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Phenotypic and Genetic Characterization of *Taraxacum kok-saghyz* Polyploids

ABSTRACT

Taraxacum kok-saghyz (Rodin) (TK) is a sexual, outcrossing diploid dandelion species which produces high quality natural rubber. We used an optimized colchicine induction method for diploid TK seeds to develop generation 0 (G0) tetraploids (4nG0). Here we crossed 4nG0 plants to determine the stability of the tetraploid phenotype and to see if the resultant G1 plants, not poisoned by colchicine, developed larger roots than the previous generation. Flow cytometry revealed that both triploid (3nG1) and tetraploid (4nG1) progeny result from 4nG0 crosses at a ratio of 6:1 respectively. 3nG1 plants were grown to maturity and we found their roots were no longer stunted, and instead shared the same size range as control diploids, both of which were significantly larger than the treated diploids and G0 tetraploids. Root rubber concentrations followed a similar pattern. A rare G1 tetraploid grew much faster than control diploids. Diurnal photosynthesis rates show that both the G0 tetraploids and G1 triploids assimilated more carbon than both control and treated diploids. However, the extra carbon did not lead to greater root weights or rubber concentrations in these plants. Cropping with G1 (or later generation) polyploid TK plants may allow more rapid growth in the field and improved yield.

INTRODUCTION

In many plant species, breeders manipulate ploidy, the number of sets of chromosomes, to increase crop vigor and yield. *Taraxacum kok-saghyz* (TK) is an emerging natural rubber crop grown in temperate areas in the United States, Canada and Europe. Rubber yield in TK is a product of both rubber concentration and root size. In an effort to increase one or both of these factors, tetraploid TK were induced by treating diploid seeds with colchicine, a chemical that interferes with meiosis.

The objective of this study was to compare root biomass, root rubber concentration and photosynthetic rates to determine if and why polyploid TK have increased vigor and/or rubber production compared to diploid TK plants.

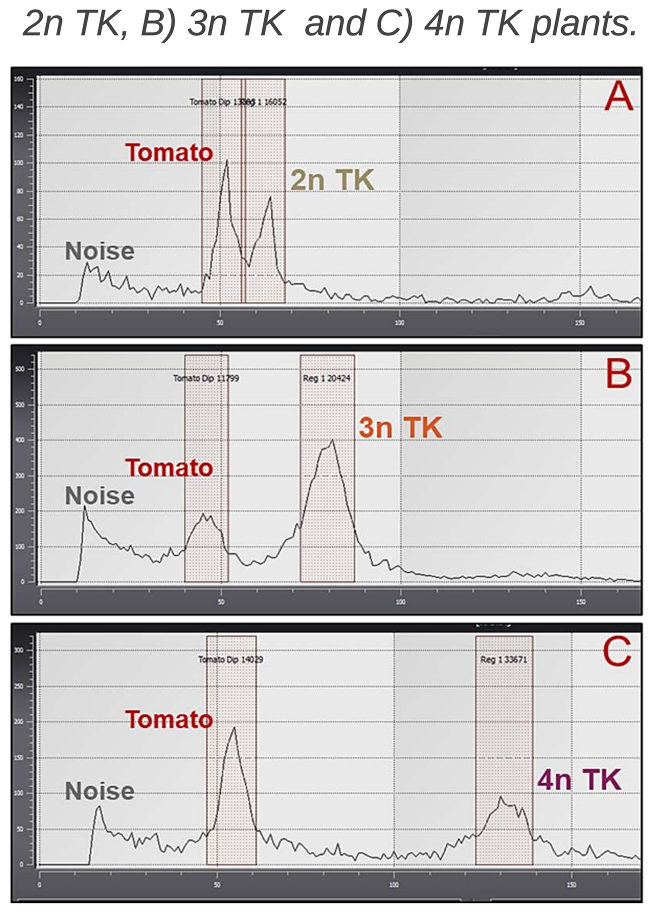
METHODS

Colchicine Treatment of TK Seeds: Diploid TK seed (germination rate > 90%) were soaked in a 0.2% colchicine solution for 24 h and planted into flats in the greenhouse. Four-week-old seedlings were visually screened for unusual leaf phenotype and selected plants were transplanted into treepots and grown for three months before tissue was sampled for ploidy analysis.

