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Temporal diversity of *Taraxacum kok-saghyz* plants reveals high rubber yield phenotypes

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[2]Department of Food, Agriculture and Biological Engineering, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, OH 44691, USA. Tel.: +1-330-263-3982; Fax: +1-330-264-3887, [♥]email address: cornish.19@osu.edu, [†]deceased

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Abstract. *Cornish K, Kopicky SL, McNulty SK, Amstutz N, Chanon AM, Walker S, Kleinhenz MD, Miller AR, Streeter JG. 2016. Temporal diversity of* Taraxacum kok-saghyz *plants reveals high rubber yield phenotypes. Biodiversitas 17: 847-856. Taraxacum kok-saghyz* is a diploid, out-crossing, rubber-producing species under development as an alternative natural rubber crop. About 17,650 seed were obtained from progeny of 20 wild collected plants. New populations of plants were developed in Ohio from a random subsample of these seed, which were then open pollinated. In November 2011, these seed were direct seeded in outdoor shallow raised beds and in high tunnel deep raised beds. Plants were harvested from the outdoor beds from July 2012 to July 2013 to provide temporal phenotypic data as plants developed and overwintered. The high tunnel beds were harvested July 2013, and provided data on 11 individual accessions, and their progeny, and on the effect of winter bed heating. Plants were highly variable. Rubber concentration in root tissue was not directly correlated with root, shoot or plant size. Across all growing conditions and developmental stage, the highest rubber yields per plant were found in plants with large roots combined with a large rosette, and an above average rubber concentration. These parameters appeared to segregate independently, and rubber concentration was heritable. Interbreeding plants selected for large root, large rosette and then high rubber concentration, should rapidly move *Taraxacum kok-saghyz* towards domestication and commercialization.

Keywords: Diversity, Kazak dandelion, natural rubber, phenotype, rubber dandelion, Russian dandelion, *Taraxacum kok-saghyz*, temporal

INTRODUCTION

Taraxacum kok-saghyz (TK, also known as rubber dandelion, Russian or Kazak dandelion) was identified as an alternative source of natural rubber in the early 1930's (Lipshitz 1934), and is suited to domestic rubber cultivation in temperate regions (Whaley and Bowen 1947; Mooibroek and Cornish 2000; van Beilen and Poirier 2007; Buranov et al. 2010). Producing *cis*-1, 4-polyisoprene rubber very similar, in terms of macromolecular structure and composition, to that from *Hevea brasiliensis* (Cornish et al. 2015), TK is an annual crop which may be managed using modern agricultural techniques. Although TK has long been known to produce rubber (Lipshitz 1934), the crop has not been domesticated in the modern era, and requires extensive agronomic optimization and genetic improvement before it can become a reliable, commercial, rubber crop.

Early cultivation of TK highlighted key issues associated with production of this emerging crop. High genetic variation was present in TK populations with root weights ranging from 2 to 150 g (Whaley and Bowen 1947). Rubber content also varied, with some TK plants producing only trace amounts of rubber, while others produced upwards of 30 percent of the total plant dry weight (Whaley and Bowen 1947). Intraspecific variation also was evident in root and leaf morphological characteristics, which seemed to be heritable, but no phenotype was identified that directly correlated with high rubber yield. By the 1940's, genetically distinct TK lines had been developed which exhibited greater uniformity in both root weight and rubber content (Krotkov,1945; Whaley and Bowen 1947). Also, yield improvements were obtained using tetraploids induced with colchicine (Warmke 1945). However, with the restored supply of low cost *Hevea* natural rubber following WWII, TK-based rubber production became economically uncompetitive, and cultivation of TK and all other alternative rubber sources ceased (Finlay 2009; Venkatachalam et al. 2013). Further, all improved TK lines, which had been maintained into the post-war era, were ultimately lost with the dissolution of the Soviet Union. A re-introduction of TK in the early to mid-2000s, which was widely distributed, was later found to be *Taraxacum brevicorniculatum*, a common seed contaminant (Kirschner et al. 2012). Because of this, our current efforts to develop TK as an alternative rubber source are based on the later 2008 USDA collection of seed from wild TK diploid outbreeding plants from Kazakhstan and Uzbekistan (Hellier 2011). This collection was guided by Dr. Alexander Sennikov, Curator of Vascular Plants, Herbarium, Russian Academy of Sciences, V. L. Komarov Botanical Institute, Russia, to ensure accurate TK identification.

In this study, we characterized phenotype diversity in a population generated by open pollination of several thousand plants grown from a subsample of 17,450 seed from 20 accessions. Diversity in growth and rubber concentration in TK plants was tracked from 7 to 20 months in outdoor shallow raised beds, and among 9-month old plants from different accessions grown in high tunnel raised beds with and without winter bed heating.

MATERIALS AND METHODS

Plant material

TK seed were obtained from the 2008 USDA collection of seed from wild TK plants in Kazakstan and Uzbekistan (Hellier 2011). The plants grown from these seed were compared with detailed descriptions of TK and *T. brevicorniculatum* (Kirschner et al. 2012). Although considerable variation existed among plants, the TK plants had thick blue-green leaves without small teeth on the leaf margins (Figure 1.A) whereas *T. brevicorniculatum* leaves were thinner and light in color (*cf.* Figure 1.A and D, and B and E). Also, the TK leaves were waxy (Figure 1.A), while the *T. brevicorniculatum* leaves were more papery (Figure 1.D). The flowers were relatively small, and had long "hornlike" bracts (Krotkov 1945; Kirschner et al. 2012) distinct from the shorter bracts on authentic *T. brevicorniculatum.* The latter species often exhibits a red/purple color on leaves and flower stalks (Figure 1.D) which almost never occurs in healthy TK (Figure 1.A-C). In addition, the genome sizes of over 1,000 TK plants were analyzed by flow cytometry (n=8, data not shown) and without exception were proved to be diploids, in contrast to the clearly triploid *T. brevicorniculatum* and *T. officinale* (common dandelion), which also were confirmed with flow cytometry (data not shown).

