



THE OHIO STATE
UNIVERSITY

COLLEGE OF FOOD, AGRICULTURAL,
AND ENVIRONMENTAL SCIENCES

A *Taraxacum*-modelled transgenic tracking system

Katrina Cornish and John Cardina

Abstract

With a growing desire for domestic and sustainable plant sources of essential resources there is increasing concern regarding the rapid adoption of wild-selected plants as crops that have the potential to become invasive pests, especially when the new crop is genetically-engineered (GE). Transgenic crops pose a special risk if they increase the tendency of a crop to go feral or have potential to hybridize with wild relatives. We propose to develop resources for identification and tracking of genetically-engineered plants using a combination of phenotypic and genetic markers. *Taraxacum* is our model because this genus includes an essential resource (rubber) producing crop plant with weedy relatives. Results will be used to guide the genetic improvement and development of this new crop in a way that is environmentally responsible and provide a template for other crops. The overall goal of this project is to develop research-based information to support the development of new, profitable, cropping systems that enhance biodiversity, while avoiding the spread of herbicide resistance, or other potentially negative traits, to weedy species. The GE tracking system will help evaluate the risk of gene flow to related species before transgenic plants are introduced into fields surrounded by weedy relatives. This research will focus on understanding how *Taraxacum* breeding systems affect gene flow and fitness-related traits in apomictic, sexual, and hybrid progeny in a genus that includes crop plants and weeds. These data will be applied to frame a broadly applicable tracking system.

Background

The problem of transgene movement to wild species will become more common with the global-scale mixing of germplasm to develop new crops for industrial, energy, nutraceutical and other novel purposes and products. Plants in the Asteraceae are of particular interest for many of these uses. The rationale for using *Taraxacum* as a model genus lies in its unique biology and historical and current use by humans. Two species, the rubber-producing *Taraxacum kok-saghyz* (TK) and the common weedy dandelion, *T. officinale* (TO), co-occur, overlap in flowering time, produce abundant pollen, and share pollinators. The TK cropping area overlaps with existing TO-infested areas, bringing exotic *Taraxacum* genes to the landscape. *Taraxacum* can be readily transformed and so can rapidly provide model GE materials for tracking system development. They can be used to predict behavior and gene flow patterns in other commercially-important recalcitrant Asteraceae, such as sunflowers.

The fact that most novel crops, like TK, are non-native in the region of intended production only adds to the risk of invasiveness. The classic example is nonnative rhizomatous perennial grass bioenergy crops that need little to no human manipulation to establish and spread. But even in more domesticated crops such as rice, brassicas, and sorghum, there are either conspecific weeds, or wild populations, or escapes that can share genes with the crop to form feral biotypes. Transgenic crops pose a special risk if they increase the tendency of a crop to go feral or have potential to hybridize with wild relatives. Herbicide resistance, the most widely introduced transgenic trait, is of obvious concern where there is potential for gene flow to wild relatives whose hybrid offspring may behave as weeds in agriculture or invade natural habitats.

Benefits of this research involve both environmental protection and economic opportunity. Information about the potential for development of genetically-modified TK will be used to help farmers avoid resistance and its spread. Developing a tracking system in a common wind-dispersed species will prevent increased use of herbicides with greater toxicity, longer persistence, and higher use rates. In addition, results will provide the foundation for making TK a viable and environmentally-sound crop that will help make U.S. agriculture the source of a sustainable domestic supply of rubber, an essential industrial resource. This will increase economic opportunities for American farmers and provide a new crop to diversify cropping systems, while reducing the need for additional toxic herbicides to manage another herbicide-resistant weed. **The validated model system will be generally and specifically applicable to other crops, especially those targeted for rapid domestication.**

Objectives

Develop germplasm and genetic resources to track potential gene flow between TK and TO

In order to assess the risk of pollen-mediated gene flow between TK and TO, robust, high throughput, non-destructive methods of detection are needed. Near Infrared Reflectance (NIR) classification (Fig.1) of fluorescent protein (FP) expression (Fig.2) will allow us to detect rare pollen-mediated hybrids from the inevitable abundance of apomictically-produced seeds in controlled crosses with more sensitivity than u/v light. FP germplasm will serve as a flexible resource, which can be used to detect pollen-mediated gene flow to additional TO genotypes and *Taraxacum* species, without an a priori molecular marker discovery and validation in these populations. In addition, FP germplasm could be introduced in the field, where NIR could be used to detect hybrids and escaped transgenics. We also will undertake molecular marker discovery, as nuclear and chloroplast markers can be used to detect interspecific gene flow. Given the expectation that most gene flow will be seed-mediated, the chloroplast markers, which are maternally inherited in *Taraxacum*, will serve as a powerful tool to detect interspecific gene flow.