Ploidy Analysis by Flow Cytometry: TK leaf tissue (1 cm^2), plus a tomato seed used as an internal standard, were chopped in nuclei lysis buffer, filtered and stained with DAPI dye. A Sysmex Ploidy Analyzer equipped with a UV light was used to detect the nuclei and sort them by size. The ratio between particle means of the tomato peak and TK peak was calculated to determine TK ploidy (Fig. 1).

Crossing G0 Tetraploids (4nG0): 4nG0 plants were directly crossed with each other at flowering and resultant seed was planted into treepots in the greenhouse. Ploidy analysis occurred when these plants were 4 months old.

Figure 1. Flow cytometric scans of A) 2n TK, B) 3n TK and C) 4n TK plants.



Diurnal Photosynthesis Measurements: A LI-COR LI-6400XT Portable Photosynthesis System was used to measure diurnal photosynthesis rates on seventeen TK plants with known ploidy. Measurements started shortly after sunrise and the LI-6400XT was moved among plants in a continuous circuit for the entire day.

Plant Harvest and Rubber Prediction: Nine-month-old plants were harvested, and plant and root fresh weights recorded. Roots were dried for two days at 50°C and ground to a powder with an analytical mill. The ground roots were scanned using the MugLite attachment of the Field Spec 3 Near Infrared Spectrophotometer (NIR) (ASD Malvern PANalytical) and root rubber concentration was predicted using our current rubber predictive model for TK (0.88 r^2).

RESULTS

Colchicine Treatment of TK Seeds: On average, 35% of colchicine treated TK seeds germinated and 20% of the seedlings survived past one month. 10% of the survivors were induced G0 tetraploids (4nG0) and the rest were treated diploids (2nT) that did not die from the colchicine poisoning.

Ploidy Analysis by Flow Cytometry: Unexpectedly, both G1 triploid (3nG1) and G1 tetraploid (4nG1) TK plants were created from crossing two 4nG0 plants. Because few 4nG1 plants were made in this round of crossing, these plants were omitted from this study due to small sample size.

Diurnal Photosynthesis Measurements: Both the 3nG1 (Fig. 2B) and 4nG0 (Fig. 2C) plants had significantly higher carbon assimilation rates ($P < 0.01$) throughout the day compared with control diploids (2nC) (Fig. 2A) and treated diploids. The polynomial curves for the 2nT plants are not shown, but fall into the same ranges as the 2nC plants in Fig. 2A.

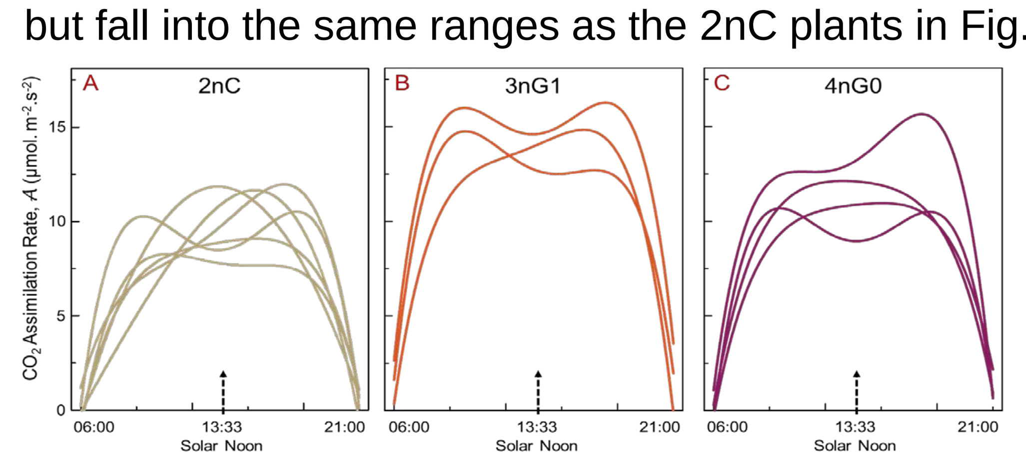


Figure 2. 4th degree polynomial curves of diurnal photosynthetic rates of A) 2nC, B) 3nG1, and C) 4nG0 TK plants. Each curve represents one plant.

