8, 10, 11, 12, 21, and 22. Following this detailed guidance, skilled artisans would have been able to similarly generate expression cassettes to deliver other AAD-12 proteins into monocot or dicot plants.

101. Dr. Silverstone states that “the scientist needs to know how to choose the appropriate gene expression cassette, including promoter, terminator, other expression regulatory sequences.” Ex. 1003 at ¶ 72. As explained above, persons skilled in the art in 2005-2006 were already familiar with suitable promoters and the other regulatory elements and routinely built functional plant expression cassettes.

And the '055 patent additionally describes using the known regulatory elements to build appropriate expression cassettes and demonstrates success in using them in the examples to express AAD-12 proteins in transgenic plant cells. Based on such guidance and examples, persons skilled in the art with prior plant transformation experience would have been able to routinely build other expression cassettes suitable for monocot and/or dicot plants.

102. Dr. Silverstone also states that “variation of protein expression between independently transformed plants with the same gene expression cassette has been observed.” Ex. 1003 at ¶¶ 72, 76, 77 (citing Ex. 1018 and the '055 patent data in Tables 20 (tobacco), 25 (soybean), and 29 (canola)). This assertion does not appear to relate to the '055 patent claims, which do not require any particular level of protein expression. To the extent Dr. Silverstone contends that variability of protein expression indicates a failure of plant transformation, I disagree.

103. Dr. Silverstone cites Ex. 1018 regarding expression variability. This exhibit, however, reports that the vast majority of the tested tobacco callus lines successfully expressed at least one of the two tested transgenes, and 32 of the 45 tested tobacco callus successfully expressed both transgenes. Ex. 1018 at Table 1. Thus, Ex. 1018 does not show that an expression cassette failed to work for any transgene. The variability in protein expression levels of the two encoded proteins (e.g., up to 175-fold) and the ratios of their expression are entirely within ranges that

a skilled artisan would have expected when transforming a series of plant tissue samples (including tobacco).

104. Regarding the '055 patent's expression data, Dr. Silverstone overlooks that the patent discloses that despite the different levels of AAD-12(v1) protein expression in Tables 20 (tobacco), 25 (soybean), and 29 (canola), those transformed plant species *all demonstrated herbicide tolerance*. Ex. 1001 at 68:21-23 (“[e]very [tobacco] event tested was more tolerant to 2,4-D than the untransformed control line”), Table 21 (showing that the T<sub>0</sub> AAD-12(v1) tobacco plants gained tolerance to 2,4-D, triclopyr, and fluroxypyr), 76:40-44 (“All AAD-12 (v1)-containing plants were clearly resistant to the herbicide application versus the wildtype Maverick soybeans”); 97:40-41 (“Twenty-four of the [forty-five transformed canola] events were tolerant to the 2,4-D DMA herbicide application”), 98:62-65 (“These results indicate that AAD-12(v1) transformed events displayed a high level of resistance to 2,4-D, triclopyr and fluroxypyr at rates that were lethal or caused severe ... malformations to non-transformed canola.”); *see also* Ex. 2013 at [00244], Table 14; Exs. 2014, 2017 at [00295], Table 21, [00328], [00424], [00433]; Ex. 2015 at [00298], Table 21, [00331], [00427], [00436]; Ex. 2016 at [00301], Table 21, [00334], [00430], [00439]. As such, variability in protein expression levels in those plant species did not render AAD-12 transformation in those plants deficient in any way.

105. Dr. Silverstone states that the location of a transgene's insertion in the plant genome (also called "position effect") and transgene copy numbers in the expression cassette can affect heterologous protein expression. Ex. 1003, ¶¶ 73, 74, 76. This opinion also does not appear to relate to the '055 patent claims, which do not require any particular transgene insertion location within a plant's genome or any particular number of copies of the transgene. While position and number of transgene copies within a transgenic plant's genome may vary, the '055 patent describes success in expressing AAD-12 enzymes in all tested plant species without requiring analysis of the transgene insertion location or copy number. Exs. 1001, 2013-2017 at Examples 7, 8, 10-12, 14, 21, and 22.

### **3. Plant Transformation**

106. As detailed in Section VI.B.3, by 2005-2006, skilled artisans routinely carried out plant transformation, and protocols for plant transformation were well established. As the '055 patent discloses, transgenic corn, soybeans, canola, cotton, sugar beets, wheat, turf, and rice had been previously successfully transformed with an herbicide resistance gene. Ex. 1001, 1:23-27; Exs. 2013, 2014, 2016 and 2017 at [0002]; Ex. 2015 at [0003].

107. Multiple plant transformation methods were readily available to skilled artisans in 2005-2006. *See* Ex. 1001 at 24:25-62 ("A large number of techniques are available for inserting DNA into a plant host cell."); Ex. 2013 at [00106]; Exs. 2014,

2017 at [00116]; Ex. 2015 at [00119]; Ex. 2016 at [00122]. For example, bombardment methods involve shooting small DNA-coated metal particles into a large number (e.g., at least thousands) of plant cells and were characterized as “a versatile and effective transformation method, *not limited by cell type, species or genotype.*” Ex. 2075 (Altpeter 2005), 305-06 (emphasis added). As the ’055 patent discloses, the use of T-DNA from *Agrobacterium* was also available. Ex. 1001 at 24:21-24; Ex. 2013 at [00106]; Exs. 2014, 2017 at [00116]; Ex. 2015 at [00119]; Ex. 2016 at [00122].

108. As summarized in Section VIII.B, the examples of the ’055 patent provide detailed descriptions of transformation processes consistent with established transformation protocols as of 2005-2006. They describe preparing plant cells in different forms from various monocot or dicot species, transforming them via *Agrobacterium*-mediated, bombardment-mediated, or WHISKER-mediated transformation methods, and regenerating the treated plant cells/tissues/segments into whole plants, with successful expression of AAD-12(v1) in the transgenic plant cells and plants. Exs. 1001, 2013-2017 at Examples 7 and 14 (describing *Agrobacterium*-mediated transformation of one-month old *Arabidopsis* plants), Example 8 (describing WHISKERS-mediated transformation of maize embryonic cells in suspension), Example 10 (describing *Agrobacterium*-mediated transformation of tobacco leaves), Example 11 (describing *Agrobacterium*-mediated

transformation of soybean explants), Example 12 (describing *Agrobacterium*-mediated transformation of cotton cotyledon segments), Example 21 (describing use of biolistics to transform suspension-derived embryogenic rice tissues), and Example 22 (describing *Agrobacterium*-mediated transformation of canola hypocotyl segments).

109. In view of the '055 patent's detailed guidance and successful working examples, a skilled artisan in 2005-2006 would have been able to use routine transformation methods to similarly deliver other AAD-12-encoding transgenes into these and other plant cells without undue experimentation.

#### **4. Regeneration of Transformed Plants**

110. As explained in Section VI.B.4, by 2005-2006, skilled artisans also routinely regenerated plant cells in various forms that underwent the transformation process into whole plants by incubating the cells/tissues/segments/explants in callus induction medium, shoot induction medium, and root induction medium to induce the cells/tissues/segments/explants to grow into plantlets with shoots and roots for planting into soil in a greenhouse. *See e.g.*, Ex. 2076 (Schroeder 1993). The '055 patent provides detailed examples of regenerating transformed *Arabidopsis*, corn, tobacco, soybean, cotton, rice, and canola into plants. Ex. 1001 at 50:28-33, 58:60-59:16, 66:64-67:27, 75:5-33, 81:23-38, 92:60-66, 95:42-96:22; Exs. 2013-2017 at Examples 7-8, 10-12, 21-22. As exemplified in the '055 patent, those T<sub>0</sub> primary

transformants could then be grown to produce seeds, which could be used to produce T<sub>1</sub> and later generation progeny plants. Ex. 1001 at 50:28-33, 62:12-14, 63:14-28, 69:13-36, 70:15-26, 76:18-31, 81:58-82:6, 93:10-21, 97:47-60; Exs. 2013-2017 at Examples 7-8, 10-12, 21-22.

111. The '055 patent discloses that selectable markers may be used to identify successful transformants while the cells that underwent transformation are regenerating into plants, or at the T<sub>0</sub> or later generations.

112. For example, the '055 patent provides detailed guidance on using the AAD-12 gene as a novel selectable marker to quickly identify successfully transformed plant cells and plants expressing AAD-12 proteins having *in planta* activity. Ex. 1001 at 53:18-39 (“AAD-12 (v1) as a Selectable Marker”), 13:2-5, 13:66-14:2, 14:13-45, Examples 7, 8, 10-12, 14, 21 and 22; Ex. 2013 at [00192]-[00193], [0053], [0060]-[0061]; Exs. 2014, 2017 at [00223]-[00225], [0058], [0065]-[0066]; Ex. 2015 at [00226]-[00228], [0061], [0068]-[0069]; Ex. 2016 at [00229]-[00231], [0064], [0071]-[0072]; Exs. 2013-2017 at Examples 7, 8, 10-12, 14, 21, and 22. Example 24 discloses using the AAD-12 gene as a selectable marker shortly after transformation in plant cells. Ex. 1001 at 100:6-102:49; Exs. 2014, 2017 at [00436]-[00451]; Ex. 2015 at [00439]-[00454]; Ex. 2016 at [00441]-[00456]. As the '055 patent's examples illustrate, relying on such guidance, a skilled artisan could similarly identify successfully transformed cells and plants at any desired time

point. Ex. 1001, 9:52-57 (“Plantlets are typically resistant throughout the entire growing cycle.”); Exs. 2014, 2017 at [0042]; Ex. 2015 at [0045]; Ex. 2016 at [0048].

**D. The Amount of Experimentation Would Not Have Been Undue or Unpredictable in 2005-2006**

113. In my opinion, it would have only required the application of routine techniques in the field of plant transformation to make and use the full scope of AAD-12 transgenic plants of the '055 patent claims. Once a skilled artisan had AAD-12 genes in hand, making transgenic plants would generally only involve: (1) preparing an expression cassette with the AAD-12 gene, (2) carrying out a known transformation technique, and (3) regenerating the transformed plant cells into plants. The time required to make transgenic plants in 2005-2006 was mainly a function of plant biology, i.e., letting plant tissues grow for transformation and regenerate into plants after transformation. It did not require extensive hands-on experimentation by skilled artisans. Nothing beyond what the '055 patent discloses was needed to perform the routine work to generate successful transformants. Accordingly, in my opinion, transgenic plant production would not have required undue experimentation in 2005-2006.

114. To produce transgenic plants, skilled artisans in 2005-2006 typically used immature plant tissues (e.g., embryos, young leaves, or pieces of young seedlings referred to as explants). Immature plant tissues are more susceptible to genetic transformation and regeneration, whereas older plant tissues may not be able

to incorporate a transgene effectively or grow new cells containing the transgene. For example, cotton cotyledon segments were often prepared from seedlings that were only 7-10 days old. Ex. 1001 at 80:7-9; Ex. 2013 at [00275]; Exs. 2014, 2017 at [00347]; Ex. 2015 at [[00350]; Ex. 2016 at [00353]. As another example, the '055 patent discloses extracting maize embryos at 9-10 days after pollination, which were then used to form callus tissue for WHISKERS-mediated transformation over 2-3 months. Ex. 1001 at 56:61-57:38; Ex. 2013 at [00210]-[00213]; Exs. 2014, 2017 at [00244]-[00247]; Ex. 2015 at [00247]-[00250]; Ex. 2016 at [00250]-[00253]. By 2005-2006, skilled artisans often skipped a pre-transformation callusing step when transforming corn because as techniques such as particle bombardment can be carried out directly on maize embryos themselves, which shortens the process. *See e.g.*, Ex. 2082 (Frame 2001).

115. Dr. Silverstone fails to acknowledge that large quantities of immature plant tissue for transformation were routinely prepared on a rapid basis in 2005-2006. The physical act of working with small embryos and other immature plant tissues was generally delegated to laboratory technicians who often did not have (and did not need) any advanced scientific education. Protocols for plant transformation were well established and thus it was advantageous to have technicians with skill and dexterity in working with small plant tissues perform these tasks. A technician, for example, could trim a small, few-days-old cotton seedling with a scalpel into

transformation-ready cotyledon segments within a matter of seconds. This amounted to a few hundred segments per hour. A technician could also extract immature maize embryos (often on the order of about one millimeter in size) from kernels rapidly, processing hundreds of 9-10 days post-pollination maize embryos per hour. These tissues were then immediately available for use in plant transformation, e.g., via particle bombardment. Some artisans in 2005-2006, however, preferred callus tissue for certain transformations because callus tissue can provide more potentially transformable cells, e.g., for WHISKERS-mediated transformation. One such example of transforming callus tissue is in Example 8 of the '055 patent. Ex. 1001 at 57:3-38; Ex. 2013 at [00212]-[00213]; Exs. 2014, 2017 at [00246]-[00247]; Ex. 2015 at [00249]-[00250]; Ex. 2016 at [00252]-[00253].

116. Skilled artisans in 2005-2006 were able to carry out many of the transformation-related tasks in parallel. Codon optimization, if desired, could be rapidly carried out within a day (or faster with software automation). Ex. 1001, 39:27-40:25; Ex. 2013 at [00141]-[00142]; Exs. 2014, 2017 at [00168]-[00169]; Ex. 2015 at [00171]-[00172]; Ex. 2016 at [00174]-[00175]. Skilled artisans could also quickly prepare plasmid expression vectors containing the AAD-12 transgenes, particularly with the '055 patent's disclosure of exemplary cassettes used with AAD-12 genes that a skilled artisan would be able to re-use. These included cassettes used to transform multiple monocot species, as well as cassettes used to transform

multiple dicot species. Ex. 1001 at 42:1-29, Table 8; Ex. 2013 at [00150], Table 7; Exs. 2014, 2017 at [00177], Table 8; Ex. 2015 at [00180], Table 8; Ex. 2016 at [00183], Table 8. The expression vectors may be incorporated into *Agrobacterium* and a population of *Agrobacterium* may be cultured in about 2-4 days, as the '055 patent discloses. Ex. 1001, 49:18-50:7; Ex. 2013 at [00177]-[00180]; Exs. 2014, 2017 at [00207]-[00210]; Ex. 2015 at [00210]-[00213]; Ex. 2016 at [00213]-[00216]. Another option was to coat gold particles with the expression vectors for biolistics-mediated transformation, which took 15-30 minutes. Ex. 1001, 92:44-59; Ex. 2013 at [00318]; Exs. 2014, 2017 at [00394]; Ex. 2015 at [00397]; Ex. 2016 at [00399].

117. As the '055 patent illustrates, performing routine transformation techniques on the prepared plant tissues (e.g., embryos, explants, etc.) was generally quick. Transforming *Arabidopsis* involved dipping the plants into *Agrobacterium* media for 15 seconds. Ex. 1001, 50:26-28; Ex. 2013 at [00182]; Exs. 2014, 2017 at [00212]; Ex. 2015 at [00215]; Ex. 2016 at [00218]. Biolistics is similarly quick: a plate of 20-30 embryos could be bombarded with a single shot of vector-coated gold particles. In 2005-2006, sophisticated biolistics systems minimized the need for human intervention, such as the Biolistic PDS-100/He<sup>TM</sup> system disclosed in the '055 patent, which is still in use today. Ex. 1001, 92:45-59 (biolistics transformation of rice); Ex. 2013 at [00318]; Ex. 2014, 2017 at [00394]; Ex. 2015 at [00397]; Ex. 2016 at [00399].

118. Post-transformation, a skilled artisan would have had AAD-12 transformed plant cells in hand, as recited in claim 1. To regenerate plants (e.g., as recited in claim 4), the necessary time was generally dictated by plant biology. Following the 15-second *Agrobacterium*-mediated transformation of *Arabidopsis*, for example, the *Arabidopsis* plants were recovered for 24 hours, washed, and then grown over four weeks. Ex. 1001 at 50:26-33; Ex. 2013 at [00182]; Exs. 2014, 2017 at [00212]; Ex. 2015 at [00215]; Ex. 2016 at [00218]. Following the biolistics-mediated transformation of rice callus, the transformed cells were selected after 14-17 days of incubation, the selected cells were then incubated for another 8-12 days, and then rice shoots were regenerated on standard regeneration media. Ex. 1001, 92:8-43; Ex. 2013 at [00316]; Exs. 2014, 2017 at [00392]; Ex. 2015 at [00395]; Ex. 2016 at [00397]. Regeneration of other crop tissues followed similar approaches, resulting in inactive downtime on the part of skilled artisans while plants grow or tissues incubate according to known, biological timelines.

119. As explained above, the '055 patent established that AAD-12 genes function effectively as selectable markers. Ex. 1001, 53:18-39, Example 24; Ex. 2013 at [00192]-[00193]; Exs. 2014, 2017 at [00223]-[00225], Example 24; Ex. 2015 at [00226]-[00228], Example 24; Ex. 2016 at [00229]-[00231], Example 24. Skilled artisans were thus well equipped to efficiently identify transformed plants or plant cells at essentially any time point after transformation

and verify their dual herbicide-degrading activity by spraying, painting, or incubating with the herbicides of interest. The '055 patent provides significant guidance and useful data regarding the variety of herbicide concentrations that may be used in the summary tables disclosed in the transformation examples. Exs. 1001 and 2014-2017 at Example 7 (Tables 11-13), Example 8 (Tables 18-19), Example 10 (Tables 20-21), Example 11 (Table 25), Example 21 (Tables 27-28), Example 22 (Tables 30-31). The '055 patent also confirms that AAD-12 selectable marker detection may occur at a variety of time points ranging from essentially immediately post-transformation, in the original  $T_0$  transformants, or in  $T_1$  or later progeny.