Cuttings from 91 different interbred TK lines developed in Ohio were transplanted into high tunnel beds in fall 2010 and overwintered. In spring 2011, bulk seed was collected by vacuum and hand harvest from 11 of the beds, forming 11 new seed accessions, and cleaned. The mean rubber content of three of the maternal parents of each accession was analyzed by accelerated solvent extraction (see method below).

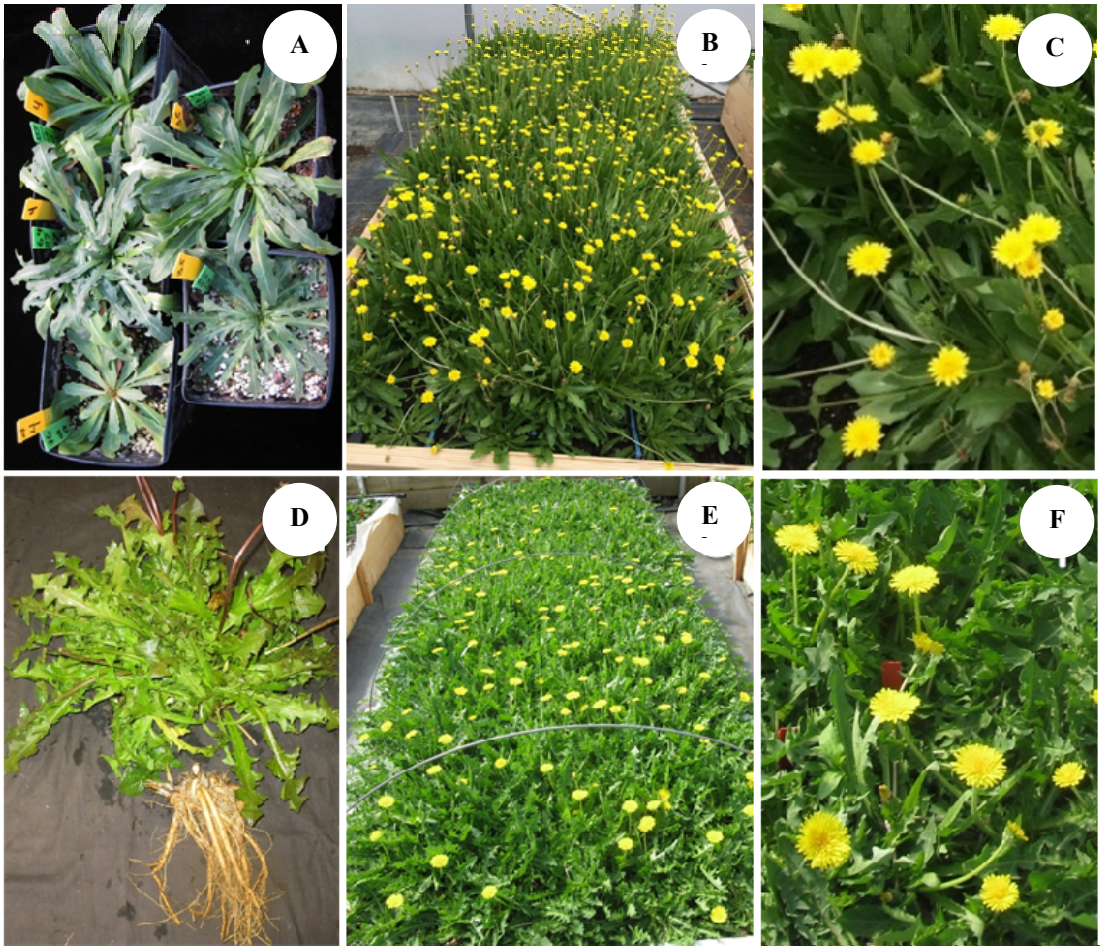

Figure 1. Photographs of *Taraxacum kok-saghyz* and *Taraxacum brevicorniculatum* plants in our germplasm collection. A. Some *T. kok-saghyz* leaf phenotypes; B. A bed of flowering *T. kok-saghyz*; C. *T. kok-saghyz* flowers; D. A typical *T. brevicorniculatum* plant; E. A bed of flowering *T. brevicorniculatum*; F. *T. brevicorniculatum* flowers

Growth and rubber content of TK in outdoor shallow beds

A subset of seed was pooled from 11 generated accessions and planted in autumn of 2011, in outdoor shallow raised beds at The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH. The shallow raised beds had a soil composition of 20% parboiled rice hulls, 30% peat moss, 25% compost from OARDC campus facilities, and 25% Wooster silt loam field soil. TK seed was mixed with a turface/sand mix, which acted as inert filler and reduced seed density, and was dispersed by hand into premade furrows in the beds. This method of seed distribution simulated direct seeding conditions. The shallow raised beds were 1.2 m wide, 9.1 m long, had a soil depth of 0.3 m and were drip irrigated. Low tunnels fashioned from clear plastic sheeting with a thickness of 0.15 mm (Klerks Hyplast, Chester, SC) were applied from November to March to provide insulation and protection from the elements. The outdoor beds were left exposed to the environment from April to October. A Hobo Pro v2 data logger (Onset Computer Corporation, Bourne, MA) was installed in the middle of a representative outdoor bed to measure both air temperature and soil temperature every 15 minutes for the duration of the experiment. The data logger was fitted with an aluminum radiation shield to deflect direct sunlight from the air temperature sensor and the soil temperature sensor probe was inserted 10-15 cm into the soil. Daily soil temperatures averaged 13°C for the duration of the experiment, with -7°C recorded as the minimum soil temperature and 44°C as the maximum soil temperature. Daily air temperatures also averaged 13°C for the duration of the experiment, with -13°C recorded as the minimum air temperature and 44°C as the maximum air temperature. The campus operated solar powered data logger and modem (Campbell Scientific, Logan, UT) inserted at 10 cm into the ground recorded an average soil temperature of 12°C during the experiments with extremes of 0°C and 29°C. This weather station also recorded air temperatures as low as -18°C in January 2012 and -17°C in January 2013.

