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Plant Symposia

P-1

Modifying Soybean Oil for Feed and Fuel. T. E. CLEMENTE. University of Nebraska-Lincoln, Center for Plant Science Innovation/Department of Agronomy & Horticulture, Lincoln, NE. Email: tclemente1@unl.edu

Our laboratory has developed soybean germplasm that produces oil with improved characteristics for food, feed and fuel applications. The first is a soybean with oil high in oleic acid (>85%) and low in palmitic acid (<4%) and polyunsaturated fatty acids (<7%). We have demonstrated that soybeans that produce such oil are not compromised in agronomic performance and provide an improved feedstock for biodiesel. Secondly, we stacked the high oleic acid/low palmitic acid phenotype with elevated stearic acid, such that we now have a soybean that produces oil with oleic acid ranging from 60% to 70% and stearic acid ranging from 9% to 19%. Oil high in oleic acid and elevated in stearic acid has utility in a number of food applications, and also may provide an improved feedstock for biodiesel, given its predictive increases in oxidative stability and ignition quality, albeit at the expense of cold flow. The third phenotype is oil that contains over 60% omega-3 fatty acids, composed of approximately 30% linolenic acid and 30% stearidonic acid. While a soybean oil with high omega-3 fatty acids would have value for direct human consumption through a variety of food applications, we are currently investigating its potential as a lipid source in aquaculture feeds.

P-2

Enhancing Soybean Seed Lipid Biosynthesis for Nutritional, Energy and Industrial Applications. ANTHONY J. KINNEY. Crop Genetics Research and Development, DuPont Experimental Station, P.O. Box 80353, Wilmington, DE 19880-0353. Email: Anthony.kinney@usa.dupont.com

Soybean seeds, which contain approximately 40% protein and 20% oil, are an important feed ingredient as well as an important source of crude vegetable oil. Our overall goal is to generate soybean seed quality traits that provide added value for feed, fuel, industrial and food applications. The first generation of traits targets soybean oil properties, specifically oxidative stability, with products such as low linolenic (LL) or high oleic, low linolenic (HO) soybeans. These new oils can address a wide range of food and industrial markets. More recently our research efforts have been directed at increasing the oil and protein content of soybean seed, as well as vitamin E fortification of soybean oil.

P-3

Camelina sativa, A Potential Oilseed Platform for the Production of High-value Industrial Oils. EDGAR B. CAHOON. Center for Plant Science Innovation & Biochemistry Department, E318 Beadle Center, 1901 Vine Street, University of Nebraska, Lincoln, NE 68588. Email: ecahoon2@unl.edu

Camelina sativa (false flax) is an emerging Brassicaceae oilseed crop in the Great Plains and Pacific Northwest of the United States. The growing interest in camelina is due largely to its potential for biodiesel production in geographic areas that are not well-suited for soybean cultivation. We are also exploring the use of camelina as a platform for the production of high-value industrial oils. Camelina appears to be a good candidate to fill this niche because it is not widely grown in the US for food use. In addition, genetic transformation of camelina can be achieved by a simple floral vacuum infiltration of agrobacterium, similar to the protocol that is widely for Arabidopsis transformation. With this method, metabolic engineering of camelina can be conducted in a rapid and non-labor intensive manner. To this end, we have initiated studies to improve the oxidative stability of camelina oil by suppression of its native *FAD2* gene. Oils from the engineered plants contain ~70% oleic (18:1) and eicosenoic (20:1) acids. We have also developed lines with seed oils that have four-fold higher levels of vitamin E antioxidants. Current efforts are directed at engineering multi-gene traits, including wax ester production, in order to obtain vegetable oils with higher value for lubricant and other industrial applications.

P-4

Using Tobacco to Teach Plant Tissue Culture and Transformation. MARGARET YOUNG[1] and Nancy Reichert[2].

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Tobacco (*Nicotiana tabacum*) has one of the highest plant regeneration and transformation rates. It also produces clones and transgenic plants in a relatively short time. This makes it ideal for developing biotechnology experiments in undergraduate labs. We have developed two plant biotechnology lab modules. In the first module, the role of plant hormones in growth and development are studied. Students make media with various concentrations of auxin and cytokinin and observe the development of shoots, roots and/or calli within 6 weeks of culture. In the second module, students generate transgenic tobacco plants in 2 mo using *Agrobacterium* transformation. Plasmid vectors used contain the *nptII* gene and students are able to differentiate between transgenic (green) shoots from non-transgenic shoots (bleached). The *gus* gene is identified using histochemical assays. In addition, students use PCR to differentiate between transgenic tobacco plants with the *gus* gene and controls.

P-5

A Classroom Exercise in Asymbiotic Orchid Seed Germination: The Whole Story. TIMOTHY R. JOHNSON and Philip Kauth. Department of Environmental Horticulture, PO Box 110675, University of Florida, Gainesville, FL, 32611-0675. Email: timjohn@ufl.edu, pkauth@ufl.edu

Many scientific reports on orchid seed germination provide germination protocols, but few provide concise descriptions of plant selection, hand pollination, and asymbiotic seed culture for use in science classroom exercises. A major limitation for conducting orchid seed germination exercises are seed and flower availability. Flower availability must be consistent throughout the year so instructors can hand pollinate flowers to produce seed on demand for laboratory exercises. However, many orchids do not flower reliably throughout the year. Seed capsule development is often a lengthy process with maturation often taking more than 100 d. An efficient and reliable classroom exercise using the orchid *Spathoglottis* to demonstrate hand pollination and subsequent asymbiotic seed germination will be discussed and demonstrated. These exercises introduce students from high school to college levels to basic skills commonly employed in plant tissue culture, plant biotechnology, and orchid seed culture.