Objective 2. Assess the risk of transgene spread via the formation of inter-specific hybrids.

Since *Taraxacum* species have mixed breeding systems, we will evaluate several possible pathways for gene flow between TK and TO. The main concern is that TO pollen will cross with a genetically-modified TK, imparting weedy characteristics in hybrids, and through repeated backcrossing could pass transgenes to a phenotype that is increasingly weedy, more competitive, and herbicide-resistant. Given the complexities of *Taraxacum* breeding systems, the potential for interspecific hybridization will be divided into three possible scenarios, each involving different germplasm and methods: 1) triploid TO acting as a pollen donor to TK; 2) TK acting as a pollen donor to diploid TO; and 3) TK acting as a pollen donor to triploid TO. *Taraxacum* gene flow potential is as complex as any of the crops for which this can serve as a model, and most crops will require only parts of these methods to be applied.

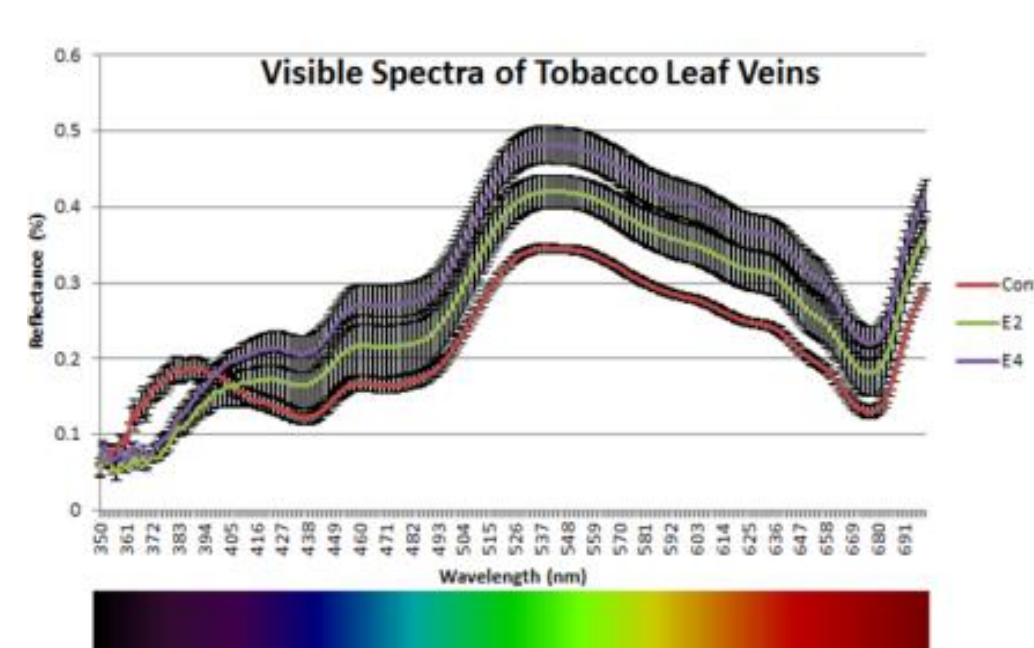


Figure 1. Visible light NIR spectra for 3 mature tobacco plants. The control has no transgene events, E2 and E4 have stable constitutive GFP expression of moderate and high GFP expression.

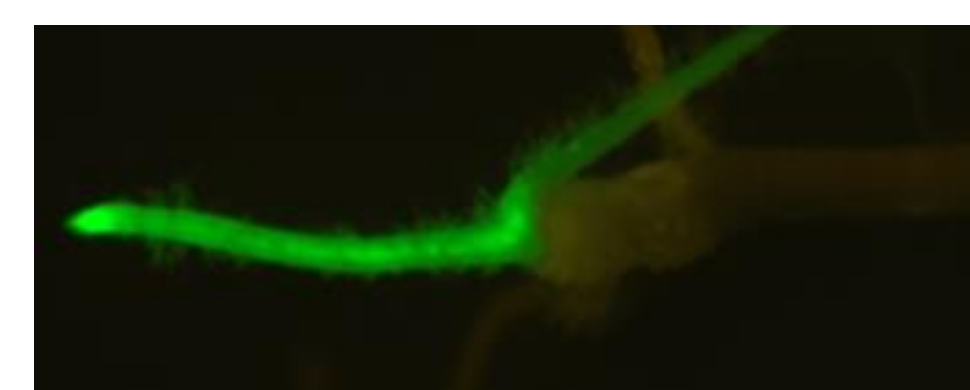


Figure 2. Stable TK A. rhizogenes-mediated GFP expression.

