

Rubber accumulation and rubber transferase activity during root development of *Taraxacum kok-saghyz* dandelion

Beenish Saba

saba.20@osu.edu

University of Nevada

Deborah J. Scott

USDA-ARS

Colleen McMahan

USDA-ARS

David Shintani

University of Nevada

Katrina Cornish

The Ohio State University

Research Article

Keywords: Natural rubber latex, dandelion, extraction, alternative rubber plant, rubber transferase

Posted Date: December 11th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-5132399/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Taraxacum kok-saghyz dandelion is a leading alternative natural rubber-producing plant that thrives in temperate climates. Latex, rubber and rubber transferase (RTase) activity of genetically identical clonal plants, produced in tissue culture, and then grown in the field for six months to one year was quantified throughout development. Induction of rubber transferase activity and rubber accumulation occurred between two and three months. RTase activity increased with age up to 16 weeks, while latex concentration increased up to week 20. When enzymatically active WRP were saturated with 5 mM [^{14}C]-isopentenyl pyrophosphate (IPP) and 12 μM farnesyl pyrophosphate (FPP), reaction velocity was 85% higher at 16 weeks than at 12 weeks of plant growth. The RTase FPP K_m was about 0.263 μM , through most of the growth period, in line with other laciferous species. High molecular weight rubber was produced in very young roots. 0.9 Mg/mol in only 6 weeks and 1.1 Mg/mol in 8 weeks.

1. Introduction

Synthetic alternatives to natural rubber have not replicated the physical properties of natural rubber, consequently it is considered a critical agricultural material throughout the globe. About 2,500 species make natural rubber but there are distinct differences among them with respect to composition, quantity and yield. Since the sole commercial source of natural rubber is *Hevea brasiliensis*, a tropical, clonal tree crop, understanding how plants make rubber and what biochemical factors affect rubber quality is important as many researchers attempt to biologically and geographical diversify the world's natural rubber supply [1–8].

One of the leading alternative natural rubber producing crops under development is *Taraxacum kok-saghyz* L. Rodin. *T. kok-saghyz* is native to the Tianshan Mountains, on the border between Kazakhstan and China [3, 9]. Natural rubber in *T. kok-saghyz* (TNR) is produced in the root laticifers. The quality of TNR produced in root laticifers is equivalent to *Hevea* rubber [10]. *T. kok-saghyz* is an excellent model system for the study of rubber biochemistry because it is an herbaceous plant with a rapid life cycle and can be grown hydroponically with a five-week root production cycle [11–12].

Rubber biosynthesis in all plants so far investigated, is catalyzed by a rubber transferase complex (RTase) integrally bound to the unilamella membrane of cytosolic, microscopic rubber particles [13]. The polymerization reaction is initiated by a single allylic pyrophosphate molecule, usually farnesyl pyrophosphate (FPP) *in vivo*, and then isopentenyl monomers, derived from isopentenyl pyrophosphate (IPP), are polymerized in a head-to-tail fashion by RTase. High quality rubber polymers commonly would have 15,000 or more monomers. Although many attempts to reconstitute RTase activity have been made, synthesis of high molecular *cis*-1,4-polyisoprene (rubber) has only been achieved in the presence of living rubber particles [13–15], except in one case with *Parthenium argentatum* (guayule), [16]. Thus, enzymatically active rubber particles have been the basis of all studies of rubber biosynthesis to date.

Studies of rubber particle-bound RTases from *H. brasiliensis*, *F. elastica*, and *P. argentatum* have shown that rubber molecular weight is significantly influenced by the ratio and specific concentrations of initiator FPP and monomer IPP. Increasing FPP concentration under limiting IPP concentrations reduces molecular weight, while increasing IPP concentrations under limiting FPP concentration has the opposite effect [17–22]. The concentration of the metal ion cofactor/activator also alters the molecular weight of the rubber produced by *H. brasiliensis*, *F. elastica*, *P. argentatum*, *L. sativa* and *T. kok-saghyz* [23–26]. Thus, the laticifer environment may play a significant molecular weight regulatory role. Of these species,

only the *P. argentatum* RTase, which makes rubber particles not in laticifers but in bark parenchyma cells, has the ability to independently regulate molecular weight [20, 27–28].