120. Accordingly, in my opinion, only a reasonable amount of experimentation would have been required in 2005-2006 to make and use the AAD-12 transgenic plants and plant cells of the '055 patent claims. The '055 patent provides extensive examples and other technical disclosures and guidance to enable skilled persons to practice the claimed subject matter, the necessary plant transformation techniques were well established in the art, skilled artisans routinely and reliably transformed large numbers of monocot and dicot plants, and the overall timeframe for plant transformation was mainly a function of plant biology that only involved waiting for plant tissues to grow and regenerate, not the efforts of a skilled artisan. For these reasons, no undue experimentation would have been required for a skilled person to carry out the claimed invention.

**E. Dr. Silverstone Incorrectly Relies on Unnecessary, Commercial Development-Level Tasks in His Enablement Analysis**

121. In his declaration, Dr. Silverstone unnecessarily complicates the routine plant-transformation steps that existed in 2005-2006 with additional commercial-level optimization tasks that a skilled artisan would have understood were unnecessary for making the AAD-12 transgenic plants and plant cells of the '055 patent claims. These unnecessary tasks include:

- a. Codon-optimizing genes for each plant species. Ex. 1003 at ¶ 72.
- b. Modifying expression cassettes for each plant species. *Id.*
- c. Performing nucleic analytic techniques to evaluate the transformation and transgene expression. *Id.* at ¶ 74.
- d. Propagating plants for several generations before herbicide tolerance can be assessed. *Id.* at ¶ 75.
- e. Testing herbicide tolerance over a range of commercially recommended (and higher) herbicide concentrations and growing environments. *Id.* at ¶ 93.
- f. Transforming a large number of transgenic plant species for each AAD-12 gene used. *Id.* at ¶¶ 73, 76.

As detailed below, I disagree that any of these tasks were necessary. Indeed, skilled artisans frequently omitted most of them in 2005-2006, particularly in early, pre-commercialization stages of transgenic crop development.

122. Dr. Silverstone's assertion that AAD-12 genes would need to be codon optimized for each "given plant species" is incorrect in the context of the '055 patent. As explained above, plant-preferred codons (e.g., high in G+C content) and codon frequency were known in 2005-2006. In practice, a single (optional) codon optimization was typically sufficient. The '055 patent illustrates this concept, as all monocot and dicot plant transformations were carried out with an AAD-12(v1) gene that was codon optimized for maize (i.e., corn). Exs. 1001 and 2014-2017, Example 2, Example 3 (Table 8); Ex. 2013, Example 2, Example 3 (Table 7). As explained earlier, characterization of AAD-12 enzymes in plant species need not rise to the level of commercial efficacy testing, which may rely on optimization techniques such as codon preference and other construct designs.

123. I also disagree with Dr. Silverstone's assertion that an expression cassette "likely requires modification for each plant species that will be transformed." Ex. 1003 at ¶ 72. Skilled artisans in 2005-2006 routinely re-used expression cassettes across different crops, as their components (e.g., promoter, transgene, etc.) generally did not require species-specific modification. As explained above, dicot promoters (e.g., CSMV or CsVMV) generally work well across dicot species and monocot

promoters (e.g., maize Ubi1) generally work well across monocot species. The '055 patent again provides good examples of this concept, illustrating that the same binary construct pDAB4101 was successfully used for WHISKERS-mediated transformation of corn and for biolistics-mediated transformation of rice, which are both monocots. Exs. 1001 and 2014-2017, Examples 8, 21, Table 8; Ex. 2013 at Examples 8, 21, Table 7. It similarly discloses using the same pDAB724 construct for *Agrobacterium*-mediated transformation of *Arabidopsis*, cotton, and tobacco, which are all dicots. Exs. 1001 and 2014-2017, Examples 7, 12, 24; Ex. 2013 at Examples 7 and 12. It also discloses using the same pDAB3759 construct for *Agrobacterium*-mediated transformation of *Arabidopsis* and canola, which are also both dicots. Exs. 1001, 2013-2017 at Examples 7, 22. Accordingly, even if a skilled artisan wished to change expression vectors for any reason, the '055 patent provides a variety of exemplary expression vectors for use.

124. Dr. Silverstone incorrectly contends that a skilled artisan would need to carry out “meticulous experimentation” to confirm proper expression in transgenic plants, including nucleic acid analytic techniques to confirm the presence of the transgene, number of copies, intactness of the inserted DNA, location in the genome, and expression of the protein. Ex. 1003, ¶ 74. None of these tasks were necessary for pre-commercial production of AAD-12 transgenic plants, however, because an easier, simpler technique was known and disclosed in the '055 patent: using a

selectable marker (e.g., AAD-12 gene) and spraying or leaf-painting with an herbicide of interest to confirm activity. Dr. Silverstone fails to mention the availability of selectable markers, even though they were the predominant method of quickly and easily evaluating successful enzyme expression in 2005-2006. The nucleic acid analyses Dr. Silverstone refers to may be required by regulatory authorities once a company is seeking deregulation (i.e., approval) of a lead commercial candidate, but those analyses were not needed (and uncommon) in earlier pre-commercialization stages of transgenic crop development.

125. Dr. Silverstone's assertion that plants must be "propagated for several generations" to assess herbicide tolerance is also incorrect. A skilled artisan would have understood that herbicide tolerance could be routinely assessed at essentially any time point, including when incubating transformed plant cells or in the T<sub>0</sub>, T<sub>1</sub>, or later plant generations. Indeed, the '055 patent explains that even young plantlets "are typically resistant throughout the entire growing cycle" and that transformed plants "will typically be resistant to new herbicide application at any time the gene is expressed." Ex. 1001 at 9:52-57; Ex. 2014, 2017 at [0042]; Ex. 2015 at [0045]; Ex. 2016 at [0048]. This was verified in the examples, where phenoxy auxin and pyridyloxy auxin herbicide tolerance were confirmed at a variety of early time points as well as in later plant generations as a skilled artisan would have expected. Exs.

1001 and 2014-2017 at Examples 7, 8, 10-12, 14, 21, 22, 24; Ex. 2013 at Examples 7, 8, 10-12, 14, 21, 22, 24.

126. I also disagree with Dr. Silverstone's position that herbicide tolerance must be evaluated across a range of commercially recommended (and higher) herbicide concentrations and growing environments. Ex. 1003 at ¶ 93. No such testing would have been necessary to verify that an AAD-12 gene has been successfully incorporated into a transgenic plant of the '055 patent claims. The '055 patent provides significant guidance as to the appropriate application rates to use with phenoxy auxin and pyridyloxy auxin herbicides. Ex. 1001 at 70:21-71:9, Table 22 (AAD-12 transformed tobacco "displayed a high level of tolerance to 2,4-D, triclopyr and fluroxypyr at multiples of commercial use rates...."); *id.* at 65:1-20, Tables 18-19 ("AAD-12 (v1) conveys high level tolerance in corn to 2,4-D, triclopyr and fluroxypyr at rates far exceeding those commercially used."); *see also* Ex. 2013 at [00251]-[00252], Table 15; Exs. 2014-2017 at Table 22, Tables 18-19. The '055 patent also discloses field testing examples, and it is thus unclear what additional growing conditions Dr. Silverstone believes skilled artisans would have to assess to evaluate whether an AAD-12 transgenic plant performed as claimed.

127. Dr. Silverstone also vaguely states that a "large number of independent transgenic plants need to be generated" for each AAD-12 gene based on position effects (e.g., where a gene may insert into the plant genome). Ex. 1003, ¶¶ 73, 76. I

disagree. In 2005-2006, skilled artisans in academia and in industry routinely transformed only a small number of plants (e.g., less than 50 plants in a typical academic lab and less than 100 plants in industry) with a single transgene while predictably achieving success. A particle bombardment plate, for example, holds about 20 to 30 embryos (each about one millimeter in size), and all are bombarded simultaneously with a single shot. The '055 patent supports my opinion, disclosing that high percentages of tested AAD-12 transgenic events exhibited herbicide tolerance. For example:

- Soybeans: “All AAD-12 (v1)-containing plants were clearly resistant to the herbicide application versus the wild type Maverick soybeans.” Ex. 1001 at 76:42-44; Exs. 2014, 2017 at [00328]; Ex. 2015 at [00331]; Ex. 2016 at [00334].
- Tobacco: “Every event tested was more tolerant to 2,4-D than the untransformed control line KY160.” Ex. 1001 at 68:21-23; Ex. 2013 at [00244]; Exs. 2014, 2017 at [00295]; Ex. 2015 at [00298]; Ex. 2016 at [00301].
- Canola: Twenty-four of forty-five T<sub>0</sub> “events were tolerant to the 2,4-D DMA herbicide application.” Ex. 1001 at 97:12-45; Exs. 2014, 2017 at [00424]; Ex. 2015 at [00427]; Ex. 2016 at [00429].

128. Accordingly, I disagree that it would have required undue experimentation in 2005-2006 to make and use the AAD-12 transgenic plants of the '055 patent claims. The field had only advanced further by the 2017 date that Dr. Silverstone relies upon, and therefore in my opinion the claims are enabled regardless of the date applied.

**X. THE DISCLOSURE OF THE '055 PATENT SUFFICIENTLY DESCRIBES THE CLAIMED INVENTION**

129. As detailed above, I understand that written description is assessed from the perspective of a skilled artisan and that factors relevant to written description include the existing knowledge in the field, the extent and content of the prior art, the maturity of the technology, and the predictability of the technology at issue. In my opinion, a skilled artisan in 2005-2006 with knowledge of plant transformation would have recognized that the inventors possessed the full scope of the claimed transgenic plants and plant cells.

130. The '055 patent specification describes transgenic plants and plant cells that express AAD-12 proteins exhibiting the recited dual herbicide degradation activity, and also provides many examples demonstrating successful transformation of monocot and dicot plants. *Supra* §§ VIII.B and IX.C. As of 2005-2006, the field of plant transformation was also advanced and known expression cassettes and transformation methods had been routinely used to successfully transform plants. A skilled artisan therefore would have considered the '055 patent's description more

than adequate to demonstrate possession of the claimed transgenic plants and plant cells.

**A. The Patent Sufficiently Describes the Claimed Transgenic Monocot and Dicot Plants Comprising the Recited AAD-12 Proteins**

131. The specification of the '055 patent provides multiple examples showing successful transformation of monocot and dicot plants through a variety of techniques with expression vectors containing an AAD-12 gene. A skilled artisan would have understood that the monocot and dicot plants used in the transformation examples were representative of transgenic monocots and dicots generally. Indeed, the dicots *Arabidopsis* and tobacco used in the patent examples are classic models of plant biology, and with success in these two models, skilled artisans expected success across dicot crops. The monocots used (corn and rice) and the other dicots used (soybean, cotton, and canola) in the patent examples are among the most widely grown crops and have been previously transformed with various transgenes to improve their use in agriculture. *E.g.*, Ex. 2075 (Altpeter 2005), 305-06 (bombardment is “a versatile and effective transformation method, *not limited by cell type, species or genotype.*”) (emphasis added); Ex. 2069 (McElroy 1991), 150 (expression vectors used in producing transgenic rice “should promote the expression of foreign genes *in most, if not all, transformed monocot cells.*”) (emphasis added); Ex. 2072 (Mitsuhara 1996), 51, 53, 58 (cassettes comprising a

core CaMV 35S dicot promoter are “representative of promoters of highest-level expression in dicotyledonous plants”).

132. Specifically, the '055 patent describes the successful transformation of corn, a monocot plant, with a corn-optimized DNA sequence AAD-12(v1), reporting that (1) two of the three tested T<sub>1</sub> AAD-12(v1) corn events “were robustly tolerant to 2,4-D and 2,4-D + imazethapyr or 2,4-D + glyphosate”; (2) “AAD-12 is heritable as a robust aryloxyalkanoate auxin resistance gene”; and (3) “AAD-12 (v1) conveys high level tolerance in corn to 2,4-D, triclopyr and fluroxypyr at rates far exceeding those commercially used.” Ex. 1001 at 62:36-55, Table 16, 63:15-29, 64:28-65:20, Tables 18-19; Exs. 2014-2017 at Table 16, Tables 18-19.

133. The '055 patent also provides data for AAD-12(v1)-transformed rice, another monocot plant species, and similarly confirms transformation and heritability of AAD-12(v1) in T<sub>1</sub> rice while also verifying high 2,4-D and triclopyr tolerance. Ex. 1001 at 93:8-94:67; Exs. 2014, 2017 at [00397]-[00409]; Ex. 2015 at [00400]-[00412]; Ex. 2016 at [00402]-[00414]. Based on data from a field tolerance trial conducted with a T<sub>1</sub> AAD-12(v1) event, the patent concludes that “[t]hese results indicate that the AAD-12(v1) transformed rice displayed a high level of resistance to 2,4-D and triclopyr...” Ex. 1001 at 94:20-64; Exs. 2014, 2017 at [00409]; Ex. 2015 at [00412]; Ex. 2016 at [00414].

134. The specification also demonstrates delivering the AAD-12(v1) transgene to multiple dicot plant species including *Arabidopsis*, soybean, cotton, tobacco, and canola, and describes successful expression of AAD-12(v1). These successful transformation experiments are summarized below:

- *Arabidopsis*: Examples 7 and 14 report that (1) the AAD-12(v1) gene “imparted herbicide resistance” to the transformed *Arabidopsis* plants compared to the wild type plants; and (2) “[t]hese results confirm that AAD-12(v1) in *Arabidopsis* provides resistance to the pyridyloxyacetic auxins tested.” Ex. 1001 at 51:59-52:11, 54:13-18, Table 13, 84:60-85:9, 70:21-71:9; Ex. 2013 at [00191], [0199], Table 12; Exs. 2014, 2017 at [00221], [00231], Table 13; Ex. 2015 at [00224], [00234], Table 13; Ex. 2016 at [00227], [00237], Table 13.
- Tobacco: Example 8 reports results that “indicate that AAD-12 (v1) transformed [tobacco] event lines displayed a high level of tolerance to 2,4-D, triclopyr and fluroxypyr at multiples of commercial use rates.” Ex. 1001 at 71:4-9, Table 22; Ex. 2013 at [00253]; Exs. 2014, 2017 at [00304]; Ex. 2015 at [00307]; Ex. 2016 at [00310].
- Soybean: Example 11 reports that the 2,4-D tolerance data from AAD-12(v1)-transformed soybean “are consistent with the fact that AAD-12 (v1) can impart high tolerance (>2x field rates) to a sensitive crop like

soybeans.” Ex. 1001 at 76:48-50, Table 25; Exs. 2014, 2017 at [00328], Table 25; Ex. 2015 at [00331], Table 25; Ex. 2016 at [00334], Table 25.

- Cotton: Example 12 reports that almost 200 callus events were screened for AAD-12(v1) expression using western blot analysis, confirming that multiple AAD-12(v1) cotton events expressed the AAD-12(v1) protein. Ex. 1001 at 81:38-56; Exs. 2014, 2017 at [00353]; Ex. 2015 at [00356]; Ex. 2016 at [00358].
- Canola: Example 22 reports that “[t]hese results indicate that AAD-12 (v1) transformed [canola] events displayed a high level of resistance to 2,4-D, triclopyr and fluroxypyr at rates that were lethal or caused severe [] malformations to non-transformed canola.” Ex. 1001 at 98:62-67; Exs. 2014, 2017 at [00433]; Ex. 2015 at [00436]; Ex. 2016 at [00438].

135. Thus, the ’055 patent demonstrates successes in transforming multiple dicot plants to express a claimed AAD-12 protein. The patent describes similarly transforming other crops with the AAD-12-encoding transgenes using known techniques and identifies exemplary additional crops that would benefit from AAD-12 transformation. Exs. 1001, 2013-2017 at Example 13.

136. Thus, in view of the ’055 patent’s disclosures and examples across a variety of monocots and dicots, a skilled artisan would have understood that the inventors possessed the AAD-12 transgenic plants and plant cells of the claims.

## **B. The State of Art Was Advanced in 2005-2006**

137. As the '055 patent acknowledges, by 2005-2006, transgenic crops resistant to herbicides had been routinely developed, and the field of generating herbicide-tolerant transgenic plants was advanced. Ex. 1001 at 1:23-27 (“Crops, such as corn, soybeans, canola, cotton, sugar beets, wheat, turf, and rice, have been developed that are resistant to glyphosate”); Exs. 2013, 2014, 2016-2017 at [0002]; Ex. 2015 at [0003]. Indeed, at that time, glyphosate tolerant soybean, cotton, corn, and canola were commercially available in the United States,” and “[m]any other glyphosate resistant species” such as sugar cane, sunflower, beets, peas, carrot, cucumber, lettuce, onion, strawberry, tomato, and tobacco were in commercial development. Ex. 1001 at 1:28-54; Exs. 2013, 2014, 2016-2017 at [0003]; Ex. 2015 at [0004].

