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13. © Exposure to gibberellins eliminates the cold stratification requirement of *Penstemon grandiflorus* seeds. Sorensen, E., Bierkamp, T., and Barry, K. In Vitro Cellular & Developmental Biology - Animal 46(Suppl):97-98. 2010.

It is well established that ethylene promotes female flower development in cucumber. However, little is known about how the gaseous hormone selectively affects female flowers, and what mechanism it uses. Previously, we found organ-specific DNA damage in the primordial anther of female cucumber flowers. This finding led to a hypothesis that ethylene might promote female flower development via the organ-specific induction of DNA damage in primordial anthers. In this study, we tested this hypothesis first by demonstrating ethylene induction of DNA damage via the ethylene signaling pathway using cucumber protoplasts. Then, using representative component genes of the ethylene signaling pathway as probes, we found that one of the ethylene receptors, CsETR1, was temporally and spatially downregulated in the stamens of stage 6 female cucumber flowers, especially along with the increase of the nodes. Furthermore, by constructing transgenic *Arabidopsis* plants with organ-specific expression of antisense CsETR1 under the control of an AP3 promoter to downregulate ETR1 expression in the stamens, we generated *Arabidopsis* "female flowers," in which the abnormal stamens mimic those of female cucumber flowers. Our data suggest that ethylene perception is involved in the arrest of stamen development in female cucumber flowers through the induction of DNA damage. This opens up a novel perspective and approach to solve the half-century-long puzzle of how gaseous ethylene selectively promotes female flowers in the monoecious cucumber plant.

P-010

Tnt1 Mutagenesis Study by In Vitro Transformation in Soybean

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Soybean (*Glycine max*) is one of the most important and widely cultivated crops in the USA and in many parts of the world. Seeds are rich sources of oil and protein, a valuable product for human and animal nutrition. Its diverse applications urges for more research to develop new varieties of this legume. For the past few decades, progress has been made in search of novel mutants in soybean. Recently, transposon mutagenesis has been widely used to generate insertional mutants to study the functions of genes. However, generation of large-scale insertional mutants is a challenge in soybean because it is still not trivial to generate such a large mutant population due to the lack of high-throughput soybean transformation process. In the present study, Tnt1, an active retrotransposon from *Nicotiana tabacum*, is used to study insertion mutagenesis in soybean. The aim of the study is to introduce multiple independent insertions (high copy number) per plant via genetic transformation to generate a population of Tnt1 insertional mutants. To achieve this, two *Agrobacterium* strains (EHA101 and AGL1) containing a vector carrying *Tnt1* gene and bar gene as a selectable marker were used to transform soybean cotyledonary explants, and transgenic events developed through organogenesis-based tissue culture. Experiments regarding generation of Tnt1 mutant soybean lines are in progress. In addition to the above experiments, explants were subjected to various levels of sucrose pretreatment, to test the impact on Tnt1 copy number in soybean. Standardized protocol with optimal sucrose pretreatment was currently used to reactivate Tnt1 copy number using T0 Tnt1 soybean seeds. More detailed studies will be presented.

P-011 #5

Exposure to Gibberellins Eliminates the Cold Stratification Requirement of *Penstemon grandiflorus* Seeds

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Penstemon grandiflorus, large beardtongue, is a native perennial plant that prefers full sun, dry conditions, and rocky or sandy soil. *P. grandiflorus* is classified as endangered in Illinois, and it is only found in the wild in a few counties of northwestern Illinois. Although *P. grandiflorus* can be grown from seed, dormant seeds require 30 d of cold stratification for germination. Embryo dormancy is often due to the presence of inhibitors such as abscisic acid (ABA) and the absence of growth promoters such as gibberellic acid (GA). Germination is often associated with a drop in the ratio of ABA to GA. We studied methods for reducing or eliminating seed dormancy by seed treatment with GA_3. Seeds were soaked in GA_3 (0, 250, 500, 1,000, and 1,500 mg/L) and then placed at 4°C for 0, 5, 10, 15, 20, 25, or 30 d in petri dishes containing moistened filter paper. As dishes were removed from 4°C, they were placed in growth chambers at 15°C or 20°C in 24-h darkness, and germination was monitored. GA_3 treatment was shown to reduce and even completely

eliminate the requirement of a 30-d cold stratification. Additionally, seeds maintained at 20°C germinated at a faster rate than the seeds at 15°C.