Unusually, *T. kok-saghyz* rubber particles coagulate over the lifetime of the plant and, at harvest, only 20–50% are in the form of individual particles that can be extracted as a latex emulsion [8, 29–30]. When extracted using a modification [31] of the *P. argentatum* latex extraction method, the latex (TNRL) proved to have excellent properties and was suitable for glove manufacture [8]. However, this coagulation behavior also means that biochemical studies are performed on a subset of uncoagulated rubber particles instead of a bulk mixture [26, 32]. In this study, the relationship of *T. kok-saghyz* RTase activity to plant age and TNR and TNRL concentration and yield was investigated with a goal of understanding how best to maximize yield and enhance the prospects of using *T. kok-saghyz* as a sustainable source of domestic rubber. Clonal plants were used to limit the genetic variation caused by the heterozygous nature of diploid *T. kok-saghyz*.

2. Materials and Methods

2.1. Tissue Culture and Plant Growth

Young leaves (2–4 inches) from existing *Taraxacum kok-saghyz* plants were harvested and surface sterilized by soaking for 10 min in 10% bleach and 0.1% Tween 20, followed by rinsing with distilled water (ddH₂O). The leaves were cut along the midrib and then into 1 cm pieces. These pieces were placed on shooting medium containing 1x Murashige-Skoog with vitamins, 2% sucrose, pH 5.7, 0.7% phytoagar, 0.2 mg/liter IAA, and 1 mg/liter BAP. The shoots were then transferred to a shoot elongation medium with 1x Murashige-Skoog with vitamins, 2% sucrose, pH 5.7, 0.7% phytoagar, 0.1 mg/liter IAA, and 0.5 mg/liter kinetin. The elongated shoots were placed on rooting medium with 0.5x Murashige-Skoog with vitamins, 2% sucrose, pH 5.7, 0.7% phytoagar, and 0.2 mg/liter IAA, with and without 0.5% charcoal [33]. Day zero was defined as the day the plantlets were transferred from the rooting medium into soil (Metro-mix 350). Plants were then grown for 55 weeks in a greenhouse environment. Plantings were sampled every 1–2 weeks until 22 weeks with a final sampling after 55 weeks.

2.2. Purification of Enzymatically Active Rubber Particles (see graphical abstract)

Plants were weighed, leaves were removed, and roots cut into 2.5 cm pieces. These root pieces were placed in a pre-chilled Hamilton blender with cold extraction buffer (100 mM Tris-HCl, pH 7.5, 5 mM MgSO₄, 1% ascorbic acid, 0.1 mM Pefabloc, 1 mM PMSF, 0.5% Antifoam A, 5 mM beta-mercaptoethanol) at a ratio of 1 ml extraction buffer to 1 g root weight. The roots were ground on high for 1 minute. The fluid mash filtered through four layers of cheesecloth into a beaker in wet ice. The cheesecloth was tightly squeezed to remove as much fluid as possible.

Pre-chilled glycerol was added to the homogenate to a final concentration of 20%. The homogenate with 20% glycerol was transferred to pre-chilled 250 ml centrifuge bottles, keeping the volume below the neck of the bottle. Using an RC-5B Sorvall centrifuge with an HS-4 swinging bucket rotor, the mixture was spun at 4°C and 2,000 x g for 10 min. The floated latex was scooped into a pre-chilled beaker with 1 ml of wash buffer (100 mM Tris-HCl, pH 7.5, 5 mM MgSO₄, 0.1 mM Pefabloc, 5 mM DTT). The spinning was continued at 4°C and 3,900 x g for 10 min. The latex was collected again and spun at 4°C and 6,900 x g for 10 min before collecting once more. All samples of floated latex were collected into the same beaker.

The partially purified latex was resuspended in half the volume of wash buffer with 20% glycerol. The mixture was spun at 1,000 x g for 10 min, and the floated latex layer was collected into a chilled beaker with a small amount of wash buffer. It was then spun at 2,000 x g for 15 min, and the latex was collected again. The process was repeated at 3,000 x g, and the latex was collected. All samples of floated latex were collected into the same beaker [33].

The collected latex was resuspended again in 50 ml cold wash buffer with 20% glycerol. The spinning steps were repeated as above, and the latex was collected in a chilled beaker without additional buffer, generating a final sample of purified (washed) rubber particles (WRP). To quantify the purified rubber particles, 25–50 μ l of the cream was pipetted onto pre-weighed filters and dried overnight. Samples of buffer were also dried and weighed, and weights were subtracted from WRP weights to determine WRP concentration. The volume of the remaining cream was measured. Enzymatically active WRP droplets dripped from a Pasteur pipette were frozen in liquid nitrogen as beads, placed in cryovials, and stored in liquid nitrogen dewars as described [34].