138. As explained in Section VI.B, by 2005-2006, plant transformation was routinely carried out using expression cassettes comprising known monocot or dicot promoters and other regulatory elements and known transformation methods. It was also known that established monocot or dicot promoters successfully drove high-level expression of a wide variety of transgenes across monocot and dicot plants, respectively. A number of transformation methods were characterized as “versatile and effective,” and “*not limited by cell type, species or genotype.*” Ex. 2075 (Altpeter 2005), 305-06 (emphasis added). The '055 patent acknowledges the advanced stage

of the field of plant transformation, describing known promoters and transformation methods that were used to build AAD-12 expression vectors and transform plants. Ex. 1001 at 27:36-28:19, 24:25-62; Ex. 2013 at [00117]-[00118], [00106]; Exs. 2014, 2017 at [00127]-[00128]; [00116]; Ex. 2015 at [00130]-[00131], [00119]; Ex. 2016 at [00133]-[00134], [00122].

139. The '055 patent describes a variety of expression vectors comprising known monocot or dicot promoters and shows that those expression vectors achieved successful expression of AAD-12(v1) in all tested monocot and dicot plant cells when used to transform those plant cells by routine methods. Exs. 1001, 2013-2017 at Examples 3, 7, 8, 10-12, 14, 21, and 22. Because those known monocot or dicot promoters and transformation methods had been reported to successfully express a variety of transgenes in different plant species and were again shown to be successful in the '055 patent examples, skilled artisans in 2005-2006 would have understood that they were suitable to similarly produce other transgenic plants and plant cells within the scope of the claims. *Supra* § VI.B. Thus, the advanced stage of plant transformation and herbicide-tolerant crop development in 2005-2006 would have further supported the '055 patent's detailed description of the AAD-12 transgenic plants and plant cells.

**C. Dr. Silverstone Incorrectly Requires *In Planta* Data for Each Plant Species and Inaccurately Describes the State of the Art**

140. In his declaration, Dr. Silverstone does not appreciate the '055 patent's multiple working examples and mistakenly requires *in planta* data for each plant species to support the claims. Ex. 1003 at ¶¶ 51-53, 78.

141. First, Dr. Silverstone states that the patent “includes five different crops (cotton, tobacco, canola, soybean and Arabidopsis)” that express a claimed AAD-12 protein variant. Ex. 1003 at ¶ 78. But importantly, he ignores that the '055 patent also demonstrates successful expression of AAD-12(v1) in rice and canola. Exs. 1001, 2013-2017 at Examples 21 and 22. Dr. Silverstone also ignores that the seven tested crops include multiple monocot and dicot plant species, which are representative of both categories of the claimed transgenic plants (i.e., monocots and dicots).

142. Second, Dr. Silverstone takes the position that it is “critical to demonstrate the activity in *each plant species* that needs to be tolerant to auxin herbicides” and that “Patent Owners did not perform all experiments with *each species* of transgenic plant.” Ex. 1003 at ¶¶ 51-53. I disagree. It was unnecessary for the '055 patent to provide test data for *each species* of transgenic plant to support the claimed invention. As explained above, the patent describes successful expression of a claimed AAD-12 protein in multiple monocot and dicot plants using

known promoters and transformation methods. Skilled artisans in 2005-2006 would have readily understood that other monocots and dicots when transformed using the same or similar procedures as described in the '055 patent, would express the claimed AAD-12 proteins using the same or similar promoters and transformation methods and exhibit similar activity. *E.g.*, Ex. 2072 (Potenza 2004), 3 (acknowledging that many promoters “drive high expression” across dicots or monocots); Ex. 2075 (Altpeter 2005), 305-06 (bombardment is “a versatile and effective transformation method, *not limited by cell type, species or genotype.*”) (emphasis added); *see also supra* §§ VI.B and IX.C.2-3.

143. Dr Silverstone asserts that the state of the art was not completely predictable, stating that:

- “[t]he field of engineering enzymes for herbicide tolerance in plants is quite complex”; Ex. 1003 at ¶ 71;
- “just because a transgene cassette functions in one plant species, does not mean that it will function equally in another plant species”; *Id.* at ¶ 55;
- “[p]lant transformation is not completely predictable.” *Id.* at ¶ 73.

144. In my opinion, Dr. Silverstone’s statements overstate the complexity of the field as of 2005-2006 and are not relevant to the sufficiency of the patent’s disclosures to skilled artisans. The '055 patent claim language does not specify any

particular level of protein expression or herbicide degradation activity. Thus, contrary to Dr. Silverstone's suggestion, the claims do not require that the claimed AAD-12 proteins exhibit an "equal" level of AAD-12 protein expression or herbicide degrading functionality in all transformed plant species. Nor would a skilled artisan be seeking equal functioning of a cassette in one species versus another, as sufficient expression is enough. Indeed, the '055 patent shows that despite the differences in the AAD-12(v1) expression levels, the recited dual herbicide-degrading activity was observed in multiple AAD-12(v1)-transformed monocot and dicot plant species. *Supra* §§ VIII.B and IX.C.2.

145. Finally, I disagree with Dr. Silverstone's position that the field of plant transformation would have had to be completely predictable for the disclosures of the '055 patent to sufficiently support the claimed invention. As explained in Sections VI.B and IX.C, regulatory elements suitable to prepare expression vectors for monocot or dicot plant transformation, including those used in the '055 patent examples, were well known and commonly used in the field in 2005-2006 to achieve successful expression of a wide variety of transgenes across various monocot or dicot plants. Skilled artisans had also developed practices of predictably achieving successful transformation by applying known transformation techniques to modest numbers of tissue samples and using straightforward approaches such as selectable markers to confirm success.

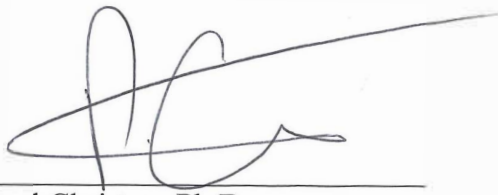
146. Thus, in my opinion, a skilled artisan in 2005-2006 would have understood that the inventors possessed the transgenic plants and plant cells of the '055 patent claims. My opinion that the '055 patent provides adequate written description support of the claimed subject matter remains the same as of the 2017 date that Dr. Silverstone relies upon.

## **XI. CONCLUSION**

147. For the reasons set forth above, it is my opinion that the '055 patent has adequate written description and enablement, particularly in view of its extensive examples and the advanced state in the field of plant transformation in 2005-2006 as well as in 2017.

148. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true. I further declare that all statements made herein were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code.

Date: January 21, 2024

By:   
Paul Christou, Ph.D.

# **Appendix A**

## *CURRICULUM VITAE*

**NAME:** Paul Christou

**DATE OF BIRTH:** July 5, 1954

**CITIZENSHIP:** USA/Cyprus

**DEGREES:** BSc. 1st Class Honours (1977)  
University of London, Chelsea College,  
United Kingdom  
Organic and natural products chemistry

PhD (1980)  
University of London, University College,  
United Kingdom  
Biosynthesis of secondary products in plant cell cultures.  
Plant Biochemistry.

### **POSITIONS HELD**

|                |                                                                                                                                                                                                   |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2019-2024      | Member of the International Scientific Advisory Board of The Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany                                                                 |
| 2012 - 2015    | Director, Agrotecnio Center,<br>University of Lleida<br>Spain                                                                                                                                     |
| 2004-present   | ICREA Professor<br>Universitat de Lleida<br>Spain                                                                                                                                                 |
| 2006           | Appointed member of ad hoc panel on GMOs for European Food Safety Authority (EFSA)                                                                                                                |
| 2001-2004      | Fraunhofer Institute of Molecular Biotechnology<br>and Applied Ecology.<br>Schmallenberg-Aachen, Germany<br>Full Professor                                                                        |
| 2000 - present | Appointed to the Scientific Advisory Board of the Center for Research and Technology Hellas (CERTH), Ministry of Development, General Secretariat of Research and Technology, Greece (June 2000). |
| 1998 - 2001    | John Innes Centre, UK<br>Head, Molecular Biotechnology Unit<br>Band 3, Rank of Full Professor                                                                                                     |
| 1994 - 1998    | John Innes Centre, UK                                                                                                                                                                             |

|             |                                                                                                                                                                             |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|             | Head, Laboratory of Transgenic Technology & Metabolic Pathway Engineering<br>Band 4, Rank of Associate Professor                                                            |
| 1997 - 2004 | Adjunct Professor<br>Mediterranean Agronomic Institute Chania, Crete, Greece                                                                                                |
| 1994 - 1995 | European Commission. Chairman<br>of Biotechnology Bridge Programme evaluation panel.                                                                                        |
| 1993 - 1997 | ABSP Technical Advisory Board Panel Member<br>Michigan State University, USA                                                                                                |
| 1988 - 1994 | Agracetus Inc. USA Senior Scientist<br>Molecular and Cellular Plant Biology<br>Secondary products<br>Medicinal and aromatic plants<br>Legume and cereal crop transformation |
| 1982 - 1987 | Agracetus. Scientist; Project Leader<br>Plant Cell & molecular biology; organic chemistry                                                                                   |
| 1980 - 1982 | University College London<br>Research Fellow - Junior Lecturer<br>Natural products chemistry; biosynthesis of natural products                                              |

## RESEARCH INTERESTS

Our programmes focus on: Transgene structure and function in genetically engineered crops; metabolic engineering and synthetic biology, production of pharmaceutical macromolecules in crop plants; engineering of multiple novel agronomic traits in crop plants. I am also interested in training and technology transfer for developing country biotechnology, intellectual property issues and regulatory and bio-safety issues of transgenic crops, focusing on developing countries. Furthermore, I am also interested in science policy issues and strategic planning covering the interphase between fundamental and applied research

Recipient of ERC Advanced Grant (2009-2014) BIOFORCE entitled “Simultaneous multi-pathway engineering in crop plants through combinatorial genetic transformation: Creating nutritionally BIOFORTified CEreal grains for food security”. Recipient of Proof of Concept Grant (2014-2015), ERC.

## MANAGEMENT COURSES

Management Practices Course Level I. Taught by R.A. Jud and Associates (New York City, May 1990).

The following topics were covered in the course: leadership, team building, motivation, communication, conflict resolution, human resource management, time management, managing change.

Advanced Management Course. Taught by Drake Beam Morin Inc. (Boca Raton, FL, November 1991).

The following topics were covered in this course: team-building, management leadership in the 90s, problem solving, decision making, effective communication skills, communication in business, conflict resolution, employee coaching and mentoring, quality management, influence management, time management.

## Other Courses

Adversarial Interviewing - TV and Radio Training, (Roger Ryan Media Management, UK; June 1999).

## **PUBLICATIONS**

### **A. BOOKS**

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#### **D. POPULAR ARTICLES AND OTHER PUBLICATIONS**

Paul Christou (1999) GM CROPS: environmental angel, consumer devil? *Helix*, September 1999, p. 6-8.

Christou P, Capell T, Bassie L, Zhu C, Naqvi S, Peremarti A, Ramessar K, Gomez S, Dashevskaya S, Yuan D, Sabalza M, Farre G, Rivera SM, Miralpeix B (2011) Canviar el gens per millorar el món. La ciencia al servei de la humanitat. Pagès Editors ISBN 978-84-9975-143-6 pp 199

#### **ISSUED PATENTS**

1. C. Zhu, S Naqvi, T.Capell and P. Christou Method for Creating a Combinatorial Population of Transgenic Plants Expressing and Accumulating a Diversity of Valuable Metabolites. ES200800121 (Spanish patent issued)
2. C. N. Burch, J. A. Moreno, E. Angulo, P. Christou. MÉTODO DE TRATAMIENTO DE COCCIDIOSIS ES P201430840 (Spanish patent)
3. P. Christou, D.E. McCabe, W.F. Swain and B.J. Martinell  
Particle-mediated transformation of soybean plants and lines  
United States Patent No. 5,015,580  
Date of Issue May 14, 1991
4. P. Christou, D.E. McCabe, W.F. Swain and B.J. Martinell  
Particle-mediated transformation of soybean plants and lines  
European Patent No. 88306613.6  
Date: July 20, 1988
5. Christou, P., T.L. Ford, M. Kofron  
Method of creating a transformed rice plant  
International Patent WO 92/20809  
Date November 26, 1992
6. Christou, P. and McCabe D.  
Plant Transformation process with early identification of germline transformation events  
European Patent Application No: 0444882A2. Date: April, 9, 1991
7. Christou, P and McCabe, D.  
Plant Transformation process with early identification of germline transformation events  
US Patent Number 5,503,998.  
April 2, 1996.
8. P Christou and L Mehlo

..... Insecticidal fusions  
..... EP1173591  
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9. P Christou and L Mehlo  
..... Insecticidal fusions  
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## **TALKS AND LECTURES**

### **A. INVITED LECTURES**

203. Christou P (2023) From single transgenes to pathway engineering and genome editing: A personal account of plant tissue culture and genetic engineering from 1980 to 2023 and beyond. XV Reunión de la Sociedad Española de Cultivo In Vitro de Tejidos Vegetales. 6-8 September, Lleida, Spain. Plenary Inaugural conference lecture.

202. P. Christou. Candidate vaccines/diagnostic reagents and recombinant antiviral lectins against HIV and SARS-CoV-2 produced in engineered plants –Current status, recent breakthroughs and perspectives. Annual General Scientific Meeting. IPK, Gatersleben, Germany (Virtual) June 16, 2021. Invited Keynote lecture

201. P. Christou Forty years of plant biotechnology 1981-2021. UCLM, Botanical Institute, Albacete, Spain. July 18, 2021. Invited Keynote lecture

200. P. Christou. Dissecting starch metabolism in rice and generation of specialty rices through genome editing. International Symposium on Rice Functional Genomics BCN November 2-4, 2021 Invited plenary lecture

199. P. Christou. Plant Biotechnology for better health and nutrition. Universidad Autónoma Madrid November 22, 2020. Biotechnology of Photosynthetic Organisms, Closing lecture

198. P. Christou, Applications of plant biotechnology in human and animal health and nutrition. Univ of Barcelona, Biotechnological Applications (Facultat de Farmàcia i Ciències de l'Alimentació, UB). November 10, 2021. Invited lecture

197. P. Christou. Dissecting starch metabolism in rice and generation of specialty rices through genome editing. International Symposium on Rice Functional Genomics BCN November 2-4, 2021 (Plenary lecture)

196. V. Aramario-Najera, T. Capell, P. Christou. Rice as a platform for the production of antibodies and vaccines against SARS-CoV-2. International Symposium on Rice Functional Genomics BCN November 2-4, 2021 (Session lecture)

195. L.M. Rubio, S. Burén, C. Baysal, W. He, X. Jiang, T. Capell, and P. Christou Expression of nif genes in rice. 14<sup>th</sup> European Nitrogen Fixation Conference September 23, 2021. Invited Lecture

194. Á. Eseverri, G. López-Torrejón, X. Jiang, S. Burén, C. Baysal, V. Medina, T. Capell, P. Christou, L. M. Rubio and E. Caro. Optimization of the production of active Nitrogenase Fe protein in the chloroplasts of tobacco and rice. 14<sup>th</sup> European Nitrogen Fixation Conference September 23, 2021. Session Lecture

193. Candidate vaccines/diagnostic reagents and recombinant antiviral lectins against HIV and SARS-CoV-2 produced in engineered plants –Current status, recent breakthroughs and perspectives. IPK, Gatersleben, Germany. June 16, 2021. Invited lecture (virtual)
192. Forty years of plant biotechnology 1981-2021. UCLM, Botanical Institute, Albacete, Spain July 8, 2021. Invited lecture (in person)
191. The creation of plant biotechnology prototypes and products for human and animal health and nutrition Universidad Autónoma Madrid November 17, 2020. Invited lecture
190. Innovation through biotechnology for human and animal health and nutrition. Univ of Barcelona, October 29, 2020 Invited lecture (virtual)
189. Of GMOs and Men. Opening lecture for the inauguration of the Food Biotechnology Master (BIOTECAL) 2019-2020, University of Girona, Spain January 31, 2020. Invited lecture.
188. Cereal endosperm as a facile platform for complex metabolic engineering. Symposium on Small Molecules in Plant Research: Chemistry and Biology Come Together, Valencia, Spain, December 10-11 2019. Invited lecture
187. Third generation plant biotechnology products to address the Food-Feed-Nutrition-Health nexus. Cost Action “European network to advance carotenoid research and applications in agro-food and health” (EUROCAROTEN)” 26-28 of November 2019, Limassol, Cyprus. Keynote opening lecture
186. From genes to products: metabolic engineering and synthetic biology for the creation of plant biotechnology products for human and animal health and nutrition Universidad Autónoma Madrid November 18, 2019. Invited lecture
185. Plant Biotechnology: From 1980 to 2060. Future prospects and constraints for the next 50 years. Mediterranean Agronomic Institute of Zaragoza (IAMZ) of the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM). June 10, 2019. Invited lecture
184. Targeted interventions to modulate and extend terpenoid metabolism in the cereal endosperm. Inst de Biol Molecular y Cellular de Plantas IBMCP, May 31, 2019, Valencia, Spain
183. Genome editing in rice for the creation of useful mutant phenotypes. The Weizmann Inst Israel May 23rd 2019. Invited seminar in the department of Plant and Environmental sciences, Rehovot, Israel.
182. Redirection of terpenoid metabolism in rice through targeted interventions: Plant Biotechnology – Genomics and Genome Editing” Tel Aviv University May 20th 2019. In memory of Prof. Zahir Eyal. Invited lecture
181. Traveling through the genes-products nexus: How fundamental science investigations can be translated to potential products for human and animal health and nutrition. Universidad Autónoma Madrid November 20, 2018. Invited lecture

180. Biofortified staple crops through metabolic engineering and synthetic biology strategies. Mini-symposium: Plant Biotechnology in the 21<sup>st</sup> Century, Opportunities and Challenges. Universidad Internacional Menéndez Pelayo & Centro de Investigaciones Biológicas (CIB) Madrid, October 29, 2018. Invited lecture.