Anticipated Outcomes

Objective 1 will produce transgenic FP expressing germplasm, which can be used to detect rare gene flow events. This germplasm will allow the risk of pollen-mediated gene flow to additional species and accessions of *Taraxacum* to be easily evaluated. This germplasm could potentially be used in the field to evaluate the risk of gene flow and the efficacy of containment strategies. The use of FP germplasm will be facilitated by robust NIR phenotyping methodologies. The development of molecular markers (SNP, CAPS, etc) is essential for the validation of hybrids and the detection of gene flow in the field. We have demonstrated our ability to detect and score nuclear and plastidic polymorphisms (Figs 3 and 4) and aim to do this work on a larger scale, to yield a larger complement of genetic resources. The development of such resources will allow for the detection of hybridization through multiple generations of backcrossing and detection of gene flow in the field. Our targeted genome reduction strategy is amenable to genotyping by sequencing of large numbers of multiplexed hybrids and genotypes of interest for future marker validation and evaluations of introgression extent.

Objective 2 will indicate whether gene flow is possible for the various combinations of reciprocal crosses, the relative contribution of parental genes in hybrids, and the breeding system involved in the crosses. We will generate and characterize hybrid germplasm, the fitness of which will be evaluated. We expect that most successful hybridization will be with TO as a pollen parent to TK mothers, but the genotyping and ploidy analysis will allow us to determine if even low rates of hybridization occur by the other scenarios. Even if limited gene flow occurs, species-specific SNPs can be found, as introgression is not equal across all areas of the genome. The general approach will be applicable to any new bioenergy, industrial or nutraceutical crop.

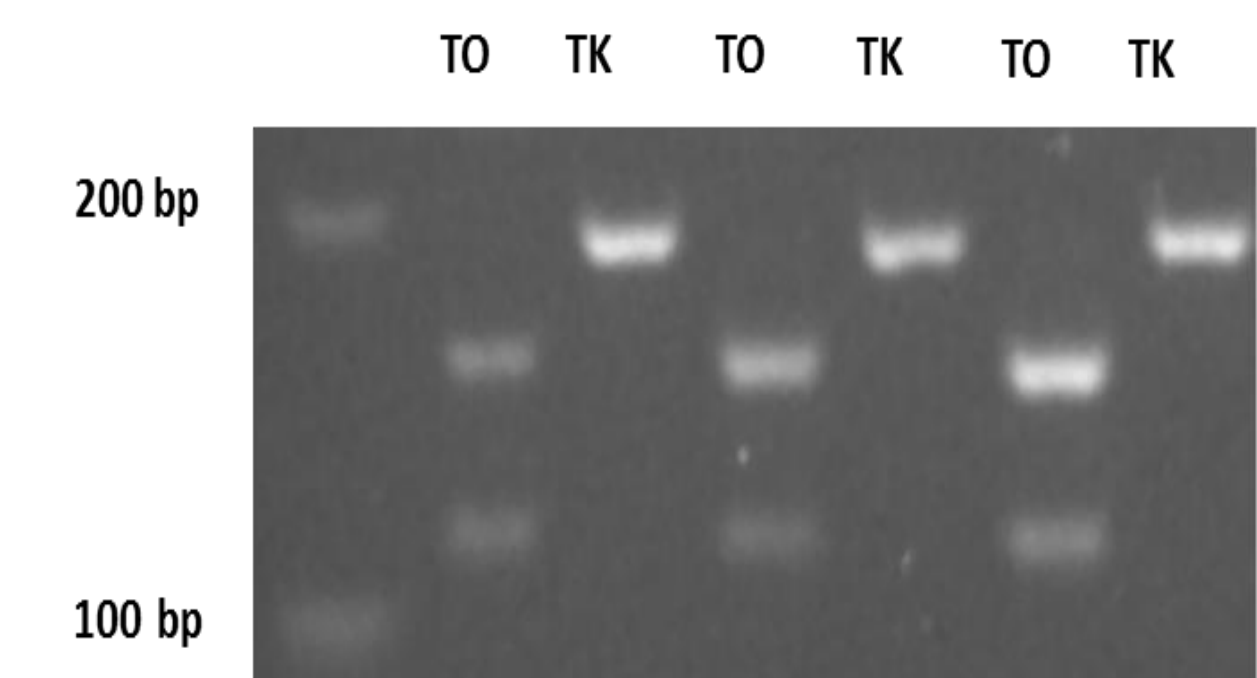


Figure 3. Visualization of SNPs within an aquaporin TIP2-1 gene..