P-012

Analysis of Stress-Induced Transcripts and Biogenic Volatile Organic Compound Emissions in Tropical *Hymenaea courbaril* and Coniferous *Pinus ponderosa*

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Changes in the composition of biogenic volatile organic compound (BVOC) emissions and stress-responsive gene transcripts were investigated in the tropical tree species *Hymenaea courbaril* and the coniferous species *Pinus ponderosa*. Heat stress and insect wounding have been associated with an increase in the release of stored and de novo synthesized BVOCs. These BVOCs are released into the atmosphere, where they can interact with nitrous oxides to create ozone and alter cloud properties via the formation of secondary organic aerosols (SOAs). To determine the source of the BVOCs being released (stored vs. de novo), we subjected both species (*Ponderosa* and *Hymenaea*) to heat stress and methyl jasmonate (MeJa) treatment. The temperature in the growth chamber containing the trees was increased from 24°C to 40°C (*Ponderosa*) or 27°C to 42°C (*Hymenaea*), and BVOCs were collected using leaf cuvette enclosures. Leaves were collected at 0 and 24 h for transcript analysis. Heat- and MeJa-responsive gene transcripts were detected using SeeGene's GeneFishing™ technology. These differentially expressed genes were then classified using BLAST analysis, and expression of 25 selected gene transcripts were examined by qPCR. These stress-inducible genes have potentials to be transferred to plants for trait enhancements.

P-013

Regeneration of Plants from Black Ash (*Fraxinus nigra* Marsh.) Hypocotyls

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Black ash is an important North American tree species with significant ecological and ethnobotanical importance to American Indians of the eastern USA. The seeds are eaten by wildlife, and the strongly ring-porous wood is preferred for making splints for basketry. Black ash has irregular seed production, immature embryos at seed set, and a complex stratification requirement, making it difficult to regenerate naturally from seed. Because of inadequate natural regeneration and the threat of the emerald ash borer (EAB), an in vitro adventitious shoot regeneration and rooting protocol would be beneficial for propagation and genetic improvement of this species. Such a system will provide the basis for an *Agrobacterium*-mediated transformation system for developing resistance to the EAB. Hypocotyls extracted from aseptic seeds were cultured for 4 wk on a Murashige and Skoog (MS) medium containing 13.3 μM 6-benzylaminopurine (BA) plus 4.5 μM thidiazuron (TDZ) for shoot induction. Shoots were then successfully regenerated on MS medium with Gamborg B5 vitamins (MSB5) plus 6.7 μM BA, 1 μM indole-3-butryic acid (IBA), and 0.29 μM gibberellic acid (GA_3), followed by transfer to MSB5 medium with 13.3 μM BA, 1 μM IBA, 0.29 μM GA_3, and 0.2 gL^{-1} casein hydrolysate for shoot elongation. Elongated shoots were successfully micropropagated using MSB5 medium with 13.3 μM BA, 1 μM IBA, 0.29 μM GA_3, and 0.2 gL^{-1} casein hydrolysate. Rooting of shoots (85%) was successful using woody plant medium containing 4.5 μM IBA plus 5.7 μM indole-3-acetic acid with a 10-d dark incubation. There was an average of 6.3 roots per shoot, and 85% of rooted shoots survived acclimatization to the greenhouse. This protocol will be used for experimental studies to produce transgenic black ash with resistance to the EAB or mass propagation for conservation.

P-014

Overproduction and Large-Scale Production of the Phytohormone Coronatine, a Methyl Jasmonate Mimic

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