2.3. Particle Size by Electron Microscopy

A bead of rubber particles in a microfuge tube was fixed for 2 h by pipetting 2.5% glutaraldehyde/2% formaldehyde at pH 8.0 with sodium cacodylate buffer (adjusted with concentrated NH_4OH) directly into the tube. The preparation was rinsed for 20 mins in the same buffer three times. Fixed particles were post-fixed in 1% aqueous osmium tetroxide for 1 h and then rinsed three times, 20 min/rinse. The preparation was centrifuged between each step to pellet the particles and then the particles were resuspended in fresh reagent.

The fixed rubber particles were collected as follows. Polycarbonate Filters (0.45 μ m pore Whatman Nuclepore PC filters) were dipped in 0.1% w/v Sigma Poly-L-Lysine so that rubber particles would stick to the filter. Filters were touched to a Kimwipe to remove excess buffer and then placed into individual 13 mm plastic filter holders (Gelman Sciences, Ann Arbor, MI, Product 4317). Rubber particles were sucked into a 1 ml disposable syringe, the filter holder was placed onto the syringe, and the particles were filtered to remove residual solution. Filters with entrained rubber particles were placed sample side up "sandwich wise" in metal holders using mesh and "O" rings to keep the filters separated. The samples were then dehydrated as a unit in a graded series of ethanol (30, 50, 70, 95, 100 x3), remaining at 20 min per concentration. The unit of filters was then critical point dried in a Tousimis Autosamdri-815 critical point dryer (Tousimis, Rockville, MD). The filters were removed from the metal holder and each filter was individually mounted onto aluminum specimen holders using double-coated Carbon tabs (Pelco, Redding, CA). The mounted filters were sputter coated with gold palladium in a Denton Desk II sputter coating unit. (Denton Vacuum, Inc., Moorestown, NJ). Samples were viewed and photographed in a S-4700 Hitachi Scanning Electron Microscope. Particle diameters were measured using the line measurement tool in Optimas 6.1 (Media Cybernetics, Silver Spring, MD) software and exported data to Excel.

2.4. Molecular Weight of Rubber

Samples of WRP were dried and stored in 1.5 ml Eppendorf tubes overlaid with N_2 at -80°C . Three samples of approximately 5 mg of rubber were dissolved in 3 ml of 0.2 micron-filtered tetrahydrofuran (THF) in 8 ml borosilicated vials with Teflon coated lids overnight. Next day, all sample solutions were filtered through 1.6-micron GF/A w/GMF Whatman filters into 12 x 32 mm clear borosilicate screw-cap vials with PTFE septa. An HPLC (HP 1100 series) with two downstream connected connectors was used under the following conditions. The first detector was a DAWN DSP Laser Photometer (Wyatt

Technologies). The second detector was an HP 1047 Refractive Index Detector. The stainless-steel tubing connecting the flow cell of the DAWN to the RI had a delay volume of 0.1833 ml. Sample vials were loaded onto the autosampler tray. The first vial was always THF (to check that the column was clean, and to locate the THF peak). 50 µl samples were injected and run through a Phenogel 5 micron Linear/Mixed Guard Column (Phenomenex) and two PL gel 10 micron mixed-B exclusion columns connected in series. Flow rate was 1 ml/min, column temperature was 35 °C and detection wavelength was 254 nm.

Chromatograms were analyzed using Astra version 4.73 (Wyatt Technologies). Analyses included the number average molecular weight (Mn), weight average molecular weight (Mw), and polydispersity (Mw/Mn).

2.5. Rubber and Resin Quantification

The resin and rubber content of freeze-dried roots of different ages was determined by sequential acetone and hexane extraction using an ASE-200 Dionex with Solvent Controller Balance Mettler Toledo AX105 Delta Range as described [35].

2.6. Rubber Transferase Activity of Rubber Particles

Rubber particles were assayed for RTase activity as previously described [33]. Routinely, 0.25 mg to 1 mg of rubber particles were assayed using 100 mM Tris-HCl, pH 7.5, 5 mM MgSO₄, 5 mM DTT. Various amounts of IPP and FPP were added. Rubber particle concentrations were determined by hemacytometry. Rubber per particle was determined by dividing the dry weight of a known volume of latex by the rubber particle concentration.