P-6

Using Plant Tissue Cultures to Demonstrate Mineral Nutrient Deficiencies: Comparison with Conventional Hydroponic Techniques. M. J. BOSELA. Department of Biology, Indiana University-Purdue University at Fort Wayne, 2001 E. Coliseum Boulevard, Fort Wayne, IN 46805. Email: boselam@ipfw.edu

Hydroponics experiments are used widely for plant science instruction, but they can be time-consuming to set up and maintain since protocols for aeration, algal growth control, and nutrient replenishment are generally required. In light of these difficulties, student researchers in my lab have evaluated tissue cultures as an alternative to conventional hydroponics techniques for the demonstration of mineral nutrient deficiency symptoms. Using a standard hydroponics medium (Hoagland's #2 Solution), supplemented with sucrose (10 g/L) and agar (7 g/L), the effects of mineral nutrient exclusion were evaluated across multiple types of tissue cultures, including seedlings cultures of tomato ('Micro-Tom'), carnation ('Cardinal'), and tobacco ('Havanna 38') and shoot cultures of hybrid aspen (*Populus x canescens* x *P. grandidentata*). The best overall results were obtained for tomato. The cultures exhibited typical deficiency symptoms for all of the nutrients evaluated (i.e., nitrogen, potassium, magnesium, calcium, and iron), the symptoms generally developed rapidly, within two to five weeks of cultures for most of the nutrients, and were expressed consistently among different seedlings (replications). This talk will provide an overview of the basic 'in vitro hydroponics' lab exercise that I have developed based on these techniques and will include a discussion of the pros and cons of using plant tissue cultures for mineral nutrient deficiency demonstration. I will also present data from more recent experiments evaluating the responses of a greater diversity of plant species to mineral nutrient deficiencies in vitro, including monocots and *Arabidopsis thaliana*, the model species for plant molecular genetics research.

P-7

Synthetic Seed Technology Demonstrated Through a Novel Teaching Exercise. M. E. KANE, S. L. Stewart, P. J. Kauth, and T. R. Johnson, Environmental Horticulture Department, University of Florida, Gainesville, FL 32611-0675. Email: micropro@ufl.edu

In higher plants, sexual reproduction involves fusion of gametes to form a single-cell *zygote* which ultimately develops into a complete plant through the process of zygotic embryogenesis. Cultured vegetative (somatic) cells can develop into non-zygotic embryos (NZE) that function like zygotes. Procedures to process NZE embryos as synthetic (artificial) seed for commercial clonal plant production have been developed. Referred to as s*ynthetic seed technology*, this application involves processing NZE embryos as synthetic seed and developing delivery systems

for mechanical planting. The most frequently used synthetic seed system involves encapsulation of hydrated NZE into beads consisting of gelled sodium alginate. The gel bead coating serves as an artificial seed coat and can enclose a synthetic endosperm core containing growth regulator, carbohydrates, and/or antibiotics. Encapsulation requires embedding the NZE in a liquid bead of sodium alginate and submersing in a calcium solution to gel the bead. The encapsulated NZE are then rinsed in water and handled like seed. A major challenge to demonstrating synthetic seed technology in the classroom has been the availability of NZE at the optimal developmental stage for encapsulation. A novel and reliable teaching exercise will be described that conceptually demonstrates synthetic seed production though encapsulation using four-day old *Arabidopsis* zygotic seedlings. The teaching exercise has several advantages for instructors. It requires only inexpensive materials and can be completed in a classroom with minimal preparation. Using *Arabidopsis* seedlings eliminates the reliance on in vitro derived NZE along with the associated tissue culture equipment and expertise required to generate them.

P-8

Freeze-tolerant Eucalyptus as a Renewable Feedstock for Industrial Applications. M. HINCHEE, C. Zhang, S. Chang, W. Rottmann, P. Raymond, D. Kaczmarek, L. Pearson, and N. Nehra. ArborGen, LLC, P.O. Box 840001, Summerville, SC. Email: mahinch@arborgen.com.

Eucalyptus, especially hybrids, such as E. grandis x E. urophylla (E.urograndis), have been developed and selected in Brazil for high productivity (greater than 12 dry tons/acre/year) as well as high cellulose yields. This productivity and wood quality make these hybrids highly suited for the production of pulp and paper. In addition, their productivity means that these trees can meet the biomass requirements for cost effective generation of bioenergy from lignocellulosic feedstocks, and can provide one of the solutions to meet the renewable fuel objectives of the United States. As these hybrids are freeze sensitive, they only can be grown in more tropical regions of the U.S., such as southern Florida. However, ArborGen has introduced a freeze tolerance gene (rd29a::Arabidopsis CBF2) and a pollen control gene into an E. urograndis elite clone EH1, and the resulting trees are tolerant to freezing temperatures of 16Â°F. These trees are in product development in northern Florida and costal regions of the Southeastern U.S.

P-9

Populus Genomics, Candidate Gene Identification and Accelerated Domestication. GERALD A. TUSKAN. Oak Ridge National Laboratory, P.O. Box 2008, MS-6422, Oak Ridge, TN 37831-6422. Email: gtk@ornl.gov

As one of the fastest growing woody perennial plants, one which can be easily clonally propagated through the use of unrooted lateral branches, *Populus* provides fiber for pulp, paper, veneer, and bioenergy feedstocks. The domestication process however has been erratic and slow due to the large size and delayed reproductive habit of this woody perennial tree. Modern genomics tools and approaches are being brought to bear on the efforts to accelerate the domestication process in *Populus*. The genome of *Populus trichocarpa* was sequenced, assembled, and annotated in 2006 through an international effort that included over 250 scientists from over 20 countries. The assembled genome contains 45,500 predicted gene models and revealed a recent whole-genome duplication that suggested the molecular clock in *Populus* is ticking at one sixth the rate seen in herbaceous annual plants such as Arabidopsis. Utilizing this information has made it possible to refine genetic maps, improve candidate gene isolation through QTL analyses and association genetics, and test hypotheses on accelerated domestication. Candidate genes related to drought tolerance, crown architecture, cell wall chemistry and disease resistance are currently being evaluated in greenhouse and field trials.