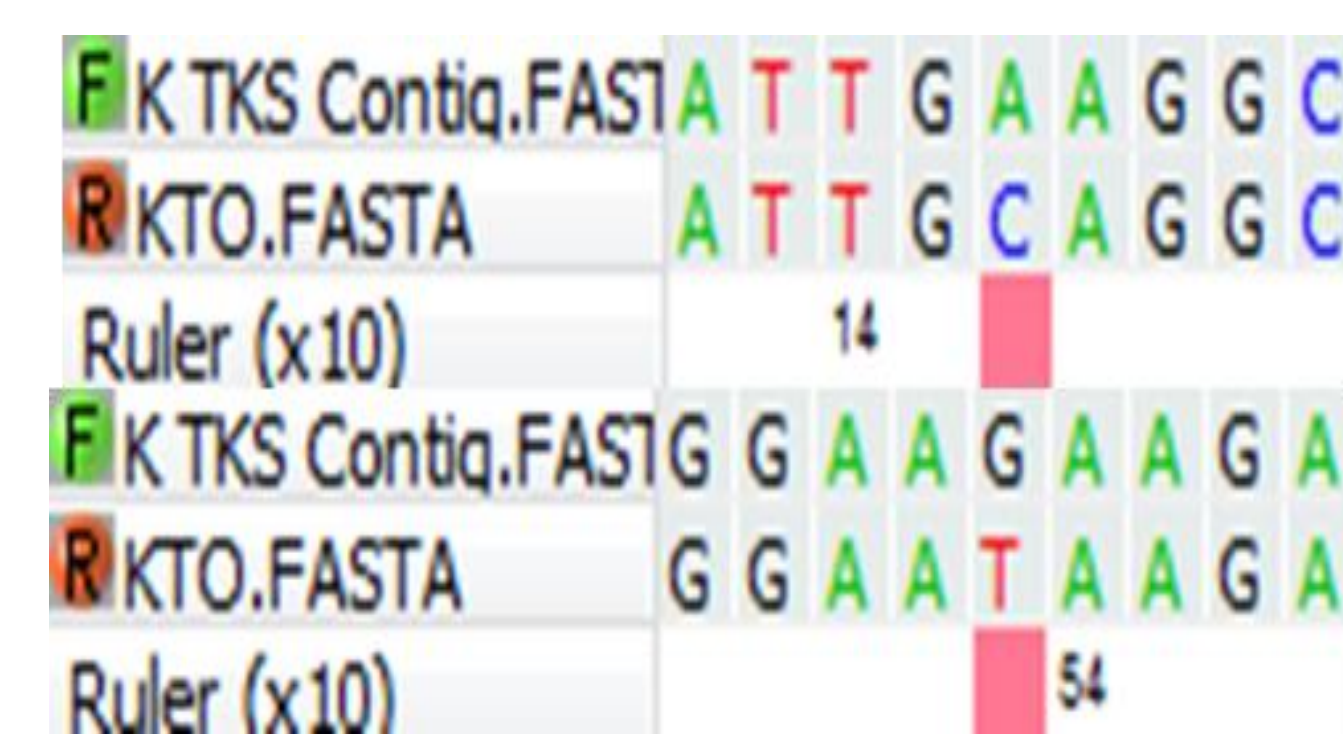


Figure 4. Two putative SNPs at the matK chloroplast gene distinguish TO from TK.

Economic Value and Potential Commercial Applications

Safe TK crops

- 1.2 MMT of NR needed for US self-sufficiency, is worth about \$2 billion for the raw material, feeds an \$18 billion industry, and requires about 2 million acres of production.
- Each 10,000 MT rubber can support >1,500 jobs, and NR self sufficiency may employ > 1 million people, many of these in new jobs.
- ~1.2 MMT CO₂ will be sequestered into rubber, and about 12 MMT into the associated crop biomass.
- The global NR and SR market in 10 years will be > 50 MMT, providing \$100 billion of rubber, serving an industry valued at ~\$1 trillion.
- NR can eventually replace most synthetic rubber, preventing the current emission of 90 MMT/year of CO₂ and 180 MMT/year in 10 years.
- The value of the carbon credits for 300 MMT CO₂, at \$6/MT, is \$1,800,000,000.
- Crop residuals can be sustainably converted to liquid biofuels and platform chemicals, replacing petroleum and creating yet more value.

General New Crops

- These methods applied to new crops, especially the large number of biofuels candidates, will evaluate potential transgene escape routes in the environment.
- This evaluation will inform risk mitigation and avoidance strategies and help ensure that harmful transgenes escapes do not happen.
- Bad publicity and possibly stiff federal and state fines will be avoided.
- Clean up expenses are not incurred.
- Helpful transgenes can be safely introduced into crops to improve yields and increase range.

GM Containment

These models will be developed and validated in confined environments. Greenhouse and field trials will be conducted under OSU IBC approvals and APHIS permits, respectively.