3. Results

3.1. Latex and Rubber Accumulation in Roots

Fresh root weight as % of plant fresh weight progressively increased during plant development, reaching 60% at 20 weeks (Fig. 1A). Latex was not measurable until soil-grown plants were six weeks old. Latex accumulation was rapid between 6 and 12 weeks and then slowed (Fig. 1A) reaching a maximum concentration at 18 weeks. Total rubber (Fig. 1B) and latex (Fig. 1C) per root increased from throughout development until at least 18 weeks. However, latex content per root and rubber per particle slightly declined between 19 and 20 weeks (Fig. 1C and D).

3.2. Analyte Accumulation Roots and WRPs

In a second series of plants, root growth increased until 10 weeks but, unlike the first series (Fig. 1B), then fluctuated greatly throughout the remaining time, indicate two cycles of root loss and regrowth although it is unclear if the loss was caused by herbivory (roots taste sweet because of their inulin content), disease or edaphic factors (Fig. 2). Rubber could be quantified in 3-week-old roots, considerably earlier than latex (Fig. 1C). Rubber accumulated throughout development as before (Fig. 1C, D) and 20-week-old roots had the highest rubber concentration (Fig. 2) as seen previously (Collins-Silva et al. 2012). In contrast, resin levels remained fairly constant with no clear trends apparent (Fig. 2). The amount of rubber extracted with hexane was more than double the amount of resin extracted with acetone (Fig. 2) across the entire 22-week growth period.

1.3. Characteristics of WRPs

Particle diameter, determined by SEM, ranged from 1.04 to 1.20 microns, with no significant changes observed during the 55 weeks of the longest growth period. However, the concentration of particles in purified latex appeared to increase up to 10 weeks and then generally declined (Fig. 3). Particle dry weight fluctuated throughout as did protein concentration with no clear trends apparent. Rubber molecular weights of the dried latex fraction surpassed 1 Mg/mol by 8 weeks and remained above this value throughout (Fig. 4). Rubber polydispersity was below 1.2 at all developmental stages tested (Fig. 4).

3.4. Rubber Transferase Activity

The velocity of RTase activity (incorporation of ^{14}C IPP amol/particle/hour) was assayed in WRP purified from roots grown for 8, 12, 16, 18 and 22 weeks, in the presence of 5 mM IPP (a saturating concentration) and varied concentrations of FPP (Fig. 5), all of which appeared to saturate the reaction. RTase activity at week 16 was more than 85% higher than at week 12 in 7.5 μM FPP and saturation was achieved at 12 μM . 30 μM FPP was inhibitory across the time course. Unexpectedly, IPP incorporation occurred in WRP isolated at all time points in the absence of additional FPP initiator molecules. The RTase FPP K_m was estimated as 0.263 using enzyme kinetics. The FPP K_m was maximum at 10 μM FPP and the ratio of V/V_{max} was 20 at week 8 (Fig. 6).

4. Discussion

Accurate measurement of RTase activity requires the initial purification of enzymatically active rubber particles from the plants. In young plants, these particles are initially scarce, making it impractical to obtain them using current purification techniques. Therefore, solvent extraction of the rubber remains the only viable option during the early stages of biosynthesis to accurately quantify rubber, but enzyme activity is, of course, lost.

Since the particle diameter in the purified latex was quite constant at 1.145 ± 0.120 and only particles with specific gravities below 1 were purified, it is unclear why the particle concentration, when purified in latex form, declined with plant age (Fig. 3). This is not due to particle coagulation *in vivo* because only uncoagulated particles were purified.

RTase is associated with rubber particles, and particle characteristics directly impact enzymatic activity [36–37]. RTase activity depends on the plant's age, increasing out to week 16 (Fig. 5) correlating with root growth and rubber accumulation but is not simply caused by changes in rubber particle number. The FPP K_m changed during root development with the greatest affinity in the youngest roots (Table 1, Fig. 6). Thus, as the roots mature, RTase complexes on older endogenous rubber particles appear to become less efficient in substrate binding rather than undergoing an instant transition from active to inactive. The range of FPP K_m is similar to that of the RTases of *H. brasiliensis* and *F. elastica* whereas the *P. argentatum* FPP K_m is much smaller [18]. This suggests that the *T. kok-saghyz* RTase is similar to those in other laticiferous species.