Five randomly selected plants were harvested by hand on approximately a biweekly basis from July 2012 to July 2013. A total of 124 TK dandelions were harvested over the course of the experiment. Immediately following each harvest, plants were wrapped in moist paper towels and transported to the lab in clear plastic bags, where data were gathered on plant size. TK roots were separated from the crown to obtain fresh root weights, then placed in paper bags, and oven dried at 53°C for two days, and weighed.

Growth and rubber content of TK in high tunnel deep planting beds

Seed of 11 accessions were planted in autumn of 2011, in high tunnel deep raised beds at The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH. The high tunnel raised beds were protected by a roof made of plastic sheeting with a thickness of 0.15 mm (Klerks Hyplast, Chester, SC) throughout the year, with retractable siding allowing airflow and temperature regulation from April to October. During the cold season, November to March, the sidewalls were closed creating a protected, unheated environment. Data loggers were installed in representative beds as described above. To further protect the plants from low temperatures and freeze/thaw, tents made from Agribon™ row cover fabric (Polymer Group, Inc., Charlotte, NC) were fitted on to the high tunnel beds from November to March. High tunnel beds had dimensions of 3.65 m by 1.2 m with a soil depth of 0.61 m. Soil in the raised beds was a mixture of 33% Pro-mix, 16.5% vermiculite, 16.5% compost from OARDC campus facilities, 16.5% Wooster silt loam soil, and 16.5% perlite. Six high tunnel beds were sectioned crosswise into 1.8-m halves forming 12 plots, and each plot was divided into 10 rows. An accession was randomly selected to be planted in each row, with no accession being repeated in a plot. Mixtures of seed and turface were dispersed into furrows in each of the 10 rows, functioning as a simulated direct seeding method. Half of the high tunnel beds were outfitted with subterranean heating cables operational from December 2011 to March 2012, maintaining an average soil temperature of 21°C, but soil temperature dropped to as low as 2°C on the coldest days of the year. Soil temperatures in the unheated beds averaged 16°C and also dropped as low as 2°C. The high tunnel *Taraxacum kok-saghyz* plants were grown until the following July (2012), then harvested. Two dandelions of each accession were harvested on each harvesting day. Plants were labelled, wrapped in moist paper towels, placed in clear plastic bags, and transported to the lab and weighed. TK roots were cut from the crown, weighed, placed in paper bags, oven dried at 53°C for two days, and reweighed before rubber analysis. A total of 240 TK dandelion samples were harvested, with 120 samples from heated beds and 120 from unheated beds.

Rubber quantification

Rubber was quantified using accelerated solvent extraction (ASE). Dry root samples were finely ground using an analytical mill (A 11 basic, IKA, Wilmington, NC) and weighed to 0.2500g ± 0.0005g. Ground material was mixed with approximately 5 ml of inert Ottawa sand (20-30 mesh), to evenly distribute the sample throughout 11 cm^3 stainless steel cells (from the Dionex ASE 200) and to improve accessibility of the solvent to sample particles during extraction. The resulting mixture was poured into the stainless steel cells fitted with cellulose filters inserted on both ends, preventing plugging during the purge cycle and increasing the flow of the dissolved analyte (Thermo Scientific, Waltham, MA). ASE cells were placed into the Dionex ASE 200 (Thermo Scientific, Waltham, MA). A sand filled blank served as both a visual and numerical control for extraction protocols. During the extraction process, hexane was injected into each cell, which were sequentially pressurized to 10.34 MPa (1,500 psi) and heated to 120°C to extract high molecular weight rubber. After a 50-minute process, including two 20-minute static cycles and two repeated 60-second purges, hexane-dissolved rubber from each cell was collected in separate glass vials. Vials containing hexane and dissolved rubber were vortexed and poured into pre-weighed aluminum

pans. Additional hexane was used to rinse analyte remnants from the vial walls using repeated vortexing. Hexane was evaporated from aluminum pans during a 24 h drying period at room temperature in a fume hood, leaving behind the rubber. Aluminum pans were then reweighed and the amounts of dried rubber determined. The following equations were used to determine rubber content in plant biomass, rounded to 0.1 mg/g dry weight.

$$Final\ Pan\ Weight\ (g) - Empty\ Pan\ Weight\ (g) = Analyte\ Weight\ (g)$$

$$\left(\left(\frac{Analyte\ Weight\ (g)}{Sample\ Weight\ (g)}\right) * 1000\right) - correction\ factor = Analyte\ Content\ \left(\frac{mg}{g}\right)$$

A correction factor was used to account for the fraction of acetone-soluble non-rubber analytes that were co-extracted with the rubber by the hexane (Pearson et al. 2013). This factor is the mean of 15.22 $\pm$ 1.73 mg/g from 78 root samples (data not shown) from sequential accelerated solvent extraction in which acetone was used as the extractant before the hexane (data not shown). Rubber extraction efficiency of this ASE method is 91% (data not shown).

RESULTS AND DISCUSSION

Outdoor shallow beds

Plant size was highly variable (Figure 2.A) and this included variation in both root (Figure 2.B) and rosette (Figure 2.C) size. Exceptionally large plants (> 3x the median) were rare in this population. Also, larger plants may be especially susceptible to plant death during the winter (Figure 2.A-C) because no very large plants were present the following spring and summer. The variation among the plants, even discounting the exceptionally large plants, was also reduced in the following spring and summer. Roots across this time course were highly variable in rubber concentration (51.4 $\pm$ 16.5 mg/g dry root, mean of 129 plants $\pm$ sd), and plants harvested on a single day varied by as much as 68 mg/g dry root (Figure 2.D).

