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Abstract

Kazak Dandelion (*Taraxacum kok-saghyz*) (TKS) produces high quality natural rubber (NR), cis-1,4 polyisoprene, by biosynthesis, and has been used historically as a source of NR during times of short supply or high prices for Hevea NR. The rubber is primarily located in root tissues along with appreciable levels of inulin, a storage carbohydrate used in foods and as a source of carbon for biobased products. Along with other temperate NR producing crops, such as guayule, TKS can be used for domestic production of natural rubber and inulin as a source of biobased raw materials for replacement of petroleum-based polymers and fuels.

In order to facilitate development of TKS in the USA, a National Plant Germplasm System (NPGS)-sponsored collection expedition took place in 2008. Twenty accessions were collected and subsequently planted at the Western Regional Plant Introduction Station in Pullman, WA for evaluation. The objective of this study was to quantify the plant phenotypes of the new NPGS collection in terms of plant biomass, root morphology, and amount of rubber and inulin stored in the root tissue.

Kazak Dandelion roots were harvested in October 2010 from plants approximately 1.5 years old grown from original seed and root pieces and shipped to the Western Regional Research Center in Albany, CA for testing. Roots were air dried and stored at -20°C until processing. Roots were categorized as tap, lateral, or a combination. A break test was conducted to give a visual score of the quantity of rubber in the root. Fructan content was measured using Megazyme Fructan Assay Kit, AOAC Method 999.03 and AOAC Method 32.32. Rubber content was determined using Accelerated Solvent Extraction (ASE), with cyclohexane solvent at 100°C.

The NR content of the roots varied from 5-19% as determined by ASE. Comparison of the break test measurements with ASE results indicated the break test was capable of distinguishing high and low rubber producers. The inulin content varied from 13-46%. No correlation was found between NR or inulin content and root morphology nor whether plants were grown from seeds vs. roots pieces. Moreover, the amount of rubber stored in the root was independent of the inulin content. With additional data collected from 2011 and 2012 root harvests, which will help to better characterize the accessions phenotypes, the next steps include correlation of plant phenotype to genotypes, and development of molecular markers for breeding lines.

Taraxacum kok-saghyz Kazak Dandelion

- An annual Composite native to central Asian Tien-Shan mountains of Kazakhstan.
- Not considered invasive, but is essentially wild type.
- Produces and stores NR (Natural Rubber) in root tissues. Inulin, at high levels, is a valuable co-product.
- Planted in 41 US States during the Emergency Rubber Project. Used commercially for tire production during 1910s and 1940s.
- Renewed interest in TKS as an industrial crop in the USA.
- Inulin (Fructan) can be used as an alternative carbon source.
- TKS provides more biobased products, jobs and land development.

Below: A. Chelak, 2009



Objectives

- Characterize the phenotypes of new TKS lines in NPGS collection.
- Determine whether TKS samples varied based on those grown from roots vs. seeds.
- Explore possible relationship between plants' inulin content and amount of rubber produced.
- Identify high and low rubber-producing lines.

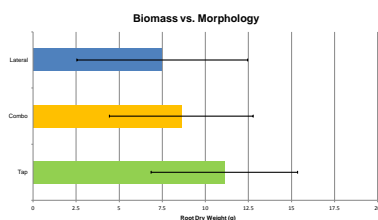
Plant materials and methods used

- Plants grown from root pieces collected in Kazakhstan. Seedlings from original seed were planted at the Western Regional Plant Introduction Station farm in Pullman, WA spring 2009.
- Plants for analysis were harvested Oct. 27th and Nov 2nd, 2010.
- Roots were washed and characterized. Data and images were collected on the plants. Roots then sent to the WRCR in Albany, CA.
- Roots were air dried; dry weight recorded prior to storing at -20°C.
- Roots categorized as either Tap, Lateral, or Combination.
- A break test was performed on dried plant roots.
- Rubber and inulin content were measured.