Table 1
Table 3. Kinetic Parameters of Rubber Transferase during root development

Age	K_m^{FPP}		V_{max}	
(weeks)	(nM)		(amol/particle/hour)	
		s.e.		s.e.
8	56.50	41.30	1.36	0.08
12	263.20	84.90	0.42	0.05
16	234.20	96.50	3.60	0.20
20	145.20	55.90	2.58	0.10
22	182.00	101.80	1.20	0.07

The rubber made in *T. kok-saghyz* roots was consistently high molecular weight and of a very low polydispersity, indicating that very uniform rubber was synthesized through development. Rubber molecular weights were consistently above 1 Mg/mol after 8 weeks and was already 0.9 Mg/mol in 6-week-old roots (Fig. 4). The ability to produce high molecular weight rubber in only six weeks of growth is unusual but could be of great value if this proves also to be the case in hydroponically-grown roots. In tissue cultured *P. argentatum*, eight-week-old plants synthesize rubber of 500–700 kg/mol while six-month-old greenhouse grown *P. argentatum* plants produce high molecular weight rubber [38]. However, in the field, neither *P. argentatum* nor *H. brasiliensis* achieves ≥ 1 Mg/mol polymer sizes until they are one or six years old, respectively [39–40].

Conclusion

The RTase FPP K_m in rubber dandelions aligns with that of other latex-producing species, and rubber molecular weight is above 1 Mg/mol suggesting that the quality of dandelion rubber is comparable to that of other rubber-producing plants and is achieved in very young tissues (6-week-old roots). Since the hydroponically grown dandelion roots can be harvested at this age, our results suggest that high molecular weight rubber could be harvested from such systems.

Declarations

Acknowledgements:

We acknowledge technical assistance from Delilah Wood, Jennifer Brichta, Jennifer Van Fleet, Benny Knuckles and Upul Hathwaik. This work was supported by funding from The National Science Foundation Plant Genome Research Program (DBI-03211690).

Conflict of Interest Statement

There is no conflict of interest between authors and funding agency.

Data Availability Statement

The data supporting the findings of this study are not publicly available but can be obtained from the corresponding author upon reasonable request.

Plant Ethics Statement

All plant material was legally obtained from the US Plant Germplasm Collection with all necessary permissions

Author Contribution

Dr. Saba wrote the manuscript and analyzed the data. Prof. Latrina Cornish Supervised the project and obtained funding along with collaborators Colleen McMahan, Deborah J. Scott, and David Shintani. All authors have equal contribution in the experiments and manuscript writing.

References

1. Pearson CH. Cornish K. McMahan CM. Rath DJ. Brichta JL. Van Fleet JE. *Ind. Crops Prod.* 2010; 31: 481–491.
2. Munt O. Aria M. Hernandez M. Ritter E. Gronover CS. Prufer D. *Ind. Crops Prod.* 2012; 36: 289–293.
3. Cornish K (2019) Rubber dandelion. *Natural History*. <https://cornishlab.cfaes.ohio-state.edu/sites/hcs-cornishlab/files/imce/Natural%20History%20Digital%20Copy.pdf>. [Accessed July 22, 2024].
4. Boon ZH. Teo YY. Ang DTC. *RSC Advances*. 2022;12: 34028–34052.
5. Piccolella S. Sirignano C. Pacifico S. Fantini E. Daddiego L. Facella P. Lopez L. Scafati OT. Panara F. Rigano D. *Ind Crops Prod.* 2023; 195: 116446.
6. Wang Y. Liao L. Wang R. Yu H. Zheng T. Lian Y. Luo M. Liao S. Liu H. Peng Z. *Ind. Crops Prod.* 2023; 202, 117070.
7. Puskas JE. Cornish K. Kenzhe-Karim B. Mutalkhanov M. Kaszas G. Molnar K. *Heliyon*. 2024; 10: E25213.
8. King-Smith NP. Davis SA. Cornish K. *Rubber Chemistry and Technology*, 2024; Submitted 2024.
9. Xie Q. Ma J. Ding G. Yuan B. Wang Y. He L. Han Y. Cao A. Li R. Zhang W. Li H. Zhao D. Wang X. *Ind Crop Prod.* 2022;181: 114776
10. Ramirez-Cadavid DA. Valles-Ramirez S. Cornish K. Michel Jr FC. *Ind Crop Prod.* 2018; 122: 647–656.
11. Benzle K. Cornish K. *Plant Methods*. 2017; 13: 37.
12. Dong G. Wang H. Qi J. Leng Y. Huang J. Zhang H. Yan J. *Gene*. 2023; 867: 147346.
13. Park JC. Ling T. