



THE OHIO STATE  
UNIVERSITY

COLLEGE OF FOOD, AGRICULTURAL,  
AND ENVIRONMENTAL SCIENCES

## TIRE TEAM

# Hybridization Between the Rubber-Producing Dandelion *Taraxacum kok-saghyz* and the Common Dandelion (*Taraxacum officinale*)

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## Abstract

*Taraxacum kok-saghyz* (TK) is a species of dandelion which can produce substantial amounts of high quality rubber in its roots; however, TK competes poorly with weeds. In order to overcome this shortcoming, transgenic, herbicide resistant TK varieties can readily be developed. The potential release of such plants raises the concern of hybridization with the ubiquitous and weedy, Common Dandelion, *T. officinale* (TO). In order to evaluate this risk, controlled crosses between the two species have been conducted. A complement of molecular markers were developed, which detected a hybridization rate of 23% among TK pollinated by TO. Hybrids exhibit TO traits, which may be explained by polyploid chromosome contributions. In order to detect natural hybrids, millions of TK produced in outdoor seed production areas heavily contaminated with TO were subjected to phenotypic and genotypic screens. No hybrids were detected in hand pollinated seed lots, while seed produced in areas amended with bees contained putative hybrids at a frequency of 0.001%. The apparent ability of TK and TO to hybridize should be considered prior to the release of transgenic TK.

## Introduction

- TK is a self-incompatible, outcrossing diploid ( $x=8$ ), while TO has a mixed breeding system, with sexual diploids as well as obligate apomict triploids ( $x=8$ ), which autonomously produce clonal seed. The former have been described in regions of Central Europe and have not been detected in North America (1,2).
- This suggests that seed-mediated gene flow may provide the most likely avenue of transgene escape, as obligate apomicts are unreceptive to pollen. While sympatric TO is expected to be triploid, it may still produce viable pollen ranging from 1 to 3x, which could fertilize TK, resulting in hybridization; such broad triploid-diploid hybridizations have previously been observed in *Taraxacum* (3).
- To evaluate this potential pathway of transgene escape, molecular tools were developed including species specific markers for both the nuclear and chloroplast genomes. These tools were then used to evaluate progeny from controlled crosses and large scale seed production areas.

## Methods

- Single Nucleotide Polymorphisms (SNPs) were mined from existing Expressed Sequence Tag (EST) resources, using the bioinformatic pipeline described by Kozik (4). Species specific SNPs were identified in complete chloroplast sequences from TK and TO (unpublished).
- Putatively fixed SNPs were validated and assayed as Cut Amplified Polymorphisms (CAPs).
- Controlled crosses were conducted, with TK serving as the maternal parent. Progeny were evaluated with a complement of five interspecific CAP markers and characterized.
- Seed from outdoor production areas were screened for weedy phenotypes, which were then queried with three species-specific chloroplast CAP markers.

## Results & Discussion

- A total of 6,200 contigs were assembled from existing TO and TK EST resources, totaling 4.2 Megabases (MB), containing 16,900 redundantly detected variants. Of these, 23 putatively species-specific SNPs were tested as CAPs in 8 lines of each species. Of these, 2 (9%) did not exhibit any diversity, 12 (52%) were polymorphic within both species and 9 (39%) were fixed between species. Results are summarized in Figure 1. These results demonstrate the utility of limited, extant EST resources and bioinformatics pipelines to yield molecular resources for emerging crops.