As the plants overwintered, there was a general overall decline in rosette weight (Figure 2.C), as many leaves senesced, and a decline in root mass also was observed (Figure 2.B). The plants in July of 2013 did not regrow to the plant, root and rosette biomass achieved a year earlier at the same time of year (Figure 2.A-C). Yet even with this variation, an overall increase in rubber concentration was apparent during the winter season, beginning in November 2012 and levelling off by February 2013 (Figure 2.D). Variability in rubber concentration among plants harvested on any single day was also greater in the spring than during previous autumn. However, when the total amount of rubber was calculated (Figure 2.E), the increase in rubber concentration (Figure 2.D) was countered by the reduction in root mass, leading to little change in rubber per plant over the year in most TK plants. It is also clear that the winter die-back affected rosettes more than roots (Figure 2.F) since the root:rosette ratio increased during the winter. However, not all plants suffered rosette senescence during the winter (Figure 2.F).

Two plants had higher rubber amounts than the general range (Figure 2.E). One of these plants (indicated by the dot and dash arrows) had a high rubber concentration in the roots (Figure 2.D) but, although above average in root biomass (Figure 2.B), was not an unusually large plant (Figure 2.A) and had a relatively small rosette (Figure 2.C). Thus, high root rubber concentration was the cause of its overall high rubber yield (Figure 2.E). The highest yielding plant in the study (indicated by the dashed arrow) had a moderate root rubber concentration (Figure 2.D), but was the largest of all the plants harvested (Figure 2.A), had the second largest rosette (Figure 2.C), and most notably, a remarkably large root mass (Figure 2.B). One other very large plant (indicated by the solid arrow, Figure 2.A) had a large rosette (Figure 2.C) but a quite small root system (Figure 2.B). Even though this plant also had an above average root rubber concentration (Figure 2.D), its small root biomass resulted in only a slightly above average plant rubber yield (Figure 2.E).

Overall there was a correlation of root fresh weight and rosette fresh weight but the high degree of between plant variation in root:rosette ratio caused a quite low correlation coefficient (Figure 3.A, $r^2 = 0.446$). No relationship of root fresh weight to the root:rosette ratio was found (Figure 3.B, $r^2 = 0.018$). However, rosette fresh weight was correlated to the root/rosette ratio in a nonlinear manner (Figure 3.C, $r^2 = 0.308$).

Rubber concentration and rubber yield per plant were then plotted against a variety of plant phenotypic parameters (Figure 4). The highest amount of rubber per plant (dashed arrow) occurred in an exceptionally large plant (Figure 4.B) with a very large root (Figure 4.D), a large rosette (Figure 4.F), and a root:rosette ratio of 0.65. In contrast, the highest concentration of rubber (dot and dash arrow) was observed in a plant with large root and, average rosette and plant biomass. However, this plant has a relatively high root:rosette ratio of 0.88.

Rubber concentration was not well correlated with plant phenotype (Figure 4.A, C, E, G) and this was confirmed by plotting concentration against root and shoot fresh weight (Figure 5). No clear trend was apparent from the weighted averages.

However, when total rubber per plant values were plotted (Figure 6.A) a clearer relationship was revealed. Small plants were invariably low rubber yielding. Plants with large rosettes and small roots had also low rubber yield. Plants with large roots and small rosettes were medium in rubber yield, although they had considerably more rubber than the first two classes described, and larger roots progressively resulted in higher total rubber. However, in this germplasm pool, plants with large root *and* large rosettes had the highest rubber yields. The weighted average plot (Figure 5.B) confirmed these trends and indicated an optimum relationship of maximum total rubber in plants with both large root and large rosette.

High tunnel deep beds

The 240 plants harvested in July 2012 from high tunnel deep beds had a wide range of root rubber concentrations (0.4 to 103 mg/g dry root). This is similar to what was

described in plants grown in outdoor shallow beds. Root rubber concentrations in individual plants were skewed towards lower rubber concentrations, but some plants had concentrations as high as twice the population mean of 51.4 mg/g dry root (Figure 7).

Plants from the 11 accessions exhibited considerable within variation for rubber concentration (Figure 8.A). However, an analysis of variance showed no significant difference among the 11 accessions (Table 1). In addition, the accessions did not significantly differ in root biomass (Figure 8.B, Table 2). Rubber concentration of mother plants did positively correlate with rubber concentration in their progeny (Figure 9, $r^2 = 0.530$).

Heating the deep beds in the winter had no effect on root rubber concentration (Figure 10.A, Table 1). However, heating had a significant effect on root biomass (Figure 10.B, Table 2), with root weight means of 6.6 and 4.7 g for the heated and unheated treatments, respectively. Maintaining the soil temperature at 21°C from December to March resulted in approximately 40% larger roots, compared to TK in unheated beds, when harvested the following July.

The relationship of plant phenotype to rubber concentration and total rubber per plant was investigated, combining data from all 11 accessions, because significant differences had not been detected among them for rubber or biomass parameters. As was found for plants grown in the outdoor shallow beds, phenotypes in the high tunnels were highly variable, and a wide range of rubber concentration and total rubber per plant was observed (Figure 11.A-F). As expected, since the outdoor shallow beds were planted with the same pool of seed, some outliers were again observed. Some plants were much larger than most (Figure 11.A and B), and had very large roots (Figure 11.C and D) or rosettes (Figure 11.E and F). In general, rubber yields increased with plant and root biomass, but rubber concentration was not correlated to biomass parameters, and the 3D plot (not shown) was similar to that in Figure 5. The overall trends in the relationship of root and rosette biomass to total rubber per plant (Figure 12) were very similar to those in the temporal study (Figure 6.B). Thus, small TK plants grown in deep high tunnel beds contained very little rubber, and the highest rubber yields were found in plants with both large roots and rosettes.