P-10

Cisgenic and Intragenic Approaches to Genetic Modification of Growth and Form in Poplar. S. H. STRAUSS[1], V. Busov[2], C. Ma[1], and K. Van Wormer[1]. [1]Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97331-5752 and [2]School of Forest Research and Environmental Science, Michigan Technological University, Houghton, MI 49931. Email: steve.strauss@oregonstate.edu

Cisgenes are generally defined as native genes from the sexually accessible gene pool, including their proximal regulatory elements, that have been inserted via asexual transformation. Intragenes are a broader category that includes cisgenes, as well as other types of genes that have been modified in their sequence or regulatory elements, but inserted via asexual transformation. Because of their familiarity as native genes and forms of gene action, and likelihood of increased public acceptance, both classes of genes may benefit from reduced regulatory scrutiny in the future, facilitating commercial use. We have been studying the value of both classes of genes in poplar for a number of genes that take part in catabolism, anabolism, or signaling through gibberellic acid pathways. The goals are strong modifications in plant growth rate and form to suit the goals of different poplar production systems, which range from

coppice "wood grass" bioenergy to solid wood forestry products. We have found striking and diverse effects from several genes in greenhouse trials, including improved growth and changes in root:shoot allocation, suggesting that this form of molecular breeding may help to speed the domestication of poplar for its diverse uses.

P-11

Phenylpropanoid Networking in *Populus*. C.-J. TSAI[1,2,3], C. An[1], R. Payyavula[1,3], and S.A. Harding[1]. [1]Warnell School of Forestry and Natural Resources; [2]Department of Genetics, University of Georgia, Athens, GA; and [3]School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI. Email: cjtsai@ uga.edu

Populus species accumulate large reserves of non-structural phenylpropanoid products, predominantly phenolic glycosides and condensed tannins, in their leaves, stems and roots. The compositions and concentrations of these reserves vary among species, genotypes and tissues, and have been postulated to impact fitness, growth, and biomass utilization. We have been investigating the plant-wide regulation of phenylpropanoid metabolism in a suite of naturally occurring *Populus fremontii* x *angustifolia* hybrids known to exhibit varying rates of growth and phenylpropanoid accrual. In general, slow-growing genotypes exhibit higher foliar concentrations of non-structural phenylpropanoids. Leaf expansion, early stem vascularization, and mature stem wood composition were found to differ in accordance with differences in the levels of non-structural phenylpropanoids. Slow-growing genotypes exhibited gene expression and metabolic responses consistent with lower leaf N-status compared to fast growers. This may be coupled to relatively strong N-demand in young stems of slow growers. From our findings, regulation of phenylpropanoid metabolism in this hybrid family appears to be linked with genetic adaptations to external N availability in the ancestral environments. Transgenic manipulation of genes involved in phenylpropanoid biosynthesis and/or homeostasis is being pursued in order to more directly test the effects of altered accumulation and composition of non-structural phenolics on tree fitness and biomass production.

P-12

Hardwood Tree Biotech Advances in the Southeast. SCOTT MERKLE. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602. Email: merkle@warnell.uga.edu

Plantations of short-rotation hardwoods offer great potential for cellulosic biomass energy and other products in the southeastern United States. Since southern hardwoods are already adapted to the climate and soils of the region and have excellent coppicing ability, these trees are outstanding candidates for rapid biomass production. We have demonstrated the feasibility of scaling-up production of varietal hardwood planting stock using hybrid breeding and somatic embryogenesis, generating large numbers of somatic seedlings of hybrid sweetgum and hybrid yellow-poplar. Embryogenic cultures were initiated from seeds derived from control pollinations between American sweetgum (*Liquidambar styraciflua*) and Formosan sweetgum (*Liquidambar formosana*) and between yellow-poplar (*Liriodendron tulipifera*) and Chinese tuliptree (*Liriodendron chinense*). We developed protocols for enhanced somatic embryo and somatic seedling production using suspension cultures of the hybrid varietal lines, resulting in hundreds of hybrid somatic seedlings being established in field tests. Trees from some clones have shown hybrid vigor in the field, with one hybrid sweetgum variety producing much higher volume than elite native sweetgum families. Since embryogenic cultures can be cryostored, hybrid varieties showing the best performance in the field can be recovered from cryostorage, re-grown and scaled-up for mass somatic seedling production. Blight-resistant hybrid backcross-derived chestnut or pure-bred American chestnut (*Castanea dentata*) varieties now being developed have a similar potential to be deployed for biomass plantations and other applications in the Southern Appalachians. To propagate these varieties, recently-developed protocols for chestnut somatic seedling production need to be further enhanced for scaled-up production. Embryogenic cultures of hybrid hardwoods and chestnuts also make excellent target material for gene transfer, opening the way for engineering value-added traits and disease and pest resistance into these highly-productive trees.

P-13

Introduction to Large Scale Liquid Micropropagation Systems. JEFFREY ADELBERG. Department of Horticulture, Clemson University, Clemson SC. Email: Jadlbrg@ clemson.edu

Bioreactor technology developed for biomass is being adapted to micropropagation. Full immersion bioreactors that work well with cell suspension, callus and hairy root culture, also are used to propagate somatic embryos. When bipolar development of shoot and root are desired, less robust agitation and a gaseous phase is needed. Partial or periodic immersion bioreactor system diminishes these deficiencies for some crop systems, allowing plantlets to develop absorbing nutrients from liquid phase, while shoots are growing aerated phase. Systems have been successfully developed for large-scale micropropagation of several tropical crops. Mechanisms

to enable the aseptic ebb/flood events vary. Complexity, cost, and economy of scale have produced several designs that will be discussed. When a successful system is found, the plants from liquid often out-perform their counterparts from agar during ex vitro nursery growth.

P-14

Transgenic Loblolly Pine and the Importance of Somatic Embryogenesis Scale-up Technologies for Future Commercialization. S. CHANG, L. Vincent, A. Perry, M. Cook, C. Zhang, W. Rottmann, N. Nehra, W. Banner, and M. Hinchee. ArborGen, LLC, P.O. Box 840001, Summerville, SC. Email: sxchang@arborgen.com

ArborGen has a research program that has successfully developed transformation and somatic embryogenesis propagation technologies for elite varieties of loblolly pine. Genes have been introduced and tested in trees, grown in multi-year field trials, that have increased growth rate, modified lignin and controlled pollen formation. The loblolly somatic embryogenesis system is the vehicle for both the introduction of genes and the large scale production of transgenic trees for product development field trials. The somatic embryogenesis process and its potential for scale up through automation and liquid culture systems will be discussed.