**Nuclear CAP markers for TO&TK**

■ NON-POLYMORPHIC ■ POLYMORPHIC ■ FIXED

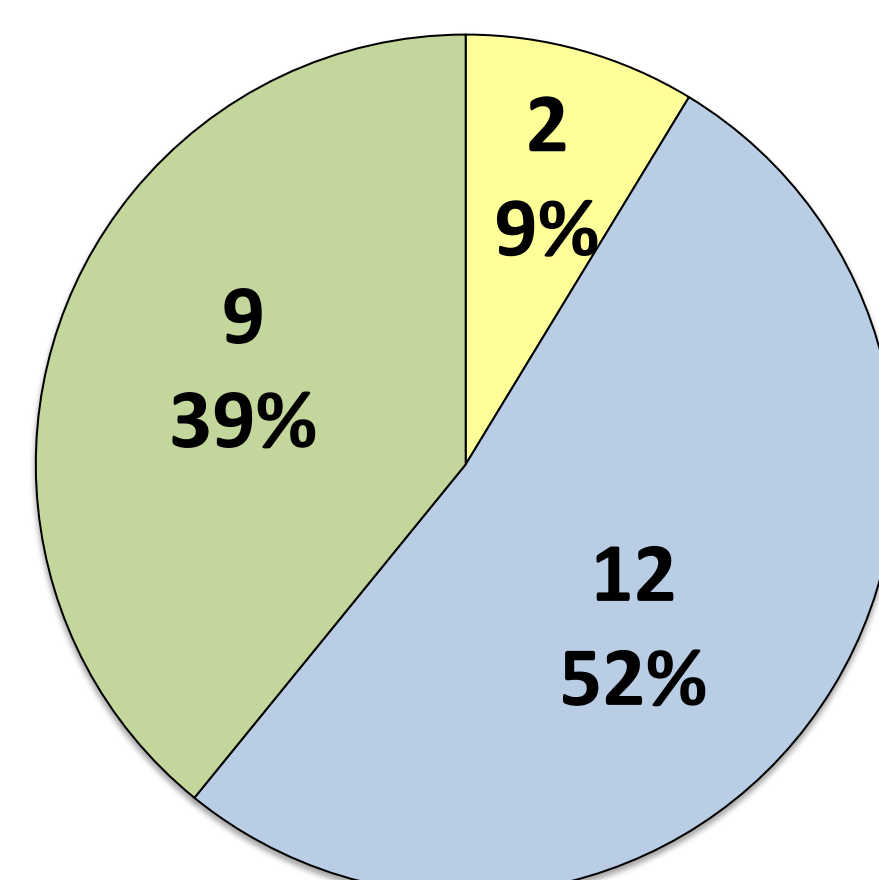


Figure 1.

- Hybrids had significantly larger genome sizes than both of their parental species. A flow cytometry histogram displaying the total genome sizes of TO (2,480 MB), TK (2,650 MB) and a hybrid (3,600 MB) is displayed in Figure 3A. This disparity of genome sizes can best be explained by redundant chromosome contributions from TO.
- The inheritance of unbalanced, interspecific genomes will likely limit the ability of hybrids to sexually reproduce and restrict transgene introgression and fitness.
- However, multiple chromosome contributions from TO impart a vigorous and potentially weedy character onto a background of TK. A juxtaposition of a hybrid (left) and TK (right) plant 2 weeks post emergence is displayed in Figure 3B.