Discussion

Wide phenotypic variation was apparent in TK grown in outdoor shallow beds and in high tunnel deep beds, as expected since 91 distinct Ohio developed TK lines made up the parent accessions, and TK is a sexual, self-incompatible diploid (Hodgson-Kratky and Wolyn 2015). This high level of variation allows for determination of some phenotypic characteristics that are, and others not, related to rubber yield. It is clear that large root biomass, not high rubber concentration alone, is currently the principle determinant of rubber yield, particularly when coupled with a large rosette (Figures 6 and 12). The lack of correlation, in both temporal and high tunnel deep bed studies, between root, rosette, plant biomass and rubber concentration, means that large plants with above average rubber concentrations are present (Figures 4 and 11) albeit in relatively small numbers. Also, it suggests that these parameters are segregating independently of each other. Thus, while rubber concentration of the population was skewed towards lower concentrations, existence of high rubber concentrations in roots of some plants indicates that selection for higher rubber concentration should be possible in large plants. These results suggest that earlier studies on season and daylength (Borthwick et al. 1943) may not be pertinent to improve growth or rubber parameters of our population.

Yield per plant is a combination of root biomass and rubber concentration parameters. There was no negative correlation apparent between root, rosette, or plant biomass and root rubber concentration, or total rubber per plant. The highest yield per plant, in our study of unimproved plants, was 0.9 g rubber in a plant established from seed in autumn 2011, by direct seeding, and then harvested in early spring 2013, a longer growing season than could be readily added to a standard crop rotation. Nonetheless, 1 million plants per acre of this phenotype would yield 2160 kg/ha, grown under these conditions in northeast Ohio. This amount is already within the rubber yield/ha/year range of tropical rubber tree plantations (800-3000 kg/ha/year and very similar to the yield from the semi-arid lands rubber crop, guayule.

Table 1. A two-way analysis of variance of accession and heat treatment on *Taraxacum kok-saghyz* rubber content.

Response: Rubber Content	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Significance (P)
	10	445	444.7	1.32	0.25
Seed lot					
Heat treatment	1	143	142.9	0.43	0.52
Interaction	1	235	235	0.70	0.40
Residuals	235	79080	336.5		

Table 2. Two-way analysis of variance of accession and heating treatment on *Taraxacum kok-saghyz* root biomass.

Response: Root Growth	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Significance (P)
	10	24.7	24.7	1.96	0.16
Seed lot					
Heat treatment	1	188.1	188.12	14.89	0.00015
Interaction	1	0.3	0.33	0.026	0.87
Residuals	235	2968.7	12.63		

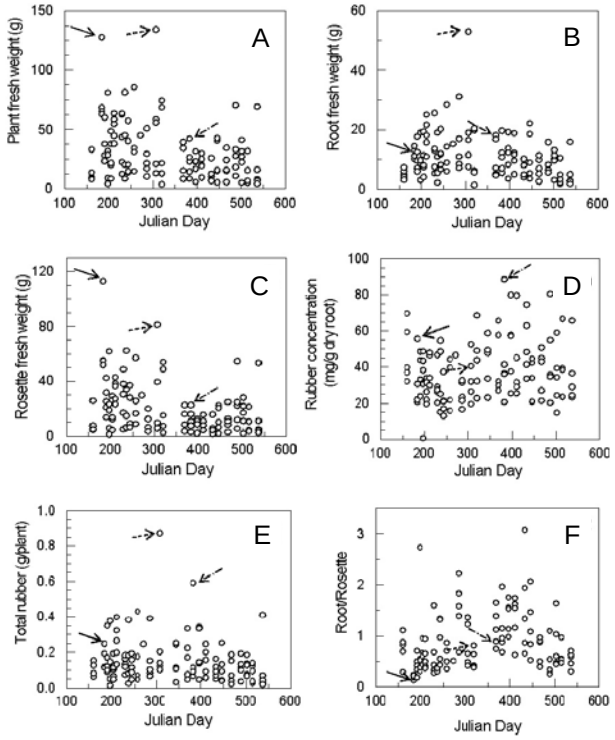

Figure 2. Phenotypic characteristics of *Taraxacum kok-saghyz* plants harvested from July 2012 to July 2013 from outdoor shallow beds. A. Plant fresh weight; B. Root fresh weight; C. Rosette fresh weight; D. Root rubber concentration on a dry weight basis; E. Total rubber per plant on a dry weight basis; F. Root:rosette ratio. Julian day #1 is 1 January, 2012. Julian day #366 is 1 January, 2013. The equinoxes during the time period shown, were September 22 (Julian day 264) December 21 (Julian day 356), March 20 (Julian day 446) and June 21 (Julian day 537). Three exceptional plants are indicated by plant-specific arrows on the different plots

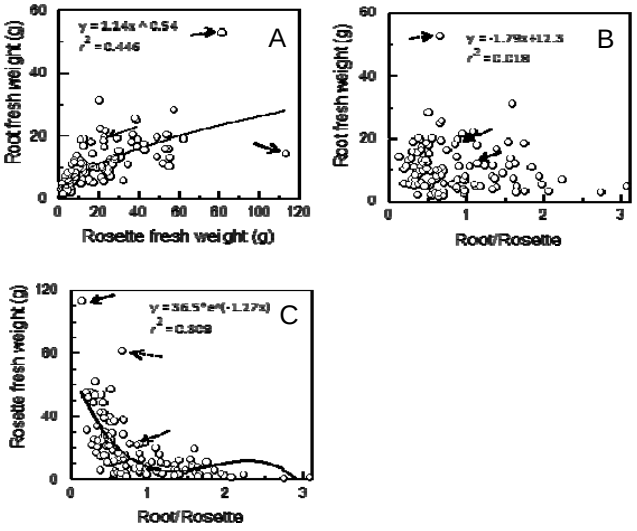

Figure 3. A. Relationship of root fresh weight and rosette fresh weight; B. Root fresh weight and the ratio of root and rosette fresh weight; C. Rosette fresh weight and the ratio of root and rosette fresh weight. *Taraxacum kok-saghyz* plants were harvested from July 2012 to July 2013, from outdoor shallow beds. The three outlier plants in Fig. 1 are indicated here by the same plant-specific arrows on the different plots.