P-15

Challenges to Large Scale Liquid Plant Micropropagation - the Arundo Story. L. MARTON and M. Czako. Department of Biological Sciences, University of South Carolina, 700 Sumter St., Columbia, SC 29208. Email: marton@mail.biol.sc.edu

Monoculture forming monocots are often able to produce more biomass than most cultivated plants. Tissue culture is prerequisite both for large scale propagation for environmental biotechnology, mutant selection and genetic improvement by introduction of key transgenes to increase their biomass quantity and quality. We have developed a universal cell culture initiation technology to establish sustained embryogenic cell cultures as well as micropropagation and genetic engineering protocols, which have successfully been used for a great number of monocots. Our technology has been fully explored with Arundo donax, (20-50 DTY/acres biomass) down to the large scale application details, including storage and transportation. An improvised lab with a maximum 200,000 sq ft nursery can produce 60-100 million propagules/year (for 10,000 ha). Labor requirements are two lab technicians plus a nursery worker for 0.5-1.0 million propagules in nursery flats/year. The sustained totipotent embryogenic cultures allowed efficient somaclonal breeding; elite lines for TCP dehaloperoxidation and salt tolerance have been selected. An Arundo stand irrigated with diluted sludge and a salt tolerant stand on a tidal creek bank (90% sea salinity) were monitored. Nutrient removal was dependent on plant age at harvest and nutrient supply producing more cellulosic biomass, using less land, without fertilizer and pesticides, than any alternative crop in Mediterranean to subtropical environments. Arundo is a second generation biofuel crop, a non-food, cellulosic biomass feedstock for bioethanol, heat, power generation and for carbon credits. Arundo may be invasive in certain riparian systems, but without rapidly flowing water to distribute stem and rhizome fragments, it is limited in its natural dispersal ability. Microscopic studies indicated there is neither pollen nor embryo resulting in total sterility (no seeds). Therefore, a weed risk management strategy needs to focus on restricting vegetative spread through selection of appropriate planting sites (not in riparian zones) and crop hygiene.

P-16

The Utilization of Temporary Immersion System (RITA) to Enhance Initial Steps in Commercial Micropropagation of Cordyline spp. C. NAVARRO and R. Vázquez. Agromod, S.A. de C.V. – Micropropagación e Invernaderos. 15a. Oriente No. 19, Colonia Centro, 30700 Tapachula, Chiapas, MEXICO. Email: lcnavarr@agromod.com

The utilization of bioreactors for mass propagation of plants is being implemented by different commercial laboratories worldwide. There are a number of challenges that must be surpassed in order to respect the key factors that make micro propagation a credible useful tool for ornamental industry and agriculture: true–to–type plants, virus–free status, homogeneity, vigor. Mass propagation in semisolid media allows us to produce plants cost effective and customer focused, with low contamination levels (<3%) during multiplication steps, respecting delivery times, volumes agreed and quality standard. In this context, the advantages of temporary immersion system (RITA) are directed towards the speed up of the process in the initial steps to rapidly increase the multiplication stock. The challenges are to produce clean cultures with vigorous true–to–type cultures that match the quality standards (specially those variegated varieties) to feed upstream the manual multiplication and rooting process.

P-17

Modular Assembly of Multi Gene Plant Transformation Vectors. V. ZEEVI, A. Tovkach, and T. Tzfira. Department of

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Most plant transformation cloning systems suffer from a limited number of tags, selection markers, promoters and terminators restricting the expression of multiple target genes on a single plasmid. We therefore developed a modular satellite (SAT) vector system that supports (i) N- and C-terminal fusions to six different autofluorescent tags, EGFP, EYFP, Citrine-YFP, ECFP, RFP, and DsRed2, (ii) expression of the target genes under the control of the 35S, ocs, nos, mas, act, and rbc constitutive promoters, (iii) RNAi-mediated gene silencing, (iv) BiFC analysis and (iv) N- and C-terminal fusions to various peptide epitopes. All these vectors carry an expanded multiple cloning site that allows an easy exchange of the target genes among autofluorescence, BiFC, and epitope tags. Furthermore, individual expression cassettes can be assembled into *Agrobacterium* binary plasmids using a set of rare-cutting enzymes, allowing the construction of up to seven expression cassettes in a single *Agrobacterium* binary vector. To further expand the very small number of commercially available rare-cutting enzymes, a set of artificial zinc finger nucleases (ZFNs) and compatible plasmids were developed. ZFNs are artificial restriction enzymes which can be custom-designed to recognize and cleave very long DNA sequences. A set of different ZFNs were produced by *de-novo* assembly of their DNA-binding regions and they were cloned into bacterial expression plasmids. A compatible set of satellite plasmids was also developed, in which each expression cassette was flanked by recognition sites of different ZFNs. Following their expression in *E. coli*, ZFNs were used to cleave their corresponding expression cassettes from the satellite plasmids, and these cassettes were then cloned into a single binary plasmid. The ability to specifically design, construct, express and use new artificial ZFNs for cloning purposes opens the way for the assembly of *Agrobacterium* binary vector with large number individual expression cassettes.

P-18

Editing the Genome of Crop Plants with Engineered Zinc Finger Proteins. PHILIP D. GREGORY[1], Fyodor D. Urnov[1],Vipula K. Shukla[2]. [1]Sangamo BioSciences, Point Richmond Tech Center, 501 Canal Blvd., Suite A100, Richmond, CA 94804 and [2]Dow AgroSciences, 9330 Zionsville Rd., Indianapolis, IN 46268. Email: pgregory@sangamo.com

Agricultural biotechnology is limited by the low efficiencies of conventional random mutagenesis and transgenesis. Classical ‘gene targeting’ has proven intractable in plants, rendering agricultural trait engineering a laborious, time-consuming, and unpredictable undertaking. A solution to this problem emerges from the application of two highly conserved biological processes: DNA recognition by zinc finger proteins and homology-driven repair of DNA double-strand breaks. Zinc finger proteins engineered to bind a DNA sequence of interest, when fused to a nuclease domain, yield a zinc finger nuclease (ZFN). These ZFNs are capable of recognizing an investigator-specified target sequence in the plant genome and inducing a double-strand break (DSB) with high efficiency and precision. The cell then employs a natural process known as homology-directed repair to heal the break. This high-frequency homology-driven process can be usurped to introduce novel genetic information, such as an herbicide resistance marker, at the target site by simultaneously providing the cell with a simple heterologous donor DNA that specifies the desired new genotype. The cell will use this exogenous DNA as the template for repair of the DSB, thereby effecting the desired genetic change. ZFNs can therefore be employed to modify endogenous loci in plants. Moreover, the site-specific nature of the approach enables “trait stacking” i.e. the ability to add multiple traits to a given crop without risk of loss via segregation through the breeding process. ZFNs can be utilized in any plant species amenable to DNA delivery, thus our results offer a new strategy in plant genetic manipulation for both basic science and agricultural application.