179. From genes to products: metabolic engineering and synthetic biology for the creation of plant biotechnology products for human and animal health and nutrition. Universitat Autònoma de Barcelona. October 10, 2018. Invited lecture.

178. Metabolic engineering through synthetic biology in cereals. Jaques Monod Conference. First International Plant Systems Biology Meeting, Roscoff, France, September, 2018. Invited lecture

177. Engineering natural products in plants for enhanced nutrition; 43rd FEBS Congress, Prague, The Czech Republic, July, 2018. Invited lecture

176. Engineering cereal crops for better health and nutrition Society for Experimental Biology Annual Meeting, Florence, Italy, July, 2018, Invited lecture.

175. From genes to products: A plant biotechnology product portfolio for human and animal health and nutrition. 3rd ISPMF Congress-International Society of Plant Molecular Farming, Helsinki, Finland. June 11-14, 2018, Invited Keynote lecture.

174. Thirty five years of plant biotechnology-Personal reflections and the importance of diversity. Second Pioneer Symposium “*Diversity in Agriculture: Faces of Plant Breeding*” Washington State University, Pullman, WA, USA, March 30<sup>th</sup>, 2018. (Invited lecture)

173. P. Christou. Development of plant biotechnology prototype products for human and animal health and nutrition. Washington State University, Pullman, WA, USA, March 28, 2018 (Invited lecture)

172. High carotenoid cereals: A plant biotechnology product portfolio for human and animal health and nutrition. German Plant Breeding Conference: Leveraging the value of genomic information. Wernigerode, Germany March 1, 2018 (Invited Keynote lecture)

171. Metabolic Engineering, synthetic biology and novel variability in plants for industrial applications. Biotechnology Master at Universidad Autónoma Madrid November 21, 2017

170. Re-wiring plant genetic circuits– Modulating the cereal carotenoid pathway as a paradigm shift from metabolic engineering to synthetic biology in plants. Reunión Nacional sobre CAROTENOIDES en Microorganismos, Plantas, Alimentación y Salud. 16th November 2017 (Conference opening Keynote lecture)

169. Re-wiring plant genetic circuits– a paradigm shift from metabolic engineering to synthetic biology in plants Technical Conference of International Council of Academies of Engineering and Technological Sciences (CAETS) Madrid 14 -15th November 2017. Invited plenary lecture.

168. Synthetic biology and metabolic engineering for the creation of complex novel plant phenotypes Univ Bordeaux-INRA, Bordeaux, France October 13, 2017 Invited seminar.
167. High carotenoid corn: A plant biotechnology product portfolio for human and animal health and nutrition. XXII Reunion de la Sociedad Española de Fisiología Vegetal, Barcelona, June 28 2017. Keynote plenary lecture
166. Genome editing in monocots: Achievements and challenges. Eight annual meeting of the EFOR network (Functional studies on Model Organisms network). Paris, France, May 2, 2017. Invited plenary lecture
165. Of GMOs and Men. University of Zurich, Switzerland. Invited seminar April 24, 2017
164. Redesigning Plant cells ofr Synthetic Biology Application. CSIC and Univ of Sevilla, Spain December 1, 2016
163. Development of a plant biotechnology prototype products for human and animal health and nutrition. Phytochemical Society Meeting, Gent, Belgium, November 23, 2016. Invited plenary lecture
162. Progress in Applied Plant Biotechnology for animal and human nutrition. Chanchun Normal Univ & Jilin Acad Agric Sci, Changchun, China, November 14, 2016
161. Plant Synthetic Biology. Progress and prospects. Northeast Normal University, Changchun, China, November 13, 2016
160. Rice as a model for plant synthetic biology applications. Capital Normal University, Beijing, China, November 12, 2016
- 159 Plant Synthetic Biology. Progress and prospects. Chinese Academy of Agric Sci., Beijing, China, November 11, 2016
158. Redesigning plant cells for synthetic biology applications. The Weizmann Inst Israel October 27, 2016
157. Applied Plant Biotechnology and its impact on the developing world. University of Barcelona, Department of Pharmacy October 19, 2016
156. Redesigning plant cells for synthetic biology applications. University of Salamanca, Spain, October 7, 2016. Invited Seminar
155. P. Christou (2016) Synthetic genotypes in plants: Boundaries between metabolic engineering and synthetic biology. University of California Davies, June 8, 2016. Invited Seminar
- 154 P. Christou & C. Zhu (2016) Field grown engineered maize hybrids with diverse carotenoid profiles reveal the complex interplay between endogenous and synthetic metabolic pathways. Gordon Research Conference on Carotenoids, Barga, Tuscany, Italy, May 22-27, 2016. Invited lecture
153. P. Christou (2016) Metabolic Engineering in maize endosperm for the creatin of novel carotenoid

chemodiversity. Hamburg, Germany May 18-20, 2016. European Maize meeting. Invited lecture

152. P. Christou (2015) Molecular Pharming and anti HIV vaccines. IrsiCaixa, Badalona, Spain February 13, 2015. Invited Seminar

151. P. Christou (2015) The role of transgenic varieties in different agricultural systems, IAMZ, Zaragoza, February 18, 2015. Invited talk (round table)

150. P. Christou (2015) Synthetic genotypes in plants: Boundaries between metabolic engineering and synthetic biology. Univ of Geneva, Switzerland, May 13, 2015. . Invited Seminar

149. P. Christou (2015) Thirty Years of Plant Biotechnology, ETSE, UdL, June 14, 201. Dept Seminar

148. P. Christou (2015) Plant Biotechnology: Applications for health and nutrition and the socio-political dimension of transgenic crop, BOKU, Vienna, Austria, June 22, 20. Invited Seminar

147. P. Christou (2015) The creation of complex genotypes in plants: from multi-gene engineering to synthetic biology Univ of Verona, Italy, September 4, 201. Invited Seminar

146. P. Christou (2015) Plant Biotechnology in Agriculture. University of Barcelona, October 29, 2015. Invited seminar.

145. P. Christou (2014). Second generation metabolic engineering in plants, the creation of synthetic genotypes, and applications in the animal feed industry. Max-Planck-Institute of Molecular Plant Physiology, Golm, Germany, November 5. Invited seminar.

144. P. Christou (2014). Third generation transgenic crops with value added traits. The Weizmann Inst of Science, October 26, Rehovot, Israel. Invited lecture.

143. P. Christou (2014). Synthetic genotypes in plants: value added traits in crops by exploring the boundaries between metabolic engineering and synthetic biology. Plant Biotechnology Achievements and Horizons. I-CORE Center of Plant Adaptation to Changing Environment. October 23, Tel Aviv University, Israel. Invited Symposium opening lecture.

142. P. Christou (2014) Synthetic genotypes in plants - Synthetic biology through multi-gene transfer. Symposium on Plant Virus Biotechnology. Spanish Congress of Plant Pathology, Lleida October 7, 2014.

141. P. Christou (2014) The creation of novel metabolic profiles in plants through the combination of multiple transgenic and endogenous pathways. BIOSPAIN 2014, September 14-26, Santiago de Compostela, Spain, Invited lecture

140. P.Christou, T Capell and C. Zhu (2014) The creation and utility of synthetic genotypes in plants: boundaries between metabolic engineering and synthetic biology, 37th Meeting of the Spanish Society for Biochemistry and Molecular Biology, Granada , Spain, September 10-12. Invited lecture

139. P. Christou (2014) Boundaries between metabolic engineering and synthetic biology: the creation and utility of synthetic genotypes in plants. Plant Genomics in China XV, Hefei, China, August 19-22. Invited lecture
138. P. Christou (2014) Second generation metabolic engineering in staple cereal crops. Spanish Federation of Biotechnologists 8th Biotech Annual Congress, Barcelona, Spain, July 10. Invited lecture
137. P. Christou (2014) Complex genotypes in plants through synthetic biology. Univ of Cape Town South Africa July 3, 2014. Invited seminar
136. P. Christou (2014). Multivitamin corn: Its creation, utility and applications. University of Western Cape, South Africa, July 1, 2013. Invited seminar
135. P. Christou (2014) Multivitamin corn in the context of food security. CSIR, Pretoria, South Africa June 30, 2014. Invited seminar
134. P. Christou (2014) Metabolic engineering and synthetic biology for the creation of synthetic plant genotypes (2014). University of Pretoria, South Africa June 27, 2014. Invited seminar
133. P. Christou (2014) The creation and utility of synthetic genotypes in plants: Boundaries between metabolic engineering and synthetic biology. University of Wageningen, The Netherlands, March 7, 2014. Invited lecture.
132. P. Christou (2014) Multi-gene engineering in plants: where does metabolic engineering end and synthetic biology begin? Plant Transformation Technologies III” Vienna (Austria), February 12-15, 2014. Keynote opening lecture
131. P. Christou (2014) Plant biotechnology in the food and feed production chain. Invited lecture at the Department of Biological Sciences, Royal Holloway, Univ of London, UK.
130. P. Christou (2013) The creation of novel high value molecules in engineered crops. 50th Symposium of the Spanish Society of Poultry Science. October 1-4 Lleida 2013
129. C. Zhu, T. Capell and P. Christou (2013) The ins and outs of multigene engineering of complex biosynthetic pathways in plants. V Central European Congress of Life Sciences EUROBIOTECH 2013. Krakow, Poland October 8-11 (keynote lecture)
128. C. Nogareda, G. Arjó, C. Pinol, J.A. Moreno, E. Angulo, M. Portero, J. Vinas, X. Matias-Guiu, X. Dolcet, J. Serrano, U. Zorilla-López, J. Berman, C. Bai, G. Sanahuja, G. Farré, T. Capell, G. Sandmann, C. Zhu, P. Christou (2013) Plant biotechnology based products for animal feed and human health. XIII Portuguese-Spanish Congress on Plant Physiology. Lisbon, Portugal July 24-27 (Keynote lecture).
127. B. Miralpeix, G. Farré, C. Bai, U. Zorilla-López, J. Berman, G. Sanahuja, T. Capell, G. Sandmann, C. Zhu, P. Christou (2013) Engineering of different terpenoid biosynthetic pathways in tobacco and corn through multigene transfer. TERPNET2013: Biosynthesis, Function and Biotechnology of Isoprenoids in Terrestrial and Marine Organisms. Crete, Greece June 1-5.
126. P. Christou Induced novel variability in carotenoid biosynthesis: moving beyond the one gene, one pathway, one technology approach. Symposium on New Frontiers in plant Biology. Madrid, Spain, January 31-February 1, 2013. Invited lecture.
125. P. Christou Trials and tribulations of transgenic crops in Europe. University of Milan Symposium on Plant Biology. Verbania, Italy October 17-20, 2012. Invited lecture

124. P. Christou Next generation nutritionally enhanced staple crops. Symposium of the Zurich-Basel Plant Science Center Trends and Advances in Plant Biology. Zurich Switzerland 19 October 2012. Invited lecture
123. P Christou Development of rice and maize with multiple essential nutrients through simultaneous multi-pathway engineering. Dialog on designer rice for the future. ICRSAT Hyderabad India 9-10 July 2012. Keynote lecture
122. P. Christou “Transgenic crops and better nutrition and health in the developing world versus political expediency and protectionism” World Science Forum, The Changing Landscape of Science: Challenges and Opportunities November 17-19, 2011 Budapest, Hungary. Invited Speaker.
121. P. Christou “Transgenic crops and better nutrition and health in the developing world versus political expediency and protectionism” 22nd General Meeting of TWAS (The Academy of Sciences for the Developing World) 21-23 November, 2011, Trieste, Italy. Invited Speaker.
120. P. Christou “Third generation nutritionally enhanced crops for human health in a European versus global context” 2nd International Conference on FOOD-OMICS, June 2011, 22-24 Cesena (Italy). Invited speaker.
119. P. Christou “Multigene constructs” Society for in Vitro Biology Meeting, June 2011, 4-8, Raleigh N Carolina. Invited Speaker.
118. P. Christou “Induced novel variability in carotenoid biosynthesis: moving beyond the one gene-one pathway-one technology approach” TERPNET 2011 meeting, May 2011, 22-26, Kalmar Sweden. Invited Speaker.
117. P Christou “Synthetic biology for the cost-effective production of useful metabolites in intact plants and in vitro plant systems” XII International Congress on Molecular Systems Biology May 2011, 8-12, Lleida, Spain. Invited speaker.
116. P Christou “Multi gene engineering for complex traits in plants” FP7-REGPOT-2008-1 SUPPORT ACTION: BPI Plant-Heal 230010 -Development of Benaki Phytopathological Institute as a Centre of Excellence in Plant Health and Crop Protection. May 2011, 5-6, Athens, Greece. Invited speaker.
115. P Christou “The role of plant biotechnology in addressing a number of the Millennium Development Goals” May 4, 2011, Universitat Pompeu Fabra, Barcelona, Spain
114. P Christou “The contribution of transgenic crops to better nutrition and health: Science & ethics versus political expediency & protectionism” April 12, 2011 National Cancer Institute, Frederick, Maryland, USA (Invited lecture)
113. P. Christou “*Trials and Tribulations of Transgenic Crops in Europe: A State of Pain or Anguish That Tests Patience, Endurance, or Belief*” April 7, 2011 Washington State University, Pullman, Washington, USA (Invited lecture)
112. P Christou “*Multi-gene and Multi-pathway Engineering for Creating Nutritionally Improved Crops*” April 6, 2011 Washington State University, Pullman, Washington, USA (Invited lecture)
111. P. Christou “Multi-gene engineering for the reconstruction and extension of complex plant biosynthetic pathways” April 5, 2011, Cornell University, Ithaca, New York, USA (Invited lecture)
110. P. Christou “Combinatorial Transformation System for Multi-gene and Multi-pathway Engineering in Plants”. Plant Transformation Technologies II Vienna (Austria) February 2011, 19<sup>th</sup> to 22<sup>nd</sup>. Invited Speaker.
109. P.Christou “Multi-gene and multi-pathway engineering for the nutritional improvement of staple crops” Carotenoids Gordon Research Conference, Ventura, California, USA, January 2010, 17-22. Invited speaker.

108. P. Christou *"Multi-step pathway engineering in plants" 10<sup>th</sup> meeting of the Spanish society of plant molecular biology Valencia, Spain, July 2010, 10-12. Invited plenary lecture.*
107. P. Christou. Better health through nutritionally enhanced transgenic crops: Multivitamin maize through genetic engineering. Asociación Española de Doctores y Licenciados en Ciencia y Tecnología de los Alimentos (ALCYTA) Universidad Complutense de Madrid April 22-24, 2009. Keynote opening lecture
106. P. Christou and T. Capell. 2nd Workshop "Biofarming and New Approaches on Plant Transformation". February 23<sup>rd</sup> – 26<sup>th</sup> 2009 Mérida, Yucatán, México. Invited lecture
105. K. Ramessar, T. Capell and P Christou. Combating Infectious Diseases in Africa: The contribution of Plant Biotechnology. African Science Communication Conferences, 18-21 February, 2009, Gauten, South Africa. Invited lecture
104. P. Christou: The development of multivitamin corn in the context of food security 8<sup>th</sup> Plant GEM Lisbon, Portugal, 7 to 10 October 2009. Invited plenary speaker
103. Changfu Zhu, Shaista Naqvi, Jürgen Breitenbach, Gerhard Sandmann, Paul Christou and Teresa Capell. Metabolic Engineering of Maize Carotenoid Content through Combinatorial Genetic Transformation. 13th International Biotechnology Symposium & Exhibition (IBS2008), Dalian, China October 12-14, 2008. Invited lecture
102. P. Christou. Combinatorial genetic transformation for the creation of plants with multiple enhanced nutritional traits. Jornada sobre Alimentación y Genes, Fundación Valenciana de Estudios Avanzados, October 15-16, 2008 Invited lecture
101. P. Christou, C. Zhu, S. Naqvi, G. Farre, T. Capell. Combinatorial genetic transformation and the generation of plant mutant libraries for metabolic engineering. International Congress on Plant Genomics (PlantGEM7) Albena, Bulgaria, September 24-27, 2008. Invited lecture
100. P. Christou, C. Zhu, S. Naqvi, G. Farre, T. Capell. Combinatorial genetic transformation for the engineering of complex biosynthetic pathways in plants. 'Transformation resources and progress in EU' 2nd Tritigen COST action FA0604 meeting Albena Bulgaria 22nd-24th September, 2008 Invited lecture
99. P. Christou. Multi-trait transfer - advances and applications of cereal transformation. The Agricultural Biotechnology International Conference (ABIC) University College Cork, Ireland 24<sup>th</sup>-27<sup>th</sup> August 2008 Invited lecture
98. P Christou, T Capell, C Zhu, L Bassie, S Naqvi, K Ramessar, S Gomes-Galera, A Peremarti, D Yuan, S Dashevskaya, G Farre, M Sabalza, B Miralpeix. Third Generation Transgenic Crops with Enhanced Multigenic traits. XVI Congress of the Federation of European Societies of Plant Biology (FESPB) Tampere Finland, 17-22 August 2008. Keynote lecture
97. C Zhu, S Naqvi, J Breitenbach, G Sandmann, P Christou and T Capell. Reconstruction and extension of the carotenoid pathway in maize through combinatorial nuclear transformation. The 15<sup>th</sup> International Symposium on Carotenoids, Okinawa, Japan 22-27 June, 2008
96. P. Christou and Teresa Capell The science and economics of plant molecular pharming-an applied perspective. Recent advances and techniques in Molecular Farming BioMurcia workshop October 15-18, 2007
95. P. Christou Molecular pharming in cereal crops and the political dimension of plant-made pharmaceuticals XXXVI Congreso de la Sociedad Española de Genética, September 18-21, Leon, Spain. Keynote lecture 2007