Root Size at Harvest: As previously observed,[1] plants treated with colchicine (2nT and 4nG0) (Fig. 3A) had stunted roots that were significantly smaller ($P < 0.01$) than the 2nC and 3nG1 plants (Fig. 3B).

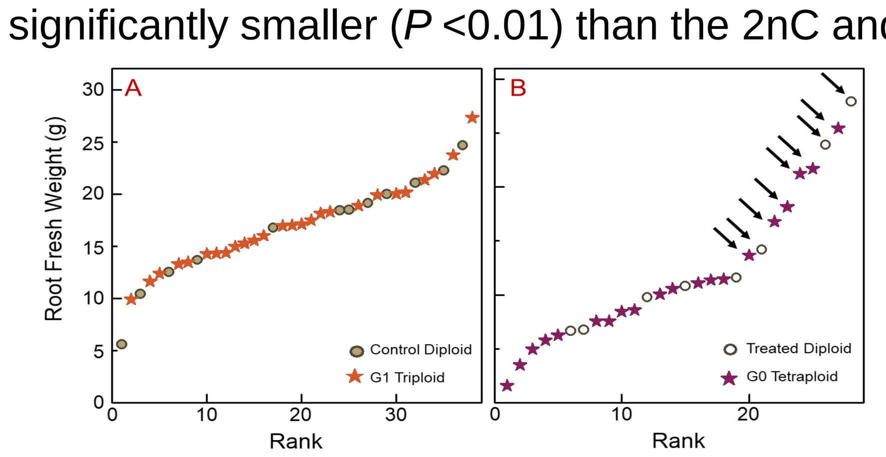
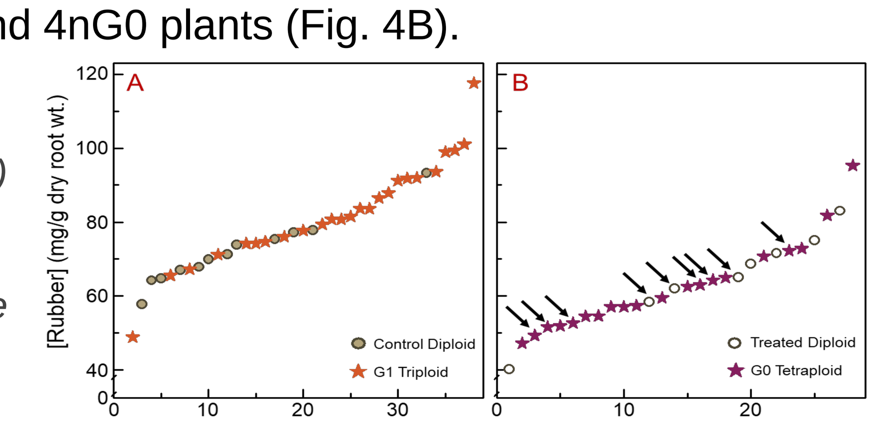


Figure 3. TK root fresh weights (g) ranked in A) 2nC and 3nG1 plants and B) 2nT and 4nG0 plants. Arrows indicate the same plants with arrows in Fig.4.

However, root weights of three 2nT and six 4nG0 plants, indicated by arrows in Fig. 3B, did not follow the trendline of the stunted roots and, instead, root weights sharply increased in these plants.

Root Rubber Concentration: Like root size, rubber concentration was significantly higher ($P < 0.01$) in the 2nC and 3nG1 plants (Fig. 4A) than the colchicine treated 2nT and 4nG0 plants (Fig. 4B).

Figure 4. TK root rubber concentration (mg/g dry root wt.) ranked in A) 2nC and 3nG1 plants and B) 2nT and 4nG0 plants. Arrows indicate the same plants with arrows in Fig.3.



The arrows in Fig. 4B follow the nine treated plants not displaying obvious stunted roots in Fig. 3B, however the increased root fresh weight exhibited by these plants did not correspond to increased root rubber concentration.