P-19

Engineered Minichromosomes in Maize. J. BIRCHLER. Division of Biological Sciences, University of Missouri, Columbia, MO 65211. Email: BirchlerJ@Missouri.edu

Engineered minichromosomes have the potential to be used as a platform for efficient stacking of multiple genes for insect, bacterial and fungal resistances together with herbicide tolerance and crop quality traits. Multiple genes conditioning complex or combined traits would be present on an independent chromosome unlinked to endogenous genes in a circumstance that would foster faithful expression. Engineered minichromosomes can be produced easily by telomere truncation with simultaneous introduction of selected genes or sites for subsequent addition to the artificial chromosome platform. Because of the near universality of the telomere sequence in the plant kingdom, one set of vectors can be used across plant species. Engineered minichromosomes have been produced from both the normal A chromosomes and the supernumerary B chromosome of maize. Truncation of B chromosomes is extremely efficient and produces a wide range of chromosome sizes that are modified in such a manner that they are indefinitely amendable for the addition or removal of genes in subsequent manipulations.

P-20

Design of Multivariate Experiments. W. C. BRIDGES[1], J. W. Adelberg[1], and R. Niedz[2]. [1]Clemson University, Clemson, SC and [2]USDA-ARS, Fort Pierce, FL. Email: wbrdgs@clemson.edu

The large number of treatment combinations necessary to run multivariate experiments often limits their application in in-vitro biology studies. The number of treatment combinations necessary can be overwhelming since it increases exponentially with the number of factors. Some strategies for picking a subset of the combinations will be discussed. The best strategy depends on several questions such as: 1) Are the factors quantitative or qualitative? 2) Is the experiment as part of a series or a one-time attempt? 3) Is the objective to compare treatments means or model a surface? etc. The exact information that is lost from the experiment and the consequences of this lost information will be examined. The design choices will be illustrated with an example, and software useful for choosing subsets will be discussed.

P-21

Optimizing Macro-nutrients for Stage II and Stage III of Micropropagation Using Response Surface Methodology. JEFFREY ADELBERG. Department of Horticulture, Clemson University, Clemson, SC. Email: jadlbrg@clemson.edu

Combining nutrients, medium volume, and amount of plant material requires an experimental design for multiple factors. Turmeric (*Curcuma longa* L.) was observed in liquid culture on an orbital shaker in a response surface design that combined a 2-component mixture (NH_4^++ K^+ amount equal to NO_3^-) where K^+ proportion ranged from 0.5 to 1.0 and NH_4^+ proportion ranged from 0.0 to 0.5 crossed with four quantitative non-mixture factors including concentration of NO_3^- (10–50 mM), sucrose (1.5– 6.0% m/v), media volume (25–45 ml/vessel) and explant density (3–9 plants/vessel). Sufficient treatment points were selected using D-optimality criteria sufficient for developing quadratic response surface models. The resulting design required 55 vessels. The effect of these factors on tandem cycles of Stage II multiplication and subsequent greenhouse growth of the plantlets was empirically quantified. The multi-dimensional maximum for multiplication requires 1) the lowest macro-nutrient concentrations with equi-molar NH_4^+:K^+; and 2) optimal growth at about 35 ml volume implies a physical (rather than nutritive) effect. The multi-dimensional maximum for rapid growth in greenhouse has a different optimization. Combining these spaces mathematically, and visualizing on a flat screen will be demonstrated. Correlative analyses showed that solutes uptake in lab predicts best quality plants for greenhouse growth.

P-22

Visualization, Interpretation, and Mining of Data from Multivariate Designs. R. P. NIEDZ. USDA-ARS-U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945-3030. Email: Randall.Niedz@ars.usda.gov

The purpose of an experiment is to understand the relationships between the factors varied and the responses measured. Linking the mathematical summary provided by the ANOVA to a visual summary provided by the graphics can clarify the apparent complexity of a multivariate experiment. Examining multiple responses in a common design space provides a rich dataset that can be dynamically mined to answer questions of interest. Using examples from in vitro experimentation, the session will show 1) the relationships between the ANOVA and the associated graphics; 2) various types of graphs suitable for visualizing 1- to n-dimensional data sets; 3) graphs for mixtures and mixture-amount experiments; 4) graphs for visualizing multi-response data in a common experimental design space; and 5) software for producing graphs.

P-23

Oilseeds as Factories for Renewable Fuels and Materials. J. JAWORSKI, J. Han, and J.-W. Nam. Donald Danforth Plant Science Center, 975 N Warson Rd., St Louis, MO. Email: jjaworski@danforthcenter.org

The major chemical companies of the world are heavily dependent on petroleum as their source of feed stocks. As the global supplies of petroleum diminish and its price rises, the cost of these petrochemicals will inevitably rise as well. The plant kingdom provides a rich source of diverse oleochemicals that have the potential to replace petrochemicals as nations move toward green economies. This presentation will discuss the progress for the production of two oleochemicals, omega hydroxy fatty acids and wax esters, in oilseed crops. In addition, there will be brief discussion of the utility of *Camelina sativa* for the production of oleochemicals.

P-24

Manipulation of Reserve Content in Cottonseeds to Influence Oil, Protein and Fiber Content. KENT D. CHAPMAN. Center for Plant Lipid Research, Department of Biological Sciences, University of North Texas, Denton, TX 76203-5017. Email: chapman@unt.edu

Transgenic cotton lines were generated using a *Brassica napus* nonfunctional delta-12 fatty acid desaturase (*FAD2*) gene under control of the phaseolin promoter. Seeds of numerous transgenic plant lines had reduced oil content compared with null segregating siblings or non-transformed seeds. Seed oil content (quantified by ^{1}H-NMR) was reduced to 12% or less of seed weight in transgenics from 20% by weight in non-transformed controls. Light- and electron-microscopic analyses of severely lowered lines, showed that lipid bodies and protein bodies were reduced in number and altered in size and distribution in embryo tissues of transgenic lines compared to those in non-transformed seeds. Coincident with the overall reduction in storage reserves, sucrose levels were significantly elevated in transgenic seeds. The overall effect of oil suppression was to selectively reduce the size of the embryo, but the seed coat and fiber properties remained unaffected in seeds. In fact there was a significant increase in lint percent in all oil suppressed lines examined, and an overall increase in fiber yield in glasshouse trials. Overall we propose that targeted alteration of storage reserves in cotton embryos can lead to a redirection of carbon reserves toward cellulose production on the seed coat surface. This represents a novel strategy to enhance fiber yield in this important oilseed crop.

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Engineering Rubber Production in Plants. M. WHALEN[1], S. Kumar[1,2], C. McMahan[1], K. Cornish[2]. [1]ARS-USDA, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710 and [2]Yulex Corporation, 37860 W Smith-Enke Road, Maricopa, AZ 85238-3010. Email: Maureen.whalen@ars.usda.gov

Parthenium argentatum Gray, commonly known as guayule, is a shrub in the Asteraceae family that is native to the southwestern United States and northern Mexico. *P. argentatum* produces high quality rubber in bark tissue. Products made from *P. argentatum* are safe for people who have type I latex allergies, induced by natural rubber proteins from the Brazilian rubber tree, *Hevea brasiliensis*. Natural rubber is essential and irreplaceable in many industrial, medical and consumer applications. As an industrial crop that grows in temperate climates, *P. argentatum* represents a viable alternative source of high quality natural rubber. One strategy for improving crops is through chloroplast engineering. Transformation of chloroplasts allows high-level production of foreign proteins because of the high number of chloroplasts per plant cell and insertions are precise and predictable. Multiple genes can be inserted at once, enhancing the efficiency of metabolic engineering. Importantly, expressing foreign proteins in the chloroplast results in transgene containment in the vast majority of plant species, in which chloroplasts are not transmitted by pollen. As the first step in this strategy, the chloroplast genome of *P. argentatum* was sequenced and analyzed. To test our metabolic engineering strategy, chloroplasts of tobacco were transformed to enhance delivery of substrate. Ultimately, improving substrate levels is expected to enhance rubber production in *P. argentatum*.

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Biomass and Industrial Oilseed Crops as Platforms for Production of Biodegradable Plastics. M. N. SOMLEVA. Metabolix, Inc., 21 Erie Street, Cambridge, MA 02139. Email: somleva@metabolix.com

Metabolix is commercializing a family of polymers made from renewable resources such as sugars and vegetable oils. Polyhydroxyalkanoate biobased plastics are high performing new materials that have the potential to put a large portion of the plastics and chemicals industry on a sustainable basis. Metabolix is also developing several crop platforms amenable for large scale production of plastics and energy, including switchgrass, sugarcane, and industrial oilseed crops. Each plant-based platform has its own distinct advantages in terms of accessible co-product (i.e., lignocellulosic biomass, sugar, or oil). The core technology that enables the expression of these multiple genetic traits in plants is being continually improved. Highlights of these efforts, and some cellular and biochemical aspects of engineering bioenergy crops for polymer production, will be discussed.

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Gene Flow in a Model GM Crop: Canola. S. I. WARWICK. Agriculture and Agri-Food Canada, K.W. Neatby Bldg, Central Experimental Farm, Ottawa, ON, CANADA K1A 0C6. Email: Suzanne.Warwick@agr.gc.ca

Large-scale use of transgenic herbicide-resistant (HR) canola (*Brassica napus* L.) provided an opportunity to estimate pollen and seed gene flow on realistic field scales. Empirical gene flow will be reviewed for this crop. Pollen-mediated gene flow (crop to crop crossing) in adjacent HR canola commercial fields occurs up to 800 m. Both pollen and seed were shown to be avenues for transgene movement and gene flow. Consequences of gene flow include the frequent presence of HR weedy/feral canola volunteers in agricultural fields (also roadsides) with multiple or stacked HR traits and contamination of pedigreed seed lots. Seed flow is the most important venue for trangene movement. Volunteer canola (seed escape) forms an important reservoir for extending the persistence

of the transgene spatially and temporally in the environment. Inter-specific hybridization is a less likely consequence of gene flow. Canola has both sexually-compatible crop and wild/weedy relatives. Gene flow between GM canola fields and Polish canola (*Brassica rapa*) and oriental mustard *(Brassica juncea*) crops occurs up to 200 m. Studies have also shown that transgenes have escaped into natural bird rape *Brassica rapa* weed populations, with hybridization frequencies averaging 10%. The transgene can persist in these populations and even be stably incorporated (introgression) into the wild species. Empirical ecological fitness studies involving transgenes are limited, and results for stacked HR canola volunteers, insect resistant Bt-canola will be reviewed. The increased fitness benefit of HR canola will be restricted to the agroecosystem but the ecological effects of "fitness-enhancing" stress-tolerant GM traits and consequences of transgene spread to non-agricultural habitats are largely undocumented.

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Strategies for Gene Confinement in Genetically Modified Perennial Plants Used for Biofuels. A. P. KAUSCH, J. Hague, A. Deresienski, and K. Nelson, Plant Biotechnology Laboratory, Department of Cell and Molecular Biology, University of Rhode Island, West Kingston, RI 02892. Email: akausch@etal.uri.edu

The utilization of dedicated energy crops, such as switchgrass, as a source of biomass for renewable biofuels is now a goal with great relevance to current economic and ecological issues on a global scale. Currently, strategies are being developed using plant genetic engineering approaches for enhancement of biofuel production. The possibilities for the use of genetic modification of biofuels crops are extensive and include a wide array of GM traits but presents significant public and regulatory challenges. Commercial-scale production of transgenic plants for biofuels could lead to undesirable environmental and agricultural consequences including transgene flow to wild and non-transgenic relatives. To realize the full potential of agricultural biotechnology for biofuels enhancement, the ecological, economic, and commercial impacts of gene flow must be addressed. Most of the plants considered for cellulosic biomass are perennial and/or have wild relatives in the areas where they will be commercially produced. Bioconfinement of engineered genes and plants used for cellulosic biofuels will likely be a prerequisite for deregulation and commercial production of these plants. Viable strategies for control of transgene flow in perennial dedicated biofuel crops include: spatial and temporal control; triploidy; transgenic male sterility; gene deletor technology; total sterility; engineered maternal inheritance and cytoplasmic male sterility; seed-based gene confinement; gene safe technology; mitigation and fail safe strategies. While some of these approaches have been tested in proof-of-concept studies, others are still theoretical. Development of these strategies will help determine the requirements for deregulation and commercial production for dedicated biofuels crops.