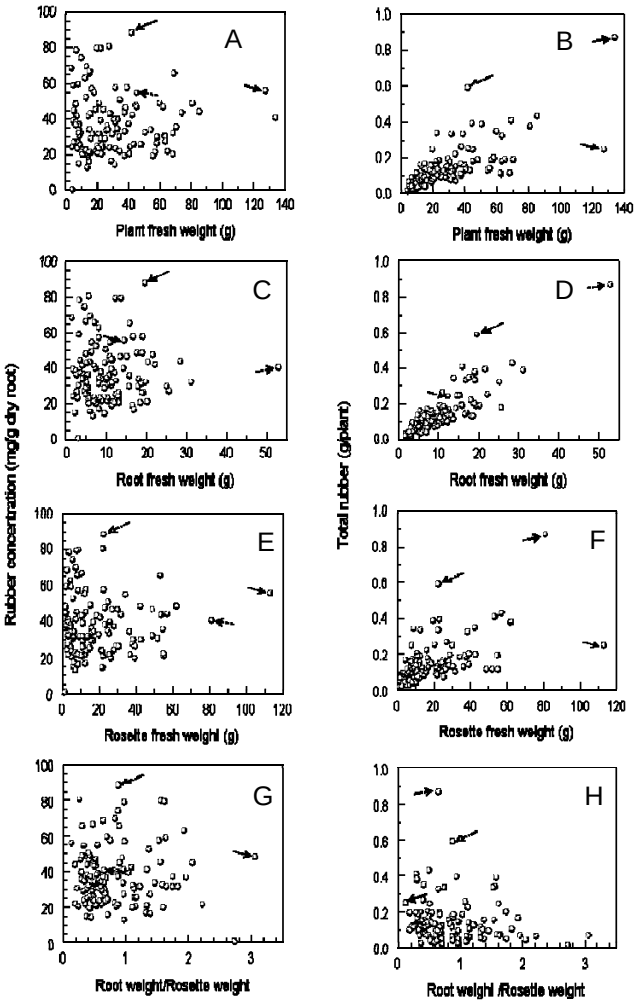

Figure 4. Relationship of root rubber concentration (A, C, E, G) and total rubber per plant (B, D, F, H) with plant fresh weight (A,B), root fresh weight (C, D), rosette fresh weight (E, F) and the ratio of root to rosette fresh weight (G, H) in *Taraxacum kok-saghyz* plants harvested between July 2012 to July 2013 from outdoor shallow beds. The three outlier plants in Fig. 2 are indicated here by the same plant-specific arrows on the different plots.

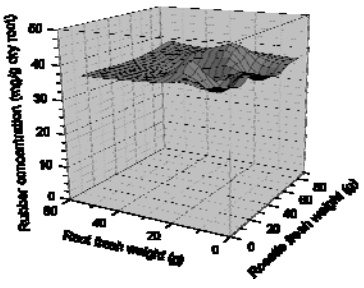

Figure 5. Surface 3D plot of root rubber concentration as a function of root and rosette fresh weights in *Taraxacum kok-saghyz*. The data were plotted as weighted averages

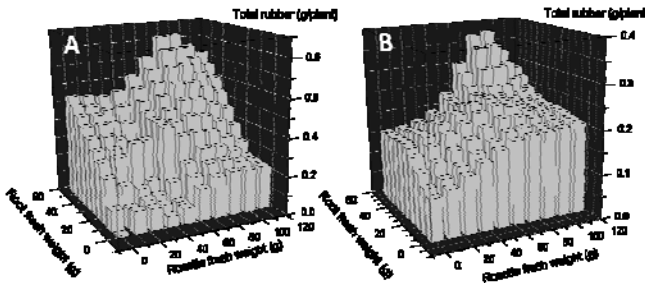

Figure 6. 3D plots of the relationship of total rubber in *Taraxacum kok-saghyz* as a function of root and rosette fresh weights by (A) linear interpolation (B) weighted average methods

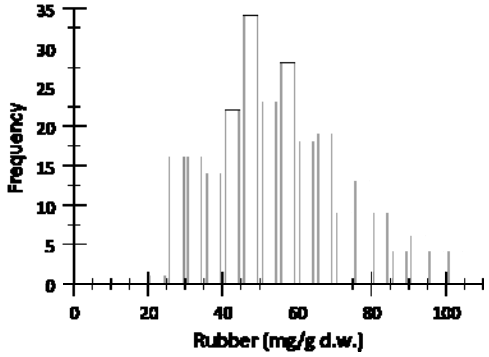

Figure 7. Distribution of rubber concentration across 10 mg intervals in 240 individual *Taraxacum kok-saghyz* root systems harvested from high tunnel raised beds during July, 2012. The plants were seeded in November, 2011

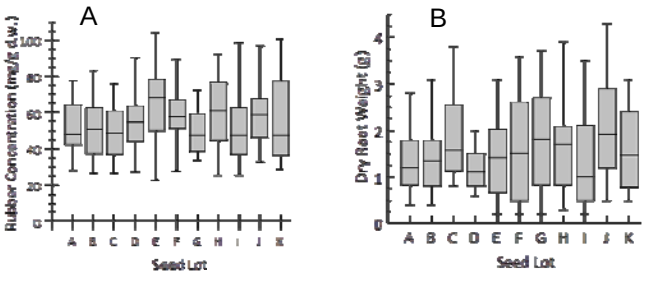

Figure 8. Box and whisker plots of (A) rubber concentration, and (B) root dry weight distribution, for the 11 *Taraxacum kok-saghyz* accessions planted from seed in November 2011 and harvested from high tunnel raised beds in July 2012. The solid boxes display the distribution of middle quartiles with the midline representing median concentrations. The extended lines display the full range representing the upper and lower quartile limits. Numbers of plants in each lot are: A-24, B-24, C-16, D-25, E-24, F-24, G-24, H-24, I-24, J-23, K-8.