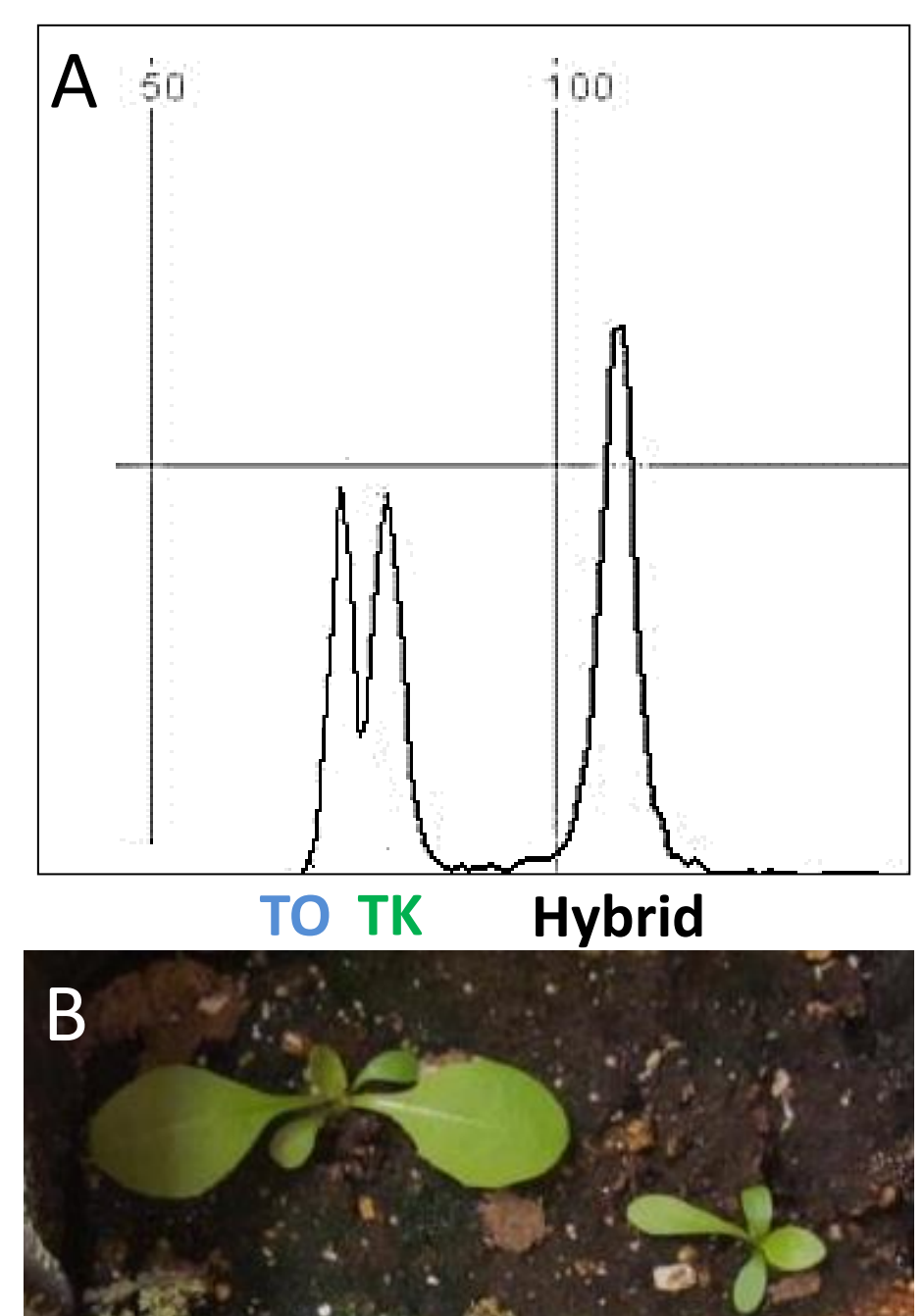


Figure 3.

- A total of 360 controlled crosses were conducted, among these 40% set seed and produced a total of 362 filled seed. This seed had a germination rate of 30%, resulting in 109 putative hybrids.
- CAP marker characterization of these individuals confirmed the formation of 25 hybridized progeny resulting from 10 crosses, yielding a hybridization rate of 23%. Hybrids exhibited vigorous growth habits and leaf morphologies characteristic of TO, of these hybrids, 60% exhibited the inheritance of apomixis, by setting seed after emasculation. Phenotypes of TK, TO and hybrids are displayed in Figure 2, as A, B and C, respectively.
- The inheritance of apomixis may allow adaptive hybrids to be proliferate in a state of heterosis; however, it may also preclude further integration of transgenes into the TO genome, by limiting sexual reproduction.



Figure 2.

- Seed produced in outdoor areas adjacent to dense populations of TO were sampled. A seed production area during a period of flowering overlap is displayed in Figure 4.
- An estimated 1.7 and 0.9 million germinants were screened, from plantings in 2013 and 2014, respectively.
- By characterizing weedy dandelions with chloroplast markers, no hybrids were detected from 2013 seed lots, while putative hybrids were detected in 2014 seed lots as rare 0.001% events.
- It is interesting that that the 2013 planting was largely generated through hand pollination, while the 2014 planting was amended with bees, this may explain the apparent absence of hybrids in 2013 seed lots.



Figure 4.

## Conclusions

These results affirm that TK can be pollinated by triploid TO to produce hybrid progeny. Molecular markers were developed, which detected a hybrids at a rate of 23% in controlled crosses. Hybrids exhibited vigorous growth habits and TO phenotypes, which may be due to multiple TO chromosome set contributions. Hybridization in outdoor seed production areas was not detected in 2013, but was detected in 2014 at a rate of 0.001%. The ability of TK to be rarely pollinated by TO demonstrates a potential risk, which should be considered when weighing the potential costs and benefits associated with the release of transgenic TK under different management practices.

## Acknowledgements

We thank Dr. David Francis<sup>1</sup> for an introduction to Linux, Dr. Matt Kleinhenz<sup>1</sup> for contributing TK germplasm, Annie Means for help conducting hybridizations, as well as Sarah McNulty and Niki Amstutz for help screening hybrids.

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