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Seed Targeted Gene Confinement Strategies. M. J. OLIVER. USDA-ARS, Plant Genetics Research Unit, University of Missouri, Columbia MO 65211. Email: Mel.Oliver@ars.usda.gov

The genetic improvement of plants using biotechnology is now centrally important to agriculture, food security, and the biofuels industry. It is also important to the continued health of the environment as the need for food (on existing arable land) and renewable energy becomes critical. New genes can be introduced into plants that confer valuable traits such as drought and stress tolerance, enhanced nutrition and yield, insect and pest resistance, vaccines and recombinant pharmaceuticals, enzymes for biomass conversion, altered cell wall properties, phyto-remediation, and a long list of others. Many of the applications will have neutral or even beneficial environmental consequences, but commercial-scale production of some of these transgenic plants could lead to undesirable environmental and agricultural consequences. The possibility that transgene escape into related crop plants (where the trait may be undesirable) or non-crop sexually compatible species (giving them a selective advantage) has to be considered. To realize the full potential of agricultural biotechnology, strategies for gene containment to prevent gene flow must be employed. Seed based gene containment strategies offer several advantages, including prevention of gene escape through pollen and seed, technology isolation, prevention of pre-harvest sprouting, and elimination of volunteers. Utilizing the GeneSafe design as a base we have designed several new strategies aimed at improving the containment of transgenes using the seed as the target for control. The new strategies offer complete protection and also allow the seed producer to remove tag-along technologies, e.g., antibiotic resistance, and ensure full expression of the technology prior to deployment in the producer's field.

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Strategies to Increase Enzyme Expression in Plants. KASI AZHAKANANDAM. Syngenta Biotechnology, Inc., Research Triangle Park, NC. Email: kasi.azhakanandam@syngenta.com

In recent years an increasing number of plant systems have been developed as potential bioreactors for the production of recombinant proteins. These include tobacco leaves, corn and rice seed, and duckweed for expression of pharmaceutical proteins. Also potato tubers and bananas, for example, have been evaluated for the production of edible vaccines. Syngenta is developing plants that express enzymes that lower the cost of biofuel production from crop polymers such as starch biomass. An example is, Corn Amylase, a speciality corn which expresses alpha-amylase in the endosperm. A key technical challenge in this effort is the generation of plants that accumulate sufficient quantities of enzymes to be commercially relevant. Significant progress has been made in expression of enzymes in desired plant tissues and subcellular compartments within those tissues. We will present recent results describing the evaluation of different expression strategies employing the molecular tool kit technology in Syngenta, with the goal to increase accumulation of enzyme expression in plants. These strategies range from the incorporation of transcriptional and translational enhancer sequences to targeting the same enzyme to more than one subcellular compartment.

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Engineering of Transgenes for High-level Protein Expression in Chloroplasts. PAL MALIGA. Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, Piscataway, NJ 08854-8020. Email: maliga@waksman.rutgers.edu

We developed a production system for the expression of recombinant proteins in tobacco chloroplasts. The production system includes transformation vectors for targeted insertion of chimeric genes into the plastid genome by linkage to selectable spectinomycin and kanamycin resistance genes. High-level expression of recombinant proteins is ensured by the availability of suitable expression cassettes consisting of the 5'-regulatory region (Promoter-Leader or PL cassette) and the 3'-regulatory region (Terminator or T-cassette). The PL cassettes were obtained by fusing the strong plastid ribosomal RNA operon promoter with translation control signals of plastid, phage and bacterial genes contained in the mRNA 5′-untranslated region and the coding region N-terminus. The T-cassettes derive from the 3'-untranslated region of plastid genes. The cassettes were standardized by expression of the bacterial enzyme neomycin phosphotransferase, which accumulated up to ~23% of the total soluble cellular protein in leaves. At the meeting examples of protein expression will be discussed for biomedical and agricultural applications. The expression system is now complete with tools for marker gene excision that involves flanking the marker genes with target sites for phage site-specific recombinases and expression of the INT or CRE recombinases when excision of the marker genes is desired.

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Deconstructing and Reconstructing Soybean Seed Protein Accumulation to Enhance Foreign Protein Yield. ELIOT HERMAN. Donald Danforth Plant Science Center, 975 Warson Rd, St. Louis. MO 63132. Email: eherman@danforthcenter.org

The ontogeny of both seed structure and seed storage substances results from a determinant genetic program that produces a population of nearly identical seeds. The seed's developmental program to accumulate storage substances can be modified by nutrient availability and environmental conditions resulting in small changes of composition. Using biotechnology techniques the synthesis of soybean storage proteins has been silenced. The resulting transgenic soybean seeds rebalance their composition restoring the seed's protein content. The transgenic soybeans were evaluated with DNA array, proteomics and metabolomics assays. The morphology of the storage cells was examined using high-pressure cryofixation and electron microscopy supplemented of the seed's organelles. The transgenic soybeans rebalance protein composition by the accumulation of an alternate protein inventory. This rebalancing of protein composition appears to occur at the post-transcriptional level. Introgression of foreign proteins into the transgenic seeds further alters protein content and results in further rebalancing and accumulation of the foreign protein. Our results indicate that soybean seeds assess the composition and quantity of reserve proteins during development and can make major adjustments in protein composition as the seed's development proceeds. How stored protein composition is regulated has broad implications for agriculture and can be used by biotechnology to produce and optimize seeds with modified composition and introduced new traits.