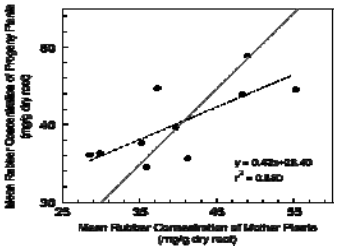

Figure 9. Correlation of rubber concentration in the progeny of 10 intrabred *Taraxacum kok-saghyz* accessions, with rubber concentration in the mother plants of the same 10 accessions. Plants were grown in high tunnel deep beds. Each progeny value is the mean of 24 plants. The dashed line is the linear regression plot. The solid line is the 1:1 ratio of progeny and maternal rubber which would occur if progeny had the same rubber concentration as their mothers

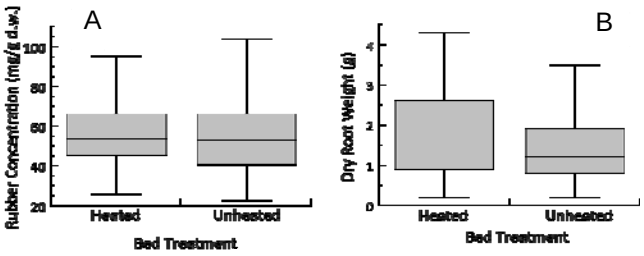

Figure 10. Box and whisker plots comparing (A) the distribution of root rubber concentrations and (B) root biomass, in 240 *Taraxacum kok-saghyz* plants of beds heated with subterranean cables from December to March, to those left untreated. The solid boxes display the distribution of middle quartiles with the midline representing median concentrations. The extended lines display the full range representing the upper and lower quartile limits

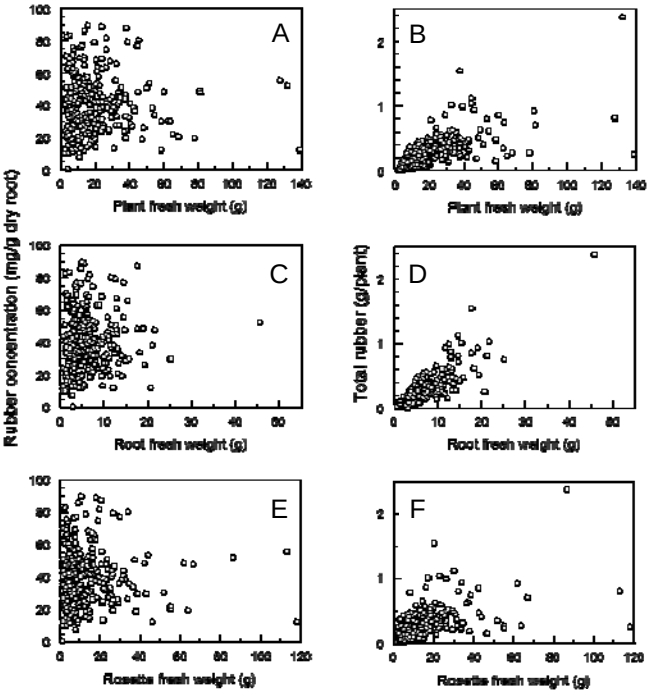

Figure 11. Relationships of root rubber concentration (A,C,E) and total rubber per plant (B,D,F) to plant fresh weight (A,B), root fresh weight (C,D) and rosette fresh weight (E,F) in 270 *Taraxacum kok-saghyz* plants grown in high tunnels and harvested in July 2012.

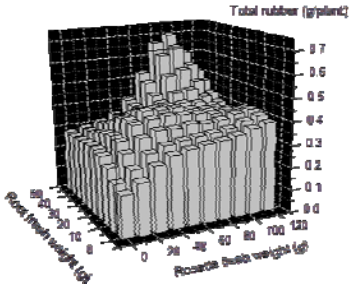

Figure 12. Relationship of rubber concentration to root and rosette fresh weights in 270 *Taraxacum kok-saghyz* plants grown in high tunnels in deep beds. Data are plotted as weighted averages

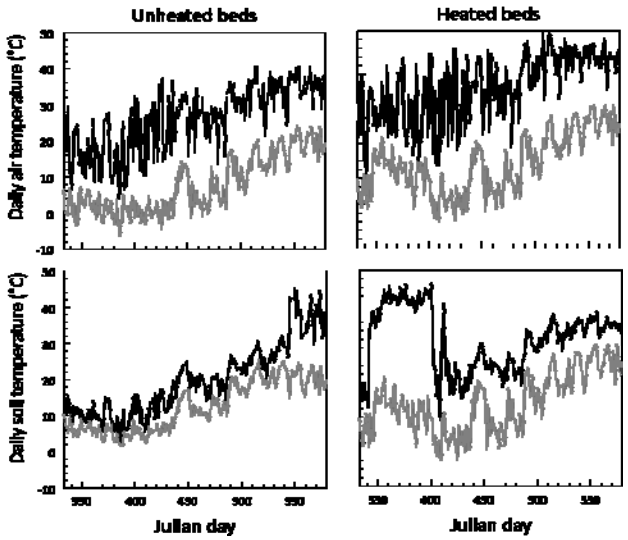

Figure 13. Maximum (black lines) and minimum (grey lines) daily air and soil temperatures in unheated and winter month-heated deep beds in high tunnels 31, from 29 November, 2011 to 31 July, 2012

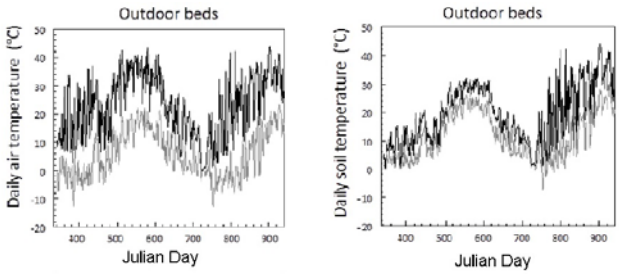

Figure 14. Maximum (black lines) and minimum (grey lines) daily air and soil temperatures in shallow outdoor beds from 29 November, 2011 to 31 July, 2013