94. P Christou and T Capell Molecular pharming in cereal crops and the political dimension of plant-made pharmaceuticals Phytochemical Society of Europe Congress August 26-29, 2007 Rantapuisto Congress Centre, Helsinki, Finland.
93. P. Christou Production of recombinant pharmaceuticals in crop plants and the political dimension of GM pharma plant regulation. Agricultural University of Athens, Greece, June 8, 2007. Invited lecture
92. P. Christou Plant Biotechnology at the UdL. IRTA Cabrils, Spain. June 12, 2007 Invited lecture.
91. P Christou European Association for Research on Plant Breeding (EUCARPIA). Cereal Science and Technology for Feeding Ten Billion People: Genomics Era and Beyond. Lleida, Spain, 13-17 November 2006. Invited lecture
90. P. Christou. Crop plants as production platforms for molecular pharming 3<sup>rd</sup> International Biotechnology Forum IGBF-3 in Athens Greece, from 5-7 October 2006. Invited lecture
89. P. Christou, Frontiers in Applied Plant Biotechnology Invited Seminar. University of Navarra, Pamplona, Spain (March 21, 2006)
88. P. Christou. Plant Biotechnology and Contemporary Agriculture-Molecular Pharming University of Costa Rica, San Jose. Invited lecture (24 February 2006)
87. P. Christou. Third generation genetically enhanced crops and their impact on agriculture in developing countries. Plant Biotechnology and Sustainable Agriculture Cultivos Genéticamente Mejorados y Bioseguridad: Oportunidades para los Países en Desarrollo, San José, COSTA RICA Keynote lecture (21-23 February 2006)
86. P. Christou Applied Plant Biotechnology-Life at the frontier Institutio Catalana de Recerca I estudis avancats, ICREA lecture (December 13, 2005)
85. P. Christou and T. Capell. Transgenic Cereals with resistance against biotic and abiotic stresses. Biotechnology Havana 2005 Havana, Cuba, Invited lecture (Nov 27, Dec 2, 2005)
- 84.P. Christou. Recent advances in applied plant biotechnology in transgenic insect pest control and molecular pharming. Symposium on plant biotechnology: retrospective and prospective views, Tel Aviv University, Tel Aviv, Israel Invited keynote lecture (October 27, 2005)
83. P. Christou. Challenges and opportunities in applied plant biotechnology. MPOB International Palm Oil Conference (PIPOC), Kuala Lumpur, Malaysia. Invited lead lecture September 24-28, 2005)
82. P. Christou. Plant Biotechnology in Contemporary Agriculture. Meeting of the Spanish Society for Tissue Culture (SECIVTV), Cordoba, Keynote address (September 12-13, 2005)
81. P. Christou. Frontiers in recombinant protein production in transgenic plants. CSIC Centro de Investigaciones Biológicas Madrid, Invited Seminar (September 8, 2005)
80. P. Christou. Recent advances in gene transfer technology. Conference on Plant-made Pharmaceuticals Montréal, QC Canada. Invited lecture (January 30- February 2, 2005)
79. P. Christou. Recent advances in transgenic insect pest control. IOBC/wprs Working Group on 'GMOs in Integrated Plant Production'. Meeting on Ecological Impact of Genetically Modified Organisms. Lleida, Spain. Keynote address (June 1-3, 2005)

78. A.Doulis, D. Gahakwa, I. Rizov, T. Rocheford and P. Christou. Genetic manipulation of oleate desaturase (FAD2) alters fatty acid and glycerolipid composition of maize leaves. Greek Lipid Society Meeting, Athens, Greece, June 13-15, 2005
77. P. Christou. Functional genomics in cereals and applications in gene discovery for abiotic stress response in rice. Invited seminar and visit At Maharashtra Seed Company, Jalna, India (March 19-24, 2004)
76. P. Christou. Plant Biotechnology in Contemporary Agriculture. UPM, ETSI Agronomos Madrid, Spain. Invited lecture (April 16, 2004)
75. P. Christou. Progress and Prospects in cereal biotechnology. University of Central Florida, Orlando, USA. Invited lecture (April 21-24, 2004)
74. P. Christou. Molecular Pharming-Therapeutics in Plants. First EU-US Bioproduct Research Workshop, USDA, ARS Western Regional Research Center (WRRC), Albany California USA, San Francisco. Invited lecture (June 25-26, 2004)
73. P. Christou. Third Generation Transgenic Plants and Applications to Developing Country Agriculture. Agriculture Congress-Innovations towards Modernized Agriculture, Kuala Lumpur, Malaysia. Keynote lecture (October 4-7, 2004)
72. P. Christou. Plant Biotechnology at the Crops genetics and Biotechnology Department at the FhIME. CSIR, Pretoria, South Africa. Guest lecture (November 5, 2003)
71. P. Christou. Applied Plant Biotechnology. Second Workshop on Green Biotechnology organised by RWTH, Aachen and FZ Julich Invited lecture. (July 17, 2003)
70. P. Christou. Transgenic plants as a vehicle for the production of pharmaceutical macromolecules and small molecular weight metabolites. In the wake of the double Helix: From the Green to the Gene Revolution. University of Bologna, Italy. Invited lecture (May 27-31, 2003)
69. P. Christou and T. Capell. Transgenic approaches towards alkaloid engineering. Bioactive Chemistry from Nature. Cambridge UK. Invited lecture. (March 31-April 2, 2003)
68. P. Christou. Metabolic Engineering in Plants. Metanomix Ghmb, Berlin. Germany Invited lecture (February 23, 2003)
67. P. Christou. University of Münster, Germany. Transgene structure and function in cereals-Functional Genomics and Epigenetics. Invited seminar (January 2003)
66. P. Christou. From the laboratory to the field, the market and beyond-The cereal paradigm. EUCARPIA, Cereal Section Meeting, Salsomaggiore, Italy, 21.25 November, 2002. Keynote lecture.
65. P. Christou and T. Capell. DNA methylation, transposons and transgene expression. Symposium on Epigenic Mechanisms and Reprogramming, DFG, Berlin 15-17 November, 2002. Invited lecture
64. P. Christou. Antibiotic and other Marker Genes and their Potential Impacts. Transgenic Rice Biosafety, National Academy of Agricultural Sciences, India, Symposium on Transgenic Rice Biosafety. 27-30 October, 2002. Invited lecture.
63. P. Christou. Third Generation Transgenic Cereals. 3<sup>rd</sup> International symposium on the molecular and cellular biology of banana. Leuven, Belgium, 9-11 September 2002. Keynote lecture.
62. P.Christou. Transposon Tagging in rice and its use for cereal functional Genomics. Maharashtra Hybrid Seed

Company Ltd, Jalna, India, September 5, 2002.

61. P. Christou. Transgenic plants and their role in fundamental scientific discovery and applications. The 19<sup>th</sup> Forum on engineering Science & Technology-Agricultural Biotechnology in the 21<sup>st</sup> Century. August 12, 2002, Beijing, Chinese Academy of Engineering. Keynote lecture.

60. P. Christou. Third Generation transgenic crops and their applications. International Symposium on Agrobiotech, Gongzhuling, China, August 5-7, 2002. Keynote lecture

59. P. Christou. Transgene integration mechanisms, transgene organisation and effect on expression. International Symposium on Agrobiotech, Gongzhuling, China, August 5-7, 2002. Keynote lecture

58. P. Christou. Transgenic rice-An ideal experimental system for fundamental science and applications. Dissemination conference of European Current research projects on rice "*RicEUconf*", Turin, Italy, 6-8 June, 2002. Invited lecture

57. P. Christou. Contemporary plant biotechnology: What makes things work. Univ. of Barcelona, Spain, January 28, 2002. Invited lecture.

56. Third Generation Strategies for the control of Insect Pests in Crops. Royal Entomological Society, University of Aberdeen, Scotland (September, 2001).

55. Third generation outputs of plant biotechnology-From fundamental science to application. University of Barcelona, Spain (April 2001)

54. University of Milan and CNR : Cereal biotechnology (January 2001)

53. Rice Biotechnology - A case study for bringing together fundamental science and applications. Invited keynote lecture, International Conference on Tropical Agriculture: Technology for Better Health and Environment. Kasetsart University, Kamphaeng Sean, Nakhon Pathom, Thailand (29 November-3 December 2000).

52. Novel strategies for broadening resistance of plants against insect pests - Transgene pyramiding and novel insecticidal fusion proteins. Paul Christou. John Innes Centre and Sainsbury Laboratory Annual Meeting, John Innes Centre, Norwich, UK (2 and 3 November 2000).

51. Transgene integration, organization and expression in cereals. Paul Christou, Eva Stoger, Ajay Kohli, Richard Twyman, Xiangdong Fu, Eva Wegel. Invited plenary lecture, Fourth International Rice Genetics Symposium at the International Rice Research Institute, The Philippines, (22-27 October 2000).

50. Transgenic cereals as a tool for unravelling fundamental molecular processes in plants and their use in the creation of valuable germplasm for commercial applications. Christou, P. Invited keynote lecture, XLIV Convegno Annuale - Società Italiana di Genetica Agraria, Università degli Studi di Bologna, Bologna (20-23 September 2000).

49. Plant Biotechnology and Crop Improvement. NAIST/JIC JSPS meeting John Innes Centre Molecular approaches to plant physiology and biotechnology. August 2000

48. Biotech Approaches to Crop Improvement. P. Christou. *Invited plenary lecture*, NAIST-JIC/JSPP Meeting - Molecular Approaches to Plant Physiology and Biotechnology. John Innes Centre, Norwich, UK (23 - 25 August 2000).

47. Genetic Engineering of the Polyamine Biosynthetic pathway in cereal grains. P. Christou, O. Lepri, N. Pham, H. Pham, L. Bassie, T. Capell. Invited plenary lecture at the International Polyamine Conference, Rimini, Italy (1-4 June 2000).
46. "An evaluation of the presently available approaches to transfer genes into plants". Invited plenary lecture, TEBIO 2000 - Plant Biotechnology in the world: an overview. International Meeting and Exhibition, Genoa, Italy (24-26 May 2000).
45. Cereal Biotechnology: From fundamental science to application. Invited lecture, University of Crete (12 April 2000).
44. Rice Biotechnology: Fundamental science and applications. Invited keynote lecture, Biotechnology Congress - Perspectives and Limitations of Biotechnology in Developing Countries, University of Costa Rica, San Jose, Costa Rica (24-28 January 2000).
43. Rice Biotechnology: From Fundamental Science to Application. Plenary Lecture (Plenary Session 4). Rockefeller Foundation - 1999 Meeting of the International Program on Rice Biotechnology, 20-24 September 1999, Phuket, Thailand.
42. The role of molecular biology to Developing Country Agricultural Systems. Fundamental science and applications. Academia Sinica, Taipei, Taiwan, 21 June 1999. Invited Seminar.
41. Secondary Product Metabolic Engineering. Invited Lecture, New York University, New York, USA, 10 May 1999.
40. Engineering of Secondary Metabolism in Medicinal Plants. The Hyoscyamus Tobacco Story. Invited Lecture, University of Barcelona, Spain, 23 April, 1999.
39. The 1998 Brighton Conference - Pests and Diseases. British Crop Protection Council Conference, Invited lecture, 16-19 November 1998.
38. First Meeting of the National Rice Biotechnology Network, Hue, Vietnam, 5-8 May 1998. Invited lecture - Plant Biotechnology and Gene Manipulation.
37. Plant Biotechnology and Gene Manipulation. Plenary lecture, International Association for Plant Tissue Culture, IX International Congress on Plant Tissue and Cell Culture, Jerusalem, Israel, 14-19 June 1998.
36. Biotechnology in the food chain: New tools and applications for Future Foods. International Symposium, organised by VTT Biotechnology and Food Research, Helsinki, Finland, in collaboration with ATO, Netherlands. Helsinki, Finland, 28-30 January 1998. Invited lecture - "Crop engineering and the creation of novel foods".
35. Mechanism of foreign DNA integration into the rice genome as a result of direct DNA transfer and the creation of rice plants expressing genes of agronomic interest. Invited plenary speaker, Rockefeller Foundation Annual Meeting on Rice Biotechnology, Malacca, Malaysia (September 1997).
34. Transgene structure, expression and organization in plants engineered through particle bombardment. Invited plenary speaker and Session Chairman, EURESCO Congress, Helsinki, Finland (September 1997).
33. Rice and Maize Biotechnology. Invited speaker, Genetic Engineering and Biotechnology Seminar, Camden and Chorleywood Food Research Association (April 1997).
32. Agricultural Biotechnology for Sustainable Productivity. Plenary speaker, ABSP - Asilomar, California, USA (April 1997).

31. Plant Genetic Engineering. Keynote address, HBBS Meeting Athens (January 1997).
30. Keynote address, Regional Rice Biotechnology Meeting, Bangkok, Thailand (November 1996).
29. Keynote address, Regional Rice Biotechnology Meeting, New Delhi, India (November 1996).
- Maize Biotechnology. Keynote address, Eucarpia Meeting, Thessaloniki, Greece (October 1996).
28. Introduction of multiple genes into elite rice varieties - Evaluation of transgene stability, gene expression, and field performance of herbicide-resistant transgenic plants. 3rd International Rice Genetics Symposium, Manila, The Philippines (October 16-20, 1995).
27. Biotechnology of Crop Legumes. Invited Lecture and Session Chairman, AEP Biotechnology Group, Copenhagen, Denmark (July 1995).
26. The role of gene transfer in plant genome research. Invited lecture. Plant Genome III. International Plant Genome Conference, San Diego, California (January 1995).
25. Strategies for variety-independent genetic transformation of important cereals, legumes and medicinal plants utilizing particle bombardment. Invited Keynote Address, Eucarpia Meeting. Cork, Ireland (September 1994).
24. Inserting genes into plants. Invited lecture, Plant Molecular Biology Congress, Amsterdam, The Netherlands (June 1994).
23. Evaluation of Transgenic Glufosinate-Resistant Rice Lines. Proceedings of 25th Rice Technical Working Group, New Orleans, LA, USA (March 5-9, 1994).
22. Universal gene transfer system for cereals, legumes and medicinal plants utilizing electric discharge particle bombardment. Invited lecture, Keystone Symposium on Improved crop and plant products through biotechnology (January 1994).
21. Multidisciplinary assessment of transgenic crop plants. Invited lecture, Organization for Economic Cooperation and Development, Crete (September 1993).
20. Towards the development of a universal gene transfer method for plants. Invited lecture, XV International Botanical Congress, Tokyo, Japan (August 1993).
19. Genetic engineering of agronomically-important legumes and cereals. Invited lecture and Session Chairman, 6th European Congress on Biotechnology, Florence, Italy (June 1993).
18. Production of transgenic rice plants in a variety-independent fashion at transformation frequencies of 10% and higher. Sixth Rockefeller Foundation Meeting on Rice Biotechnology, Chiang-Mai, Thailand (February 1993).
17. Transformation of recalcitrant crops using electric discharge particle acceleration. Invited lecture, World Congress of Cell and Tissue Culture, Washington D.C. (June 1992).
16. Methods of Plant Transformation: Current Status. Invited Keynote lecture at the Canadian Section of the International Association of Plant Tissue Culture, Guelph, Ontario Canada (June 1992).
15. Rice as a model for developing variety-independent transformation systems for monocotyledonous plants. Keystone Symposium, Crop Improvement via Biotechnology: an International Perspective. Invited lecture, Keystone, Colorado (April 1992).

14. Variety-independent transformation of rice. Invited lecture, 24th Rice Technical Working Group meeting, Little Rock Arkansas (February 1992).
13. Commercial production of transgenic soybean and rice plants from elite cultivars using electric discharge particle acceleration. Invited lecture, Miami Bio/Technology Winter Symposium, Miami Beach, Florida (January 1992).
12. Genotype-independent transformation of rice using electric discharge particle acceleration. Fifth Annual Meeting, Rockefeller Foundation International Program on Rice Biotechnology, Tucson, Arizona (1991).
11. Transformation via electric discharge microprojectile bombardment as a tool for studying developmental events in higher plants. Invited lecture, Gordon Research Conference in Plant Cell and Tissue Culture, Plymouth, New Hampshire (1991).
10. Invited keynote lecture on Agricultural Biotechnology. Biotech U.S.A., Washington D.C. (1990).
9. Applied soybean genetic engineering. Workshop Chairperson, International Association of Plant Tissue Culture Meeting, Amsterdam, The Netherlands (1990).
8. Commercialization of a process for the recovery of transgenic soybean cultivars from elite varieties expressing novel agronomical traits. National Soybean Breeding/Entomology Workshop, St. Louis, Missouri, USA (1990).
7. Soybean transformation by electric discharge particle acceleration. Invited lecture, American and Canadian Societies of Plant Physiology, Toronto, Canada (1989).
6. Particle acceleration in the transformation of soybean. Invited lecture, Gordon Research Conference on Plant Cell Tissue Culture, Plymouth, New Hampshire, USA (1989).
5. Transgenic soybean plants and their progeny using electric discharge particle acceleration. Invited lecture, American Society of Agronomy, Anaheim, California, USA (1988).
4. Crop Engineering. United States Department of Agriculture Working Group on Biotechnological Applications in Agriculture, Madison, Wisconsin, USA (1988).
3. Electric discharge particle acceleration - A new method for the recovery of transgenic plants. European Molecular Biology Organization: Gene Transfer to Plants: A Critical Assessment. Zurich, Switzerland (1988).
2. Genetically engineered soybean. World Conference on the Molecular and Cellular Biology of the Soybean. Ames, Iowa, USA (1988).
1. Anti-cytokinin activity of substituted phenethylamines in in vitro cultures. Phytochemical Society of North America. Tampa, Florida, USA (1987).