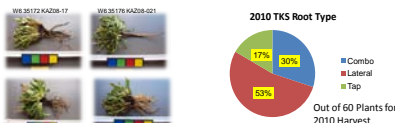
Root Characteristic Data

Root characteristic data for summary of Taraxacum kok-saghyz plants harvested in fall 2010										
ACP	ACNO	collection number	total # plants harvested	# plants from root pieces	root width (mm)					
					avg. total plant weight (g)	avg. tap root weight (g)	avg. # of lateral roots	avg. # of total roots	avg. total length (mm)	avg. total fresh weight (g)
W6	35156	KAZ08-001	6	1	67.0	22.5	7.0	9.2	26.3	25.7
W6	35159	KAZ08-004	1	0	98.6	23.0	7.0	10.0	22.5	33.7
W6	35160	KAZ08-005	3	1	37.0	21.3	4.0	10.0	23.8	9.7
W6	35162	KAZ08-007	1	0	63.7	19.0	10.0	22.1	9.9	20.8
W6	35164	KAZ08-009	2	2	302.4	43.5	7.0	17.0	27.2	77.1
W6	35165	KAZ08-010	4	0	56.4	22.5	6.5	9.8	21.6	17.4
W6	35166	KAZ08-011	5	1	96.2	28.4	5.8	13.0	30.7	21.8
W6	35168	KAZ08-013	5	1	196.3	31.0	6.4	17.6	27.0	55.5
W6	35169	KAZ08-014	5	4	109.0	30.2	7.0	13.0	27.8	40.5
W6	35170	KAZ08-015	3	0	121.6	26.7	5.7	12.0	28.7	35.3
W6	35172	KAZ08-017	3	1	131.9	30.7	7.0	13.7	29.7	37.0
W6	35173	KAZ08-018	5	0	92.3	28.6	5.2	8.8	22.2	28.5
W6	35176	KAZ08-021	2	0	82.7	24.0	7.0	9.5	18.5	18.9
W6	35177	KAZ08-022	3	0	100.7	21.7	5.7	8.3	24.0	22.2
W6	35178	KAZ08-023	2	0	77.6	21.0	8.5	8.0	28.5	31.5
W6	35179	KAZ08-024	3	1	129.9	28.0	6.3	16.7	28.3	36.4
W6	35180	KAZ08-025	1	0	72.4	20.0	6.0	21.0	16.0	16.8
W6	35181	KAZ08-026	3	0	138.0	21.7	6.0	14.3	25.0	35.5
W6	35182	KAZ08-027	3	0	86.7	20.0	5.7	11.3	28.3	21.5
W6	35183	KAZ08-028	3	0	104.1	22.7	7.7	15.3	28.0	30.6

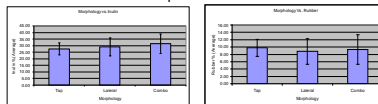
Root Biomass



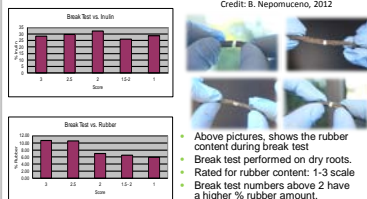
Root Type& NR/inulin production



- No relationship between root type and inulin or rubber produced was observed.



Break Test



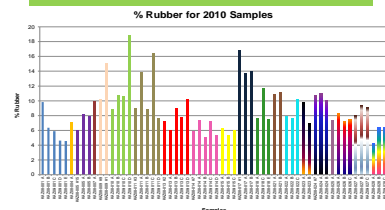
Credit: B. Nepomuceno, 2012

Testing for rubber content: ASE extractions

- Volunteer samples were initially tested to determine the best conditions to yield the highest % rubber.
- 250 mg ground root tissue per test. Samples tested in triplicate.
- Best conditions: cyclohexane solvent at 120 °C, with 9 cycles. (After the first 6, cells are broken down and refilled)



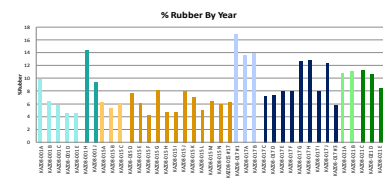
2010 Rubber Results



2010 Rubber Results Summary

- No significant differences between rubber content in plants from seed compared to plants from root pieces, due to the variability in rubber content seen across the entire plant harvest.
- Variability within a line observed.
- Plant tissues from W6 35156 KAZ08-001, W6 35170 KAZ08-015, W6 35172 KAZ08-017, W6 35176 KAZ08-021 in the July 2011 harvest were also tested for comparison.
- Average % rubber for all samples is 8.64.

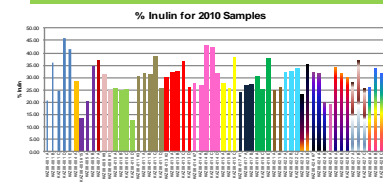
% Rubber October 2010 and July 2011 harvested roots



Testing for Inulin

- Roots were cryoground in Retsch ball mill and 100 mg of tissue was used.
- Samples tested in duplicate.
- Sucrose is hydrolysed to D-fructose and D-glucose using sucrose enzyme. Sugars reduced to sugar alcohols with treatment of alkaline borohydride. Excess borohydride removed with addition of dilute acetic acid. Fructan is hydrolysed to D-fructose and D-glucose with fructanase (exo-inulinase) and sugars are measured (410 nm) with PAHBAH

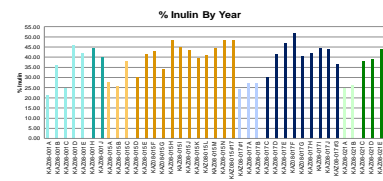
2010 Inulin Results



Inulin Test Summary

- Roots inulin content ranged from 13-33%, seeds inulin content ranged from 18-46%.
- Transplanted root tissues likely used stored sugars for growth. Plants harvested in Fall 2010 from seed are less likely to have used stored sugars.
- For all samples, average % of inulin is 29.7 %.
- Lines 1, 15 and 17, 21 from 2011 harvest tested for comparison

% Inulin October 2010 and July 2011 harvested roots



Summary

- The NPGS 2008 TKS Collection:
 - Rubber content: 5-19%
 - Inulin content: 13-46%
- Line comparison (2010 vs. 2011): For both harvests, line 15 lower in rubber; line 21 higher in rubber content. Lines 1 and 17 results mixed. % inulin generally higher in Summer (July 2011) harvest compared to Fall (October 2010).
- Testing continuing...
- High variability in root morphology observed.
- All plants confirmed diploid genotype.

Acknowledgements

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