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Viral Suppression of RNA Silencing: Toward Mechanism. VICKI VANCE, Matt Endres, Zhihuan Gao, Amy Wahba, Sizolowenksi Mlotshwa, Xin Ge, and Lewis Bowman. Department of Biological Sciences, University of South Carolina, Columbia, SC 29208. Email: vance@biol.sc.edu

RNA silencing is a sequence-specific RNA degradation pathway that serves as an antiviral defense in plants. The double-stranded RNA (dsRNA) trigger of silencing is processed into primary siRNAs which direct destruction

of complementary RNAs. The process can be amplified by transitive silencing, which produces secondary siRNAs from dsRNA generated via cellular RNA-dependent RNA polymerase(s). Here we report that two unrelated plant viral suppressors of silencing require an /Arabidopsis thaliana/ transcription factor, /RAV2///EDF2/, to block silencing induced by a hairpin transgene. Neither potyviral P1/HCPro nor TCV p38 can block target RNA degradation directed by primary siRNAs in /rav2/edf2/ mutant plants, although both still block secondary siRNA accumulation. These results suggest that /RAV2///EDF2/ is a key regulator of silencing pathways and a focal point of viral counter-defensive strategies. The biotechnological implications of the work will be discussed.

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The North Carolina Research Campus: A Transdisciplinary Approach to the Science of Wellness. MARY ANN LILA. North Carolina Research Campus, North Carolina State University, Kannapolis, NC 28081. Email: mlila@ncsu.edu

The Southeast has a new tour de force devoted exclusively to research on optimization of human health, via biotechnology and discovery of plant-based foods as a source of both nutrients and bioactive phytochemicals: The North Carolina Research Campus (NCRC). The NCRC, which officially opened in October 2008, was developed as a public-private partnership at the impetus of Mr. David H. Murdock, owner of Dole Food Company Inc, who invested $1.5B to launch this model of collaborative scientific inquiry. The 350 acre campus juxtaposes seven public university members of the UNC system (North Carolina State University [Plants for Human Health Institute], UNC-Chapel Hill [Nutrition Research Institute], UNC-Charlotte [Bioinformatics Research Center], UNC-Greensboro [Center of Excellence in Bioactive Food Components], A&T University [Center of Excellence for Post-Harvest Technologies], North Carolina Central University [Nutrition Research Program], and Appalachian State University [Human Performance Laboratory]), as well as the privately held Duke University [Translational Medicine Institute]. Scientists from each institution all have a common mandate: to join together in a transdisciplinary teamwork approach to tackle the most complex research challenges in health, disease prevention and treatment, and nutrition. A 311,000 sq ft state of the art Core Laboratory facility is the hub of the campus, which hosts next generation sequencing, cutting edge confocal imaging, a comprehensive portfolio of precision instrumentation, and a suite of nuclear magnetic imaging instruments including the world's first actively shielded 950 MHz NMR. High resolution / high sensitivity / high throughput analytical instrumentation coupled with multivariate statistical and data mining techniques generates fingerprints or profiles of given samples to enable differentiation from similar plants or microbes, identification of impurities, or a comparison to determine pharmacological variations occurring due to different preparations, geographical locations or harvesting times. Of particular interest to SIVB scientists, the NCSU's Plants for Human Health Institute will link discovery and translational research in plant genomics/systems biology, metabolomics of bioactive phytochemicals, metabolic pathway engineering, biochemistry, pharmacogenomics, phytochemistry, breeding and postharvest attributes towards development of mainstream fruit and vegetable produce with enhanced health benefits, and introduction of new or underappreciated crops and products, allowing consumers to make proactive, responsible dietary choices that benefit their own, and their family's, health.

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Apomixis in Crops: Hope or Hype? P. OZIAS-AKINS, J. A. Conner, and Y. Zeng. Dept. Horticulture, University of Georgia Tifton Campus, Tifton, GA 31793-0748. Email: pozias@uga.edu

Asexual reproduction through seeds, known as apomixis, is achieved through several developmental pathways that impact the sporophyte and/or female gametophyte generations. Sporophytic apomixis is termed adventitious embryony, a process that occurs in parallel with sexual reproduction. The alternative, gametophytic apomixis, can replace sexual reproduction, and when obligate, leads to the dominance of one genotype. Most apomicts, however, display at least some facultative sexual reproduction that provides an avenue for genetic recombination to occur. Expression of apomixis (facultativeness) can be modified in different genetic backgrounds. Our study of gametophytic apomixis in the genus Pennisetum has shown that the formation of aposporous female gametophytes is ostensibly under simple genetic control, yet the transmission of a large portion of a chromosome arm is necessary for expression of the trait. This chromosomal region, the Apospory-specific Genomic Region (ASGR) is densely populated with retrotransposons but also contains transcribed genes. The lack of recombination in the ASGR limits the ability to efficiently determine which genes or non-coding regions may be essential for apomixis. The potential and limitations for developing apomictic crops will be discussed.

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Generation and Risk Assessment of Apomictic, Transgenic Turf and Forage Grass (*Paspalum notatum* Flugge). F.

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The commercially important bahiagrass cultivar "Argentine" reproduces apomicticly which produces uniform seed progeny and might reduce the risk of unintended gene dispersal by pollen. We investigated pollen mediated gene transfer from apomictic, tetraploid bahiagrass to sexual, diploid or tetraploid apomictic bahiagrass using glufosinate resistance as a marker. Glufosinate resistant and fertile transgenic bahiagrass was generated by biolistic gene transfer and used as pollen donor in a field trial (USDA-Aphis permit # 05-365-01r) in Marianna, Florida with two replications. Seeds were harvested from non-transgenic bahiagrass surrounding the transgenic glufosinate resistant bahiagrass in 1 m distance. More than 30,000 seedlings were sprayed with glufosinate to determine the gene transfer frequency. Very low hybridization frequencies were observed between the apomictic transgenic and wild-type diploid or tetraploid bahiagrass even if pollen receptor and donor are grown in close distance (1 m) in the field. Bar gene integration and expression in primary transgenics, seed progeny and hybrids were confirmed by Southern blot and immuno-chromatographic analysis and herbicide resistance, respectively. All of the analyzed hybrids harvested from diploid pollen receptors were confirmed as triploids or aneuploid triploids by both flow cytometry and root-tip chromosome counting. Data describing vigor, productivity and fertility of hybrids will be presented.