## **B. PARTICIPATION IN INTERNATIONAL WORKSHOPS AND MEETINGS**

Scientific Committee member. Plants for Human Health in the Post-Genome Era. Phytochemical Society of Europe, Helsinki 26-29 August, 2007

Panel discussion chairman: Constraints and limitation of plant biotechnology in developing countries. Perspectives and Limitations of Biotechnology in Developing Countries, Costa Rica (24-28 January 2000).

Panel review member for Institute of Agrobiological Sciences, Academia Sinica, Taipei, Taiwan. Site visit and programme evaluation, June 21-24, 1999.

Alternative uses of crop plants. Workshop funded by EU FAIR. Organizing committee (Norwich, UK, June 1996).

Metabolic Engineering of secondary products. Workshop funded by the EU BIOTECH Unit. Organizer and Chairman. (Norwich, UK, April 15-16, 1996).

Panel member and session chairman on Workshop "Uncertainty, Precautions and Decision-Making: The release of GMOs into the Environment". Organized by the Green Alliance and the University of Lancaster (London, September 18th, 1995).

South and Central American Plant Genetic Engineering workshop on tropical crop biotechnology (Ixtapa, Mexico, 1992).

International particle bombardment workshop - Organizer and Chairman (Madison, Wisconsin, 1990)

Gene transfer into plants-EMBO workshop (Bud-Schoenbrunn, Switzerland, 1989)

#### **Other Activities:**

External Examiner PhD candidate Univ of Helsinki

External Examiner PhD candidate Univ India.

Panel Member - evaluation for Greek Ministry of Science and Technology (1999).

External examiner for PhD candidate at the Institute for Biological and Experimental Technology, (IBET), Oeiras, Portugal (January 15, 1999).

External examiner for PhD candidate at Leiden/Amsterdam Center for Drug Research, Leiden University, Leiden, Netherlands (April 14, 1999).

Organizing Committee and Session Chairman, International Association for Plant Tissue Culture, IX International Congress on Plant Tissue and Cell Culture, Jerusalem, Israel (June 14-19, 1998)

Member of Advisory Committee. Biotechnology in the food chain: New tools and applications for Future Foods. International Symposium, organised by VTT Biotechnology and Food Research, Helsinki, Finland, in collaboration with ATO, Netherlands. Helsinki, Finland, (28-30 January 1998)

Organizing Committee for EURESCO Congress, Helsinki, Finland (September 1997)

External examiner for PhD candidate at De Montfort University (1997).

Graduate course organizer at Institute of Molecular Biology and Biotechnology and University of Crete (1997)

Co-organizer of EU-sponsored workshop on use of plants for alternative uses, including vaccines, antibodies and secondary products (Institute of Food Research, Norwich, UK; June 1996).

Organizer of EU-Funded Secondary Product Engineering Workshop (John Innes Centre, Norwich, UK; April 15-16, 1996)

Position paper on Molecular Farming and Secondary Product Engineering for FAIR (1996).

Position paper on Secondary Product Engineering for BIOTECH (1996).

### **Courses Taught:**

Master course

Master course in "Applied Plant Molecular Biology-Green Biotechnology". University of Crete, Heraklion, Greece, November 15-18, 2021

Master course in "Applied Plant Molecular Biology-Green Biotechnology". University of Crete, Heraklion, Greece, November 18-20, 2020

Master course in "Applied Plant Molecular Biology-Green Biotechnology". University of Crete, Heraklion, Greece, November 13-15, 2019

Master course "Biotechnological Applications" (Facultat de Farmàcia i Ciències de l'Alimentació, UB). Plant Biotechnology: Applications for health and nutrition. University of Barcelona, Barcelona, Spain. November 7, 2019

Applied Plant Biotechnology and Genome Editing. International Master in plant Breeding, XXII. Mediterranean Agronomic Institute of Zaragoza (IAMZ) of the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM). Zaragoza, Spain. January 14-17, 2019.

Intensive course on Plant Biotechnology and Applications, CIHEAM, Zaragoza, 24 February to 4 March, 2011

Transgenics in our life: Agriculture and food, health and industry. Universidad Internacional Menéndez Pelayo, Santander, Spain August 3-8, 2009

Intensive course on Plant Biotechnology and Applications, International Centre for Advanced Mediterranean Agronomic Studies, Mediterranean Agronomic Institute of Chania, Crete, Greece, 30 March-4 April, 2009.

Intensive course on Plant Biotechnology and Applications, CHEAM, Zaragoza, 16-19 February, 2009.

Applied Biotechnology Course, University of Crete, Iraklion, Crete, Greece, 11-13, November 2008

Intensive course on Plant Biotechnology and Applications, International Centre for Advanced Mediterranean Agronomic Studies, Mediterranean Agronomic Institute of Chania, Crete, Greece, 4-8, June, 2007.

Applied Biotechnology Course, University of Crete, Iraklion, Crete, Greece, 12-14, November 2007.

Intensive course on Plant Biotechnology and Applications, International Centre for Advanced Mediterranean Agronomic Studies, Mediterranean Agronomic Institute of Chania, Crete, Greece, 15-19, May, 2006.

Intensive course on Plant Biotechnology and Applications, CHEAM, Zaragoza, 7-9 March, 2005.

Intensive graduate course on applied plant biotechnology, Universidad de Lleida, January 2005.

Applied Biotechnology Course, University of Crete, Iraklion, Crete, Greece, 15-19, November 2004.

Intensive course on Plant Biotechnology and Applications, International Centre for Advanced Mediterranean Agronomic Studies, Mediterranean Agronomic Institute of Chania, Crete, Greece, 6-12, May, 2004.

Applied Biotechnology Course, University of Crete, Iraklion, Crete, Greece, 20-24, November 2003.

Intensive course on Plant Biotechnology and Applications, International Centre for Advanced Mediterranean Agronomic Studies, Mediterranean Agronomic Institute of Chania, Crete, Greece, May, 2003.

Applied Biotechnology Course, University of Crete, Iraklion, Crete, Greece, November 2002.

Intensive course on Plant Biotechnology and Applications, International Centre for Advanced Mediterranean Agronomic Studies, Mediterranean Agronomic Institute of Chania, Crete, Greece, May, 2002.

Applied Biotechnology Course, University of Crete, Iraklion, Crete, Greece, November 2001.

Intensive course on Plant Biotechnology and Applications, International Centre for Advanced Mediterranean Agronomic Studies, Mediterranean Agronomic Institute of Chania, Crete, Greece, June, 2001.

University of East Anglia, Norwich, School of Biological Sciences - Plant Transformation. Postgraduate Course. 6 December 2000.

Applied Biotechnology Course, University of Crete, Iraklion, Crete, Greece, November 2000.

Visiting lecturer, San Jose University, Costa Rica, 4 day course on principles and applications of plant biotechnology, 31 January 31- 3 February 2000.

University of East Anglia, Norwich, School of Biological Sciences - Plant Transformation. Postgraduate Course. 8 December 1999.

University of East Anglia, Norwich, Centre for Continuing Education. Presentations given as part of course - Science and Management of the Food Chain: Crop Biotechnology, held 9 - 11 September 1999.

Intensive course on Plant Biotechnology and Applications, International Centre for Advanced Mediterranean Agronomic Studies, Mediterranean Agronomic Institute of Chania, Crete, Greece, 24 - 28 June, 1999.

University of East Anglia, Norwich, School of Biological Sciences - Applied Plant Biology Course. 1 and 2 March, 1999.

University of East Anglia, Norwich, School of Biological Sciences - Plant Transformation. Postgraduate Course. 4 December 1998.

Heraklion, Crete, Greece. Plant Biotechnology, Applied and Fundamental Science Aspects. University of Crete. 26-29 October 1998.

Chania, Crete, Greece. Biotechnology (Natural Products). Mediterranean Agronomic Institute of Chania. 25-29 May 1998.

University of East Anglia, Norwich, School of Biological Sciences - Plant Transformation. Postgraduate Course. 4 December 1997.

Bogor, Indonesia - Genetic Transformation Workshop (teaching), Puslitbang bioteknologi-LIPI, Cibinong (August 1997)

Chania, Crete, Greece. Biotechnology. Mediterranean Agronomic Institute of Chania. 19-23 May 1997.

University of East Anglia, Norwich, School of Biological Sciences - Plant Transformation. Postgraduate Course. 5 December 1996.

Lisbon, Portugal - EMBO course on Applied Biotechnology (September, 1996)

Chania, Greece. Instructor on course on biotechnology. Mediterranean Agronomic Institute (May, 1996).

Hangzhou, China - South East Asia Rice Biotechnology (April, 1996).

John Innes Centre/Norwich Research Park - Graduate course organizer on Transformation methods (February, 1996).

Canberra, Australia - South East Asia Rice Biotechnology (October, 1995).

Brasilia, Brazil. Transgene expression and analysis. South American Biotechnology Association (August, 1995).

Genetic Engineering and Plant biotechnology. EMBO course, Heraklion, Greece (June, 1995).

## **C. OTHER MAJOR TALKS AND LECTURES**

Invited Seminars 1995-1999

26. The Role of Molecular Biology to Developing Country Agricultural Systems. Fundamental science and applications. Invited Seminar, Academia Sinica, Taipei, Taiwan (21 June, 1999).

25. Transgene organization in engineered crops and metabolic pathway engineering. Plant Genetics Systems, Gent, Belgium (July, 1997).

24. Alternative uses of cereals and pathway engineering. Kellogg Company, Battle Creek, Michigan, USA (May, 1997).

23. Current Progress in Agricultural Biotechnology. Hercules, Inc. (March, 1997).

22. Plant Improvement by Transgenesis. De Montfort University, Leicester, UK (February, 1997).

21. Humboldt Institute of Biodiversity for Medicinal Plants. Invited lecture, Bogota, Colombia (1996)
20. Organization of transgenes in cereals and legumes, CIAT, Cali, Colombia. Invited lecture (1996)
19. Transgenic legumes and cereals. Invited lecture, CIAT, Cali, Colombia (1996).
18. Transgene structure, expression and organization in genetically engineered cereals and legumes. EMBRAPA, Brasilia, Brazil (1996).
17. Transgene expression. Brasilia, Brazil (1996)
16. Association of Cereal Food Manufacturers. Technical Committee Meeting. Status of the genetic engineering of crops used in breakfast cereal manufacturing, London, UK (1996).
15. Association of Cereal Food Manufacturers. Executive Committee Meeting. Status of the genetic engineering of crops used in breakfast cereal manufacturing (London, 1996).
14. Genetic Engineering of Cereal Crops-Workshop on Accessible Biotechnology. HGCA, Gloucestershire (London, 1996)
13. Transgene organization in cereals. PBI, Cambridge (1996). Rice Biotech. CAMBIA/CSIRO Australia (1996)
12. Genetic engineering. Plenary lecture, IAEA Vienna (1996)
11. Secondary product metabolic engineering. University of Cambridge, Department of Biochemistry (1996)
10. Secondary product metabolic engineering. Xenova Ltd., UK (1996)
9. Metabolic pathway engineering in transgenic plants. Cambridge Biotechnology Association (1996)
8. Rice biotechnology. Rockefeller Foundation Meeting, Manila, The Philippines (1995).
7. Crop biotechnology. Agricultural University of Athens (1995)
6. Secondary product metabolic engineering. University of Leiden, The Netherlands (1995)
5. Secondary product metabolic engineering. ATC, Cambridge (1995)
4. Crop biotechnology. Vanderhave, The Netherlands (1995)
3. Metabolic Pathway Engineering. Hercules Inc., Delaware, USA (1995)
2. Transgene structure and function in engineered crops. University of Vienna, Austria (1995)
1. Secondary product metabolic engineering. University of Helsinki, Finland (1995)

**D. PRESENTATIONS OF PhD STUDENTS AND POSTDOCS**

## AT NATIONAL AND INTERNATIONAL MEETINGS

Saba-Mayoral A (2022) Biolistic-mediate transformation of the Spanish elite rice genotype Bomba. 6<sup>th</sup> International Conference on “Transgenic plants and transformation technologies” Vienna International Science Conferences and Events Association VISCEA pp 22 July 6-7 Vienna, Austria. INVITED ORAL COMMUNICATION

He, W (2022) A crucial first step in engineering nitrogenase in cereals crops: expression of functional Fe protein (NifH) in rice mitochondria. 6<sup>th</sup> International Conference on “Transgenic plants and transformation technologies” Vienna International Science Conferences and Events Association VISCEA pp 22 July 6-7 Vienna, Austria. INVITED ORAL COMMUNICATION

Huang X (2022) reconstitution of a heterologous crocin biosynthetic pathway in Nicotiana. 6<sup>th</sup> International Conference on “Transgenic plants and transformation technologies” Vienna International Science Conferences and Events Association VISCEA pp 22 July 6-7 Vienna, Austria. INVITED ORAL COMMUNICATION

Sobrino-Mengual G, Cerdá-Bennasser P, Capell T, Christou P, Alvarez D (2022) Engineering rice endosperm with an ectopic cytosolic MEP pathway induces profound metabolite and transcriptome changes. 6<sup>th</sup> International Conference on “Transgenic plants and transformation technologies” Vienna International Science Conferences and Events Association VISCEA pp 22 July 6-7 Vienna, Austria. Poster presentation.

Sobrino-Mengual G, Cerdá-Bennasser P, Capell T, Christou P, Alvarez D (2022) The role of cis-acting elements in the control of gene expression: restoring functionality of the rice *psi1* gene promoter to accumulate endosperm carotenoids. 6<sup>th</sup> International Conference on “Transgenic plants and transformation technologies” Vienna International Science Conferences and Events Association VISCEA pp 22 July 6-7 Vienna, Austria. Poster presentation.

Vargheese A, He W, Armario-Najera V, Nölke G, Schillberg S, Capell T, Christou P (2022) Introducing an algal Carbon Concentration Mechanism into rice to enhance photosynthetic efficiency and productivity. 6<sup>th</sup> International Conference on “Transgenic plants and transformation technologies” Vienna International Science Conferences and Events Association VISCEA pp 23 July 6-7 Vienna, Austria. Poster presentation.

Vargheese A, He W, Armario-Najera V, Nölke G, Schillberg S, Capell T, Christou P (2022) Introduction of bacterial glycolate catabolic pathway into rice to enhance photosynthetic efficiency of rice. 6<sup>th</sup> International Conference on “Transgenic plants and transformation technologies” Vienna International Science Conferences and Events Association VISCEA pp 23 July 6-7 Vienna, Austria. Poster presentation.

He W, Vargheese A, Nölke G, Schillberg S, Capell T, Armario-Najera V and Christou P (2022) Towards the engineering of a novel ectopic oxygen scavenging pathway to enhance the photosynthetic efficiency of rice. 6<sup>th</sup> International Conference on “Transgenic plants and transformation technologies” Vienna International Science Conferences and Events Association VISCEA pp 22 July 6-7 Vienna, Austria. Poster presentation.

Huang X, Zhu C, Christou P, Capell T (2022) Towards CRISPR/Cas9 mediated enhancement of  $\beta$ -carotene in maize endosperm. 6<sup>th</sup> International Conference on “Transgenic plants and transformation technologies” Vienna International Science Conferences and Events Association VISCEA pp 26 July 6-7 Vienna, Austria. Poster presentation.

Armario-Najera V, Sobrino-Mengual G, Saba-Mayoral A, Capell T, Bardor M and Christou P (2022) Glycosylation analysis of SARS-CoV-2 receptor binding domain (RBD) produced in rice. 6<sup>th</sup> International Conference on “Transgenic plants and transformation technologies” Vienna International Science Conferences and Events Association VISCEA pp 22 July 6-7 Vienna, Austria. Poster presentation.

Armario-Najera V Blanco-Perera A, Shenoy SR, Sun Y, Marfil S, Muñoz-Basagoiti J, Perez-Zsolt D, Blanco J, Izquierdo-Useros N, Capell T, O'Keefe BR, Christou P (2022) Microbial activity of the Scytovirin domain 1 (SD1) produced in rice against HIV-1. 6<sup>th</sup> International Conference on "Transgenic plants and transformation technologies" Vienna International Science Conferences and Events Association VISCEA pp 22 July 6-7 Vienna, Austria. Poster presentation.

P. Cerdá Bennasser, T. Capell, P. Christou, C. Zhu. Differentiation-state dependent metabolomic and transcriptomic profiling of rice callus and endosperm engineered with an ectopic MEP pathway. Symposium on Small Molecules in Plant Research: Chemistry and Biology Come Together, Valencia, Spain, December 10-11 2019. Poster presentation.