DISCUSSION

Colchicine treatment of diploid TK seeds produces 4nG0 and 2nT plants that have stunted roots and produce less rubber than 2nC and 3nG1 plants. However, these results conflict with two previous studies in polyploid TK. One study reported that 4nG0 plants produced larger roots with higher rubber concentrations than diploids,[2] and the other reported that 4nG0 plants had stunted roots but above average rubber concentrations.[1] We believe the three conflicting results to be the result of a yet not understood effect of colchicine poisoning of the plant cells. The sharp increase in root fresh weight of a subset of both 2nT and 4nG0 plants in Fig. 3B indicates that the lingering effects of the colchicine on the plant may be as important as the ploidy of the plant. Here, 3nG1 plants, not treated by colchicine had larger roots and more rubber that the 4nG0 plants but were not significantly different from the 2nC plants. Since these plants are progeny of 4nG0 plants, perhaps some toxicity is remaining in this G1 generation. However, 4nG1 plants seem to be faster to establish, develop and flower than 2nC (Fig. 5) and 3nG1 (not shown).

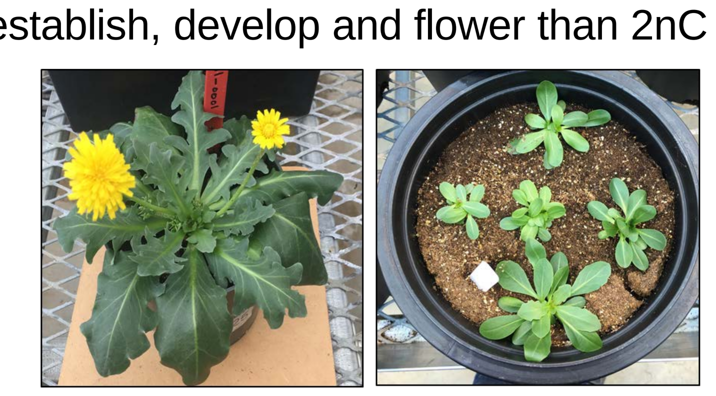
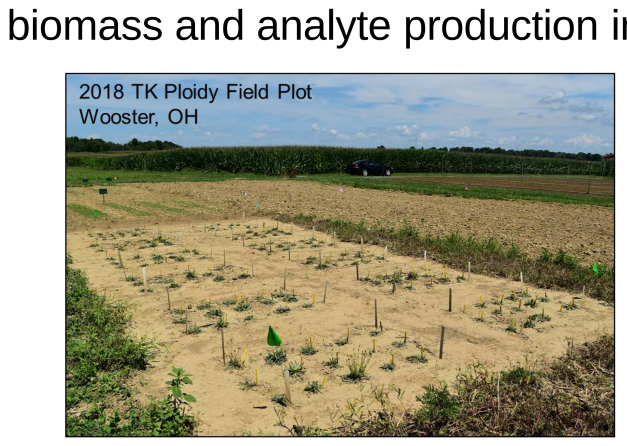


Figure 5. Growth rate comparison of A) A 2-month-old 4nG1 TK (already flowering!) growing in a 13 cm diameter pot, and B) Five 2-month-old 2nC plants growing in a 15 cm diameter pot.

Diurnal photosynthesis rates of 3nG1 plants, while on average higher than 4nG0 plants, did not significantly differ from them ($P = 0.10$) (Fig. 2). The destination of the excess carbon in the 4nG0 plants is still unknown but may have gone to increased rosette size, reproduction, or inulin production. A field trial planted in May 2018 (Fig. 6) includes 2nC, 2nT and 4nG0 plants. Plant and root biomass, root rubber and root inulin concentrations will be measured at harvest (November 2018). These data will better allow us to understand the effects of the colchicine toxicity on biomass and analyte production in TK.

Figure 6. Randomized block design of 2nC, 2nT and 4nG0 TK plants growing in the field August 2018 in Wooster, OH.



CONCLUSIONS

Although G0 TK tetraploids are still exhibiting effects of colchicine toxicity, these plants and their progeny show promising potential to increase TK rubber yields. Data from the field study and 4nG1 and 3nG2 plants growing in the greenhouse will allow further characterization of the performance of polyploid TK plants.

ACKNOWLEDGEMENTS

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