The correlation between rubber concentration in parental accession and progeny (Figure 9), indicates some degree of heritability of rubber concentration. So interbred large high rubber plants may generate progeny with improved rubber yield. Our results indicate that the root system should be at least 30 g fresh weight and that these plants need to have a rosette fresh weight of at least 60 g to support high rubber production in roots (Figures 9 and 12). As plants are selected and bred for high biomass and high rubber concentration, it is possible that higher yielding plants may have different rosette and root biomass requirements to those described here. It is also possible that, because rubber is a metabolic end product, increasing rubber concentrations eventually may begin to inflict an unsustainable drain on plant resources and that these yields will plateau, as for other phenotypic traits in domesticated crops (Meredith 2000). Interestingly, rubber quantities above 300 mg rubber/g plant have been previously reported (Whaley and Bowen 1947). In our current study, highest rubber concentrations in these essentially wild plants were below 100 mg/g dry root, which would be less than 30 mg per average sized plant and less than 10% of the highest value reported. Nonetheless, since this study was performed, we have observed rubber concentrations in roots of up to 220 mg/g dry root in large field plants grown in Ohio.

Historically, TK has been tested as an annual crop and as an overwintered crop, and both of these can be considered with either a fall planting or a spring planting, and with direct seed or transplants as propagules. A high density direct seeded crop will be preferable for commercial viability due to the high cost of transplants. It has previously been shown that TK roots will more than double in biomass between spring and autumn (Cornish et al. 2013) in their first year of growth, but after the major flowering and seed set the next spring, field-grown plants were highly prone to summer dormancy, die back of rosettes, and root senescence after flowering, as was also described previously (Scarth et al. 1947).

There are many advantages to establishing TK as an annual production crop using an autumn planting scheme with a harvest the following autumn, especially with respect to maximizing growing days. Unfortunately, autumn-planted field plants, established in Wooster, Ohio with transplants or with direct seed, have not yet been carried through *en masse* into the spring because of the very high level of young plant death caused by frost-heaving. It is possible that a nurse crop will facilitate overwintering of young plants, but preliminary trials have not been encouraging in our region. Until overwintering of young plants is validated, a spring planting seems the only viable option, and this should be initiated as early as possible to maximize the growing season and to generate the large plants needed to produce a high rubber yield. Spring crop establishment is also needed for TK seed crops. Such plants are sufficiently large at the onset of winter that they can survive frost heaving that would otherwise kill younger plants. Overwintering allows these plants to vernalize and ensures good seed set in the following spring (Hodgson-Kratky et al. 2015).

Cold induction of rubber biosynthesis in TK has been reported (Cornish et al. 2013), and was observed in some plants in the outdoor shallow beds (Figure 2.D), but the threshold temperature for induction has not been established. Similar rubber concentrations in plant roots produced in high tunnel deep beds to those grown in

outdoor shallow beds, clearly indicate that cold temperatures are not required (Figures 13 and 14). This conclusion is supported by the high tunnel study, in which roots in heated and unheated beds produced very similar rubber concentrations. The high tunnel plants in heated beds did not become dormant like the plants in unheated beds (Figure 10) and so were able to grow more rubber-producing root tissue than the other plants. Since they maintained the same concentration of rubber as the roots grew, this resulted in a higher rubber yield per plant. This suggests that rubber concentration is genotype specific (a genotype is a genetically-unique individual or set of identical clones), is not inhibited by warm soil temperatures, and that the growing region for TK may extend to milder climates than those found in Ohio. However, variability in rubber concentration increased among the different plants harvested during the winter (Figure 2.D). This indicates that some plants were more responsive to cold induction during the winter than others (Figure 2.D). These data suggest that only some genotypes are capable of responding to cold by increasing rubber biosynthesis. Selecting these responsive genotypes may be key to establishing effective postharvest cold storage practices, which can be used to increase rubber content in stored fresh roots (Cornish et al. 2013). The presence of ambient and cold-inducible components of rubber production is similar to another species, *Parthenium argentatum* (guayule) which also has both features (Downes and Tonnet 1985; Madhavan et al. 1989; Cornish and Backhaus 2003).

In the outdoor shallow beds, rubber concentration increased during the winter, but roots did not become larger on average (Figure 2.B). Root weight per plant decreased during the winter and the resultant rubber yield per plant in the spring was essentially the same as in the previous autumn (Figure 2.E) indicating no overall yield benefit from the additional months in the outdoor beds. The additional months also pose a risk of plant death and yield loss in severe winters. In the severe "polar vortex" winter of 2013/14, in which temperatures dropped to -26°C, half of the plants grown outdoors (field and outdoor raised beds) died (unpublished results). A previous report described roots that were harvested in the autumn and then stored at 4°C in the dark after clipping off their rosettes. Root inulin reserves were catabolized during the next month, and new rubber biosynthesis then occurred, approximately doubling the rubber content of roots above 10g fresh weight in 45-60 days (Cornish et al. 2013). This method may prove to be a viable production practice for cold-responsive genotypes, especially as no roots are lost during storage.

In conclusion, clear initial selection targets have been identified - plants with large roots, large rosettes and high root rubber concentration. Even though rubber concentration and root biomass are not correlated, the relative abundance of target plants in the TK population suggests that utilization of small plants with high rubber concentration as parents and then recovering large biomass through backcrossing is unnecessary. These targets hold true independent of season for plants between 6 and 18 months of age. Rubber concentration does appear to be heritable and interbreeding selected target plants has considerable promise in rapidly converting this species into a domesticated crop.

ACKNOWLEDGEMENTS

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