X. Huang, P. Cerdá Bennasser, Ma V. Armario-Najera, A. Schiermeyer, T. Capell, S. Schillberg, P. Christou, C. Zhu. Modulation of maize root architecture by altering strigolactone content and composition through genome editing. Symposium on Small Molecules in Plant Research: Chemistry and Biology Come Together, Valencia, Spain, December 10-11 2019. Poster presentation.

X. Huang, Ma V. Armario Najera, P. Cerdá Bennasser, T. Capell, P. Christou, C. Zhu. Enhancement of  $\beta$ -carotene content in maize endosperm by genome editing. Symposium on Small Molecules in Plant Research: Chemistry and Biology Come Together, Valencia, Spain, December 10-11 2019. Poster presentation.

M. Lobato, L. Gómez-Gómez, O. Ahrazem, T. Capell, C. Zhu, P. Christou, D. Álvarez. Towards the engineering of the saffron biosynthetic pathway in rice. Symposium on Small Molecules in Plant Research: Chemistry and Biology Come Together, Valencia, Spain, December 10-11 2019. Poster presentation.

D. Álvarez, M. Lobato, T. Capell, P. Christou, C. Zhu. Dissection of the early endogenous carotenoid biosynthetic pathway in rice endosperm. Symposium on Small Molecules in Plant Research: Chemistry and Biology Come Together, Valencia, Spain, December 10-11 2019. Poster presentation.

C. Zhu, T. Capell, P. Christou. The subcellular localization of two isopentenyl diphosphate isomerases in rice suggests a role for the endoplasmic reticulum in isoprenoid biosynthesis. Cost Action "European network to advance carotenoid research and applications in agro-food and health" (EUROCAROTEN)" 26-28 of November 2019, Limassol, Cyprus. Invited oral presentation

C. Zhu, X. Jin, C. Bai, T. Capell, P. Christou. Regulation of carotenoid biosynthesis and accumulation in cereal crops National Plant Breeding & Biotechnology Symposium (April 13-16, 2019) Linzhi, Tibet, China. Invited Oral Presentation

C. Zhu, X. Jin, C. Bai, T. Capell, P. Christou Regulation of  $\beta$ -carotene biosynthesis and accumulation in maize endosperm Carotenoids Gordon Research Conference: Carotenoids, Apocarotenoids and Retinoids: From Nature to Bedside (June 17-22, 2018) Grand Summit Hotel at Sunday River Newry, ME, USA

Capell T, Vamvaka E, Farré G, Molinos-Albert LM, Evans A, Canela-Xandri A, Twyman RM, Carrillo J, Ordoñez RA, Shattock R, O'Keefe BR, Clotet B, Blanco J, Christou P (2018) A triple combination microbicide against HIV-1 expressed in rice. 3<sup>rd</sup> ISPMF Conference in Helsinki, June (11<sup>th</sup> – 14<sup>th</sup>) Selected oral communication.

C. Zhu, X. Jin, C. Bai, G. Farre, T. Capell, P. Christou Regulation of  $\beta$ -Carotene biosynthesis and accumulation in maize endosperm The 3<sup>rd</sup> Maize Biology Conference of China (April 25-28, 2018) Qingdao, China

Nogareda C, Moreno JA, Angulo E, Garcia-Curbelo Y, Sandmann G, Castellani M, Capell T, Zhu C, Christou P (2018) Maíz transgénico enriquecido con carotenoides en la alimentación de los pollos les protege de los coccidios y de la pododermatitis plantar VI Congreso Internacional de Producción Animal Tropical 2018 "Por la Resiliencia de los sistemas ganaderos" 29 Oct al 2 Nov La Habana, Cuba

C. Baysal, L. Bortesi, C. Zhu, G. Farré, V. Medina, S. Schillberg, P. Christou CRISPR/Cas9 mutation of the rice starch branching enzyme iib gene (*OsBEIIb*) does not affect the closely related potential off-target osbeiiia and results in mono and biallelic knockouts in the T1 generation XXII Reunión de la Sociedad Española de Fisiología Vegetal/XV Spanish Portuguese Congress of Plant Physiology (June 26-29, 2017) Barcelona, Spain

X. Jin, C. Bai, J. Berman, G. Farré, T. Capell, P. Christou, G. Sandmann, C. Zhu Regulation of  $\beta$ -carotene biosynthesis and accumulation in maize endosperm EUROCARTEN 4th Management Committee & 3rd Working Groups Meetings & Workshop Sustainable Production of Carotenoids: From Biosynthesis to Biotechnology (October 16-18, 2017) Trogir, Croatia. Selected Oral Presentation

J. Díaz-Gómez, C. Nogareda, J.A. Moreno, E. Angulo, G. Sandmann, T. Capell, P. Christou and C. Zhu (2017) High-carotenoid biofortified maize as feed component. CaroRed meeting (Valencia, 16-18 November) pp 47.

X. Jin, C. Bai, J. Berman, G. Farré, T. Capell, P. Christou, G. Sandmann, C. Zhu Regulation of maize (*Zea mays* L.)  $\beta$ -carotene hydroxylase 2 (*ZmBCH2*) gene expression Reunión Nacional sobre Carotenoides en Microorganismos, Plantas, Alimentación y Salud (November 16-17, 2017) Valencia, Spain. Selected Oral Presentation

J.A. Moreno, J. Diaz-Gomez, E. Angulo, B. Serrano-Pérez, G. Sandmann, T. Capell, C. Zhu, P. Christou, C. Nogareda Carotenoid-enriched corn protects poultry against coccidiosis APICOWPLEXA 4th International Meeting on Apicomplexa in Farm Animals (October 11-14, 2017) Madrid, Spain

J.A. Moreno, E. Angulo, G. Sandmann, T. Capell, C. Zhu, P. Christou, C. Nogareda Los pollos que consumen maíz enriquecido con carotenoides mejoran los niveles de pododermatitis, úlceras plantares y coccidiosis XXII Simposio Anual de AVEDILA (November 16-17, 2017) Valladolid, Spain

J.A. Moreno, J. Diaz-Gomez, E. Angulo, B. Serrano-Pérez, G. Sandmann, T. Capell, Zhu C, P. Christou, C. Nogareda High-carotenoid biofortified maize as feed component Reunión Nacional sobre Carotenoides en Microorganismos, Plantas, Alimentación y Salud (November 16-17, 2017) Valencia, Spain

C. Zhu, X. Jin, C. Bai, G. Farré, T. Capell, P. Christou Regulation of  $\beta$ -carotene hydroxylase 2 gene promoter in maize The 18<sup>th</sup> International Symposium on Carotenoids (18<sup>th</sup> ICS) (July 9-14, 2017) Lucerne, Switzerland. Selected Oral Presentation.

C. Zhu, C. Bai, X. Jin, J. Berman, G. Farre, T. Capell, P. Christou (2016) Regulation of  $\beta$ -carotene biosynthesis and accumulation in maize endosperm. XIX International Botanical Congress (July 23-29, 2017) Shen Zhen, China. Oral presentation.

Y. Sheng, X. Ni, X. Jin, L. Shi, G. Farre, T. Capell, P. Christou, C. Zhu (2016) Molecular cloning and functional analyses of two genes encoding dihydroflavonol 4-reductase (DFR) from orange-flower gentian (*Gentiana lutea* L. var. *aurantiaca*) XIX International Botanical Congress (July 23-29, 2017) Shen Zhen, China. Oral presentation

P. Diaz-Benito, B. Fernández, R. Banakar, S. Rodríguez, P. Christou, R. Pereiro, J. Abadía, A. Álvarez-Fernández. (2016). Localization of Metals and Metal Ligands in Rice Seeds Overexpressing Nicotianamine Synthase and/or Barley Nicotianamine Amino Transferase 18th International Symposium on Iron Nutrition and Interaction in Plants, Madrid, May 30-June 3

R. Banakar, Teresa Capell, Paul Christou (2013) Heterologous expression of barley YS1 transporter in rice improves iron uptake and seed iron levels EPSO Conference, Greece September 6-9.

E. Vamvaka, Murad A.M, E. Rech, P. Christou, T. Capell (2013) Proteomic analysis and functional characterization of the anti-HIV antibody 2G12 in rice seeds. XIII Portuguese-Spanish Congress on Plant Physiology. Lisbon, Portugal July 24-27 (poster presentation)

E. Vamvaka, E. Arcalis, Melnik S, Altmann F, E. Stoger, P. Christou, T. Capell (2013) Expression, purification and glycosylation profile of the 2G12 anti-HIV antibody produced in rice seeds. XIII Portuguese-Spanish Congress on Plant Physiology. Lisbon, Portugal July 24-27 (selected short oral presentation)

Arjo G, Zhu C, Capell T, Matias-Guiu, Piñol C, Christou P (2012) genetically engineered multivitamin corn feeding assessment. Congress 22<sup>nd</sup> IUBMB and 37<sup>th</sup> FEBS from Single Molecules to Systems Biology September 4-9 th Sevilla Spain P 18-22

Yuan D, Masip G, Buntru M, Kreuzaler F, Capell T, Christou (2012) reconstruction of glycolate dehydrogenase in rice: a first essential step towards reducing photorespiration in C3 plants. Plant Biology Congress 2012 FEBS and EPSO, 29 July to 3 August, Albert-Ludwings-University Freiburg, Germany pp 157

Farre G, Rivera S, Canela R, Zhu C, Capell T, Christou P (2012) Transcriptional-metabolic networks in corn plants expressing transgenes from the carotenoid biosynthesis pathway. Plant Biology Congress 2012 FEBS and EPSO, 29 July to 3 August, Albert-Ludwings-University Freiburg, Germany pp 640

Sanahuja G, Farre G, Bassie L, Zhu C, Capell T, Christou P (2012) Ascorbate biosynthesis and recycling enzymes are subject to complex and genotype-dependent feedback regulation during maize endosperm development. Plant Biology Congress 2012 FEBS and EPSO, 29 July to 3 August, Albert-Ludwings-University Freiburg, Germany pp 668

Berman-Quintana J, Farre G, Rivera S, Bai C, Canela R, Sandmann G, Zhu C, Capell T, Christou P (2012) Transgenic maize and rice expressing a heterologous Orange (Or) gene exhibits enhanced accumulation of carotenoids. Plant Biology Congress 2012 FEBS and EPSO, 29 July to 3 August, Albert-Ludwings-University Freiburg, Germany pp 627

Sabalza M, Ramessar K, van Dolleweerd C, O'Keefe B, Ma J, Capell T, Christou P (2012) Plant based microbicides containing antiviral lectins for HIV prevention. Plant Biology Congress 2012 FEBS and EPSO, 29 July to 3 August, Albert-Ludwings-University Freiburg, Germany pp 617

Zorrilla-Lopez U, Farre G, Zhu C, Capell T, Christou P (2012) Transgenic rice with altered vitamin E content through metabolic engineering. Plant Biology Congress 2012 FEBS and EPSO, 29 July to 3 August, Albert-Ludwings-University Freiburg, Germany pp 287

Banakar R, Gomez-Galera S, Capell T, Christou P (2012) Multigene engineering of rice for iron biofortification Plant Biology Congress 2012 FEBS and EPSO, 29 July to 3 August, Albert-Ludwings-University Freiburg, Germany pp 121

Arjó G, Capell T, Christou P, Piñol C (2011) Multivitamin corn: toxicity and allergenicity safety assessment. 5th International Symposium on Recent Advances in Food Analysis (RAFA 2011) November 1 - 4, Prague, Czech Republic. Poster presentation

Banakar R, Capell T, Christou P (2011) A novel mechanism for oxidative stress tolerance in plants mediated by the Arabidopsis ATP-sulfurylase gene. Conference Molecular bases of Plant Stress, 21-23 September. Varna, Bulgaria. Poster presentation

Sanahuja G, Farre G, Bassie L, Zhu C, Capell T, Christou P (2011) Elucidation of rate limiting steps of ascorbate biosynthesis in maize. International Phytochemical society of Europe (PSE) Symposium. Photochemicals in nutrition and health 27-30 September 2011 Giovignano (BARI), Italy. Poster presentation. pp 86

Miralpeix B, Hoefer R, Werck D, Dong L, Bouwmeester HJ, Capell T, Christou P (2011) Metabolic engineering of terpenoid biosynthesis in transgenic tobacco plants. International Phytochemical society of Europe (PSE) Symposium. Photochemicals in nutrition and health 27-30 September 2011 Giovinazzo (BARI), Italy. Poster presentation pp 89

Bai C, Rivera SM, Medina V, Sandmann G, Canela R, Zhu, Capell T, Christou P (2011) Metabolic engineering of carotenoid biosynthesis in rice endosperms. International Phytochemical society of Europe (PSE) Symposium. Photochemicals in nutrition and health 27-30 September 2011 Giovinazzo (BARI), Italy. Poster presentation. pp 76

Peremarti A, Villegas D, Christou P, Capell T, Royo C (2011) Molecular and physiological characterization of a light-green durum wheat mutant. Mexico September

Yuan D, Capell T and Christou P (2011). Engineering rice with glycolate dehydrogenase – a first step in addressing limitations in carbon assimilation in rice. The Gordon Research Conference on CO<sub>2</sub> Assimilation in Plants: Genome to Biome held May 29, 2011 – June 3, 2011 at Les Diablerets Conference Centre in Les Diablerets, Switzerland. Poster Presentation.

Arjo G Capell T Piñol C Christou P (2011) Preliminary safety assessment of two genetically engineered corn lines expressing carotenoids and other vitamin biosynthetic genes. 2011 Meeting of the Society for In Vitro Biology June 4-8 Raleigh, North Carolina, USA poster presentation pp 6

Sabalza M, van Dolleweerd C, Capell T, O'Keefe B, Ma J, Christou P (2011) Antiviral lectins as components of plant based microbicides for HIV prevention. 2011 Meeting of the Society for In Vitro Biology June 4-8 Raleigh, North Carolina, USA poster presentation pp 11

Vamvaka E, Sabalza M, O'Keefe B, Ma J, Capell T, Christou P (2011) A transgenic rice seed based platform for the production of next generation multi-component microbicides against HIV. 2011 Meeting of the Society for In Vitro Biology June 4-8 Raleigh, North Carolina, USA poster presentation pp 11

Dashevskaya E, Horn R, Zimmermann D, Chudobova I, Schilbert S. Capell T, Christou P (2011) Identification of rice genes induced by abiotic stress by proteomics and qRT-PCR. 2011 Meeting of the Society for In Vitro Biology June 4-8 Raleigh, North Carolina, USA poster presentation pp 15.

Gemma Farré, Sol M Rivera, Ramon Canela, Gerhard Sandmann, Changfu Zhu, Teresa Capell and Paul Christou (2011). Transgenic corn plants engineered with the Arabidopsis homologue of the Orange (Or) gene. TERPNET 10th International Meeting: Biosynthesis and function of isoprenoids in plants, microorganisms and parasites. May 22-May 26, 2011, Kalmar, Sweden. pp.

B Miralpeix R Hoefer D Werck L Dong H Bouwmeester T Capell P Christou (2011) Towards engineering of the early steps of terpenoid Indole Alkaloid pathway in tobacco plants. TERPNET 2011, 10th International Meeting: Biosynthesis and function of isoprenoids in plants, microorganisms and parasites. May 22-May 26, 2011, Kalmar, Sweden. pp.

Bai C, Rivera SM, Medina V, Sandmann G, Canela R, Zhu C, Capell T and Christou P (2011) An effective in vitro functional expression assay for carotenogenic genes in rice. TERPNET 2011, 10th International Meeting: Biosynthesis and function of isoprenoids in plants, microorganisms and parasites. May 22-May 26, 2011, Kalmar, Sweden. pp.141.

Zhu C, Farre G, Shi L, Bai C, Sanahuja G, Capell T, Sandmann G and Christou P (2011) Cloning and functional characterization of carotene hydroxylase genes from maize (*Zea mays* L.) endosperm. TERPNET 2011, 10th International Meeting: Biosynthesis and function of isoprenoids in plants, microorganisms and parasites. May 22-May 26, 2011, Kalmar, Sweden. pp.143.

C. Bai, S. Maïam Rivera V. Medina, R. Canela, G. Sandmann, T. Capell, P. Christou, and C. Zhu (2010) Exploring the mechanism of carotenoid accumulation in rice (*Oryza sativa*) endosperm through multi-gene engineering. First Global Conference in Biofortification Washington DC USA, 8-11- November

Gemma Farre, Shaista Naqvi, Gerhard Sandmann, Teresa Capell, Changfu Zhu Paul Christou (2010) Multivitamin biofortification of an elite south african corn inbred through combinatorial nuclear transformation. First Global Conference in Biofortification Washington DC USA, 8-11- November

G. Farre, S. Naqvi, G. Sandmann, T. Capell, C. Zhu, P. Christou (2010) The development of nutritionally enhanced crops for human health and food security: high zeaxanthin corn. First Global Conference in Biofortification Washington DC USA, 8-11- November

Sabalza M, Ramessar K, Van Dolleweerd C, Capell T, O'Keefe B, Ma J, Christou P (2010) next generation combination microbicides to combat HIV COST ACTION FA0804 Molecular Farming Annual meeting 6-8 October Vico Equence (Naples) Italy Poster p24

Miralpeix B, Avesani L, Capell T Pezzoti M, Christou P Ramessar K (2010) expression and analysis of recombinant human glutamic acid decarboxylase (GAD) autoantigens in transgenic maize to prevent insulin-dependnet diabetes mellitus. COST ACTION FA0804 Molecular Farming Annual meeting 6-8 October Vico Equence (Naples) Italy Poster p13

Miralpeix B Rimini 14 International Biotechnology Symposium and Exhibition 15-19 September Rimini Italy

Farre G, Sanahuja G, Naqvi S, Sandman G, Capell T, Christou P, Zhu C (2010) The development of nutritionally enhanced crops for human health and food security: high provitamin A, multi-vitamin and high zeaxanthin corn. Annual Meeting of the American Society of Plant Biologist, Montreal, Canada 31 July to 4 August

Dashevskaya S, Haensel U, Horn R, Zimmermann D, Chudobova I, Shillberg S, Capell T, Christou P, von Koskull-Doring P (2010) Novel strategies for the identification of inducible molecular markers for abiotic stress response in rice Annual Meeting of the American Society of Plant Biologist, Montreal, Canada 31 July to 4 August

Sabalza M, Ramessar K, van Dolleweerd C, Capell T, O'Keefe B, Ma J, Christou P (2010) next generation combination microbicides to combat HIV Modern Mucosal Vaccines, Adjuvants and Microbicides: preventing transmission of pathogens 28-30 April The Alexander Hotel Dublin Ireland

Zhu C (2010) The role of genetic background in determining qualitative and quantitative carotenoid profiles in corn endosperm In the 12<sup>th</sup> World Congress of the International Association for Plant Biotechnology (**12<sup>th</sup> IAPB**) and the 2010 In Vitro Biology Meeting of the Society for In Vitro Biology (**SIVB**) held in St. Louis, MO, USA, June 6-11.

Bai C, Maïam Rivera S, Medina V, Canela R, Sandmann G, Capell T, Christou P, Zhu C (2010) metabolic engineering of carotenoid accumulation in rice endosperm through combinatorial genetic transformation. In the 12<sup>th</sup> World Congress of the International Association for Plant Biotechnology (**12<sup>th</sup> IAPB**) and the 2010 In Vitro Biology Meeting of the Society for In Vitro Biology (**SIVB**) held in St. Louis, MO, USA, June 6-11.

Bai C, Maïam Rivera S, Medina V, Canela R, Sandmann G, Capell T, Christou P, Zhu C (2010) Carotenoid biofortification of rice endosperm through multiplex gene transformation. Gordon Research Conference in Carotenoids January 17-22 Ventura Beach Marriot, Ventura CA, USA.

Zhu C, Naqvi S, Sandmann G, Capell T, Christou P (2010) Enhancement of zeaxanthine levels in corn by combining transgenesis and conventional breeding. Gordon Research Conference in Carotenoids January 17-22 Ventura Beach Marriot, Ventura CA, USA.

S Naqvi, G Farre, G Sanahuja, C Bai, T Capell and P Christou. (2009) Simultaneous multi-pathway engineering in crop plants through combinatorial genetic transformation: creatin nutritionally biofortified cereal grains for food security. Event Planners Australia del 10/08 al 14/08 Tropical North Queensland (Australia)

S Naqvi, Ch Zhu, G Farre, J Breitenbach, G Sandmann, P Christou and T Capell (2009) Creation of carotenoid-enriched corn mutant series through combinatorial genetic transformation. Gordon Research Conference on Plant Metabolic Engineering. Waterville Valley, NH United States, July 12-17 Poster presentation

G Farre, S Naqvi, K Ramessar, L Bassie, J Breitenbach, D Perez, G Ros, G Sandmann, T Capell, P Christou and Ch Zhu , (2009) Multivitamin biofortification of an elite South African maize inbred through combinatorial nuclear transformation IPMB (international congress of plant and molecular biology) 21/10 al 30/10. St. Louis (EEUU)

S Gomez-Galera, D Sudhakar, Ch Zhu, A Pelacho, T Capell, P Christou (2009) Expression of the barley *iron-phytosiderophore transporter 1 (HvYS1)* gene in rice for enhanced tolerance to alkaline soils and iron biofortification. IPMB (international congress of plant and molecular biology) 21/10 al 30/10. St. Louis (EEUU)

M Sabalza, K Ramessar, T Capell and P Christou (2009) Expression and analysis of recombinant anti-hiv monoclonal antibodies in transgenic corn. IPMB (international congress of plant and molecular biology) 21/10 al 30/10. St. Louis (EEUU).

A Peremarti, L Bassie, Ch Zhu, P Christou and T Capell (2009) Molecular characterization of the arginine decarboxylase (Adc) gene family in rice. IPMB (International Congress of Plant and Molecular Biology) 21/10 al 30/10. St. Louis (EEUU).

Yuan D, Peremarti A, Bassie L, Zhu C, Christou P, Capell T (2009) Cloning and characterization of two novel polyamine gene promoters from rice (*Oryza sativa*) Gordon Research Conference on Plant Metabolic Engineering. Waterville Valley, NH United States, July 12-17, Poster presentation

G Sanahuja, G Farre, S Naqvi, J Breitenbach, G Sandmann, C Zhu, T Capell and P Christou (2009) Enhanced levels of vitamin E in transgenic corn trough metabolic engineering. 14 th. European Meeting on Fat Soluble Vitamins. Potsdam (Germany)\_

G Farre,S Naqvi, J Breitenbach, D Perez Conesa, G Ros, G Sandmann, T Capell, P Christou and C Zhu (2009) Biofortification of corn with enhanced vitamins through combinatorial genetic transformation. 14 th. European Meeting on Fat Soluble Vitamins. Potsdam (Germany)\_

C.Bai, C Zhu, T Capell, J Breitenbach, G Sandmann, P Christou (2009) Metabolic engineering of carotenoid accumulation in rice endosperm through combinatorial genetic transoformation. 14 th. European Meeting on Fat Soluble Vitamins. Potsdam (Germany)\_

Ariadna Peremarti, Ludovic Bassie, Dawei Yuan, Ana Pelacho, Paul Christou, Teresa Capell (2009).Transcriptional regulation of the rice *arginine decarboxylase (Adc1)* gene by methyl jasmonate. XVIII-National Meeting of Spanish Society of Plant Physiology and XI-Congress Spanish-Portuguese of Plant Physiology. Zaragoza. Spain. pp. 302. Abstract.

Qunrui Li, Dawei Yuan, Chao Bai, Qingjie Yang, Teresa Capell, Gerhard Sandmann, Paul Christou and Changfu Zhu (2009). Cloning and characterization of a chromoplast-specific zeaxanthin epoxidase gene promoter from *Gentiana lutea*. XVIII-National Meeting of Spanish Society of Plant Physiology and XI-Congress Spanish-Portuguese of Plant Physiology. Zaragoza. Spain. pp. 138. Abstract

Sonia Gómez-Galera, Duraialagaraja Sudhakar, Changfu Zhu, Ana M. Pelacho, Teresa Capell, Paul Christou (2009) Genetic engineering of rice with a barley iron-phytosiderophore transporter XVIII-National Meeting of

Spanish Society of Plant Physiology and XI-Congress Spanish-Portuguese of Plant Physiology. Zaragoza. Spain. pp. 186. Abstract

Svetlana Dashevskaya, Ulrike Haensel, Ruth Horn, Denise Zimmermann, Ivana Chudobova, Stefan Schillberg, Teresa Capell, Paul Christou, Pascal von Koskull-Döring (2009). Identification of molecular markers for bioregulators that enhance rice productivity and quality. XVIII-National Meeting of Spanish Society of Plant Physiology and XI-Congress Spanish-Portuguese of Plant Physiology. Zaragoza. Spain. pp. 134. Abstract

Ludovic Bassié, Eduard Perez-Massot, Ariadna Peremarti, Changfu Zhu, Paul Christou, Teresa Capell (2009). Cloning and characterization of the wheat arginine decarboxylase gene. XVIII-National Meeting of Spanish Society of Plant Physiology and XI-Congress Spanish-Portuguese of Plant Physiology. Zaragoza. Spain. pp. 291. Abstract

M. Sabalza, K.Ramessar, T.Capell, P.Christou (2009) HIV neutralizing monoclonal antibodies expressed in maize seeds. XVIII-National Meeting of Spanish Society of Plant Physiology and XI-Congress Spanish-Portuguese of Plant Physiology. Zaragoza. Spain. pp. 346 Abstract

Chao Bai, Changfu Zhu, Teresa Capell, Jurgén Breitenbach, Gerhard Sandmann and Paul Christou (2009). Exploring the mechanism of carotenoid accumulation in rice endosperm through multi-gene engineering. XVIII-National Meeting of Spanish Society of Plant Physiology and XI-Congress Spanish-Portuguese of Plant Physiology. Zaragoza. Spain. pp. 337. Abstract

Gemma Farre, Shaista Naqvi, Georgina Sanahuja, Changfu Zhu, Teresa Capell, Paul Christou (2009) Transgenic corn with enhanced levels of vitamin E through metabolic engineering. XVIII-National Meeting of Spanish Society of Plant Physiology and XI-Congress Spanish-Portuguese of Plant Physiology. Zaragoza. Spain. pp. 341. Abstract

Qunrui Li, Gemma Farre, Shaista Naqvi, Gerhard Sandmann, Teresa Capell, Paul Christou and Changfu Zhu (2009). Cloning and characterization of two carotenoid isomerase (CRTISO) genes from maize (*Zea mays* L.) endosperm. XVIII-National Meeting of Spanish Society of Plant Physiology and XI-Congress Spanish-Portuguese of Plant Physiology. Zaragoza. Spain. pp. 139. Abstract

Gemma Farre, Shaista Naqvi, Teresa Capell, Gerhard Sandmann, Paul Christou and Changfu Zhu (2009). Characterization and functional analyses of two individual beta-carotene hydroxylase (BCH) genes from maize (*Zea mays* L.) endosperm. XVIII-National Meeting of Spanish Society of Plant Physiology and XI-Congress Spanish-Portuguese of Plant Physiology. Zaragoza. Spain. pp. 135. Abstract

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## EDITORIAL WORK

1. Plant Journal - Editorial Advisory Board (1990-1996)
2. Plant Science - Editorial Advisory Board (1994-1998)
3. Molecular Breeding - Editorial Board (November 1998-2002)  
**Chief Editor** (April 2002-2020)
4. Transgenic Research - Editorial Board (1998 -1999)
5. **Transgenic Research - Chief Editor** (1999 – 2022)

# **Appendix B**

## Materials Considered

| Exhibit | Description                                                                                                                                                                                                              |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1001    | US Patent No. 11,371,055 (“the ’055 patent”).                                                                                                                                                                            |
| 1003    | Dr. Silverstone’s declaration                                                                                                                                                                                            |
| 1018    | Cindy Peach and Jeff Velten, <i>Transgene expression variability (position effect) of CAT and GUS reporter genes driven by linked divergent T-DNA promoters</i> , 17 PLANT MOLECULAR BIOLOGY, 49-60 (1991).              |
| 2010    | Paine et al., <i>Improving the nutritional value of Golden Rice through increased pro-vitamin A content</i> , 23(4) NATURE BIOTECHNOLOGY 482–87 (2005) (“Paine”).                                                        |
| 2013    | Provisional U.S. App. No. 60/731,044.                                                                                                                                                                                    |
| 2014    | Certified File History for U.S. App. No. 12/091,896.                                                                                                                                                                     |
| 2015    | Certified File History for U.S. App. No. 13/647,081.                                                                                                                                                                     |
| 2016    | Certified File History for U.S. App. No. 14/491,197.                                                                                                                                                                     |
| 2017    | WO 2007/053482 A2, published May 10, 2007.                                                                                                                                                                               |
| 2057    | Twyman et al., <i>Foreign DNA: Integration and Expression in Transgenic Plants</i> , 24 GENETIC ENGINEERING 107–36 (2002) (“Twyman 2002”).                                                                               |
| 2058    | Vaeck et al., <i>Transgenic plants protected from insect attack</i> , 328 NATURE 33–37 (1987) (“Vaeck”).                                                                                                                 |
| 2059    | Block et al., <i>Engineering herbicide resistance in plants by expression of a detoxifying enzyme</i> , 6(9) THE EMBO JOURNAL 2513–18 (1987) (“Block”).                                                                  |
| 2060    | Owen, <i>Current use of transgenic herbicide-resistant soybean and corn in the USA</i> , 19 CROP PROTECTION 765–71 (2000) (“Owen”).                                                                                      |
| 2061    | Twyman et al., <i>Molecular farming in plants: host systems and expression technology</i> , 21(12) TRENDS IN BIOTECHNOLOGY 570–78 (“Twyman 2003”).                                                                       |
| 2062    | Potenza et al., <i>Targeting Transgene Expression in Research, Agricultural, and Environmental Applications: Promoters Used in Plant Transformation</i> , 40(1) IN VITRO CELL. DEV. BIOL. PLANT 1–22 (2004) (“Potenza”). |
| 2063    | Kim et al., <i>Handbook of Plant Biotechnology</i> , Vol. 1 Ch. 5 WILEY 71–80 (2004).                                                                                                                                    |
| 2064    | Perlak et al., <i>Modification of the coding sequence enhances plant expression of insect control protein genes</i> , 88 PROC. NATL. ACAD. SCI. USA 3324–28 (1991) (“Perlak”).                                           |

| Exhibit | Description                                                                                                                                                                                                                                             |
|---------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2065    | Murray et al., <i>Codon usage in plant genes</i> , 17(2) NUCLEIC ACIDS RESEARCH 477–98 (1989) (“Murray”).                                                                                                                                               |
| 2066    | Verdaguer et al., <i>Functional organization of the cassava vein mosaic virus (CsVMV) promoter</i> , 37 PLANT MOLECULAR BIOLOGY 1055–67 (1998) (“Verdaguer”).                                                                                           |
| 2067    | De Bolle et al., <i>Analysis of the influence of promoter elements and a matrix attachment region on the inter-individual variation of transgene expression in populations of Arabidopsis thaliana</i> , 165 PLANT SCIENCE 169-179 (2003) (“De Bolle”). |
| 2069    | McElroy et al., <i>Construction of expression vectors based on the rice actin 1 (Act1) 5’ region for use in monocot transformation</i> , 231 MOL. GEN. GENET. 150–60 (1991) (“McElroy”).                                                                |
| 2070    | Christensen and Quail, <i>Ubiquitin promoter-based vectors for high-level expression of selectable and/or screenable marker genes in monocotyledonous plants</i> , 5 TRANSGENIC RESEARCH 213–18 (1996) (“Christensen”).                                 |
| 2071    | Mascarenhas et al., <i>Intron-mediated enhancement of heterologous gene expression in maize</i> , 15 PLANT MOLECULAR BIOLOGY 913–20 (1990) (“Mascarenhas”).                                                                                             |
| 2072    | Mitsuhara et al., <i>Efficient Promoter Cassettes for Enhanced Expression of Foreign Genes in Dicotyledonous and Monocotyledonous Plants</i> , 37(1) PLANT CELL PHYSIOL. 49–59 (1996) (“Mitsuhara”).                                                    |
| 2074    | Bhat and Srinivasan, <i>Molecular and genetic analyses of transgenic plants: Considerations and approaches</i> , 163 PLANT SCIENCE 673–81 (2002) (“Bhat”).                                                                                              |
| 2075    | Altpeter et al., <i>Particle bombardment and the genetic enhancement of crops: myths and realities</i> , 15 MOLECULAR BREEDING 305–27 (2005) (“Altpeter”).                                                                                              |
| 2076    | Schroeder et al., <i>Transformation and Regeneration of Two Cultivars of Pea (Pisum sativum L.)</i> , 101 PLANT PHYSIOL. 751–57 (1993) (“Schroeder”).                                                                                                   |
| 2077    | Engvall and Perlmann, <i>Enzyme-linked immunosorbent assay, ELISA, III. Quantitation of Specific Antibodies by Enzyme-Labeled Anti-Immunoglobulin in Antigen-Coated Tubes</i> , 109(1) THE JOURNAL OF IMMUNOLOGY 129–35 (1972) (“Engvall”).             |
| 2078    | Burnette, <i>“Western Blotting”: Electrophoretic Transfer of Proteins from Sodium Dodecyl Sulfate-Polyacrylamide Gels to Unmodified</i>                                                                                                                 |

| Exhibit | Description                                                                                                                                                                            |
|---------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|         | <i>Nitrocellulose and Radiographic Detection with Antibody and Radioiodinated Protein A</i> , 112 ANALYTICAL BIOCHEMISTRY 195–203 (1981) (“Burnette”).                                 |
| 2079    | Zhang et al., <i>The use of glufosinate as a selective agent in Agrobacterium-mediated transformation of soybean</i> , 56 PLANT CELL, TISSUE AND ORGAN CULTURE 37–46 (1999) (“Zhang”). |
| 2080    | Brasileiro and Aragão, <i>Marker Genes for In Vitro Selection of Transgenic Plants</i> , 3(3) JOURNAL OF PLANT BIOTECHNOLOGY 113–21 (2001) (“Brasileiro”).                             |
| 2081    | WO 1997/013402 A1, published April 17, 1997.                                                                                                                                           |
| 2082    | Frame et al., <i>Genetic Transformation Using Maize Immature Zygotic Embryos</i> , 710 PLANT EMBRYO CULTURE METHODS AND PROTOCOLS 327–41 (2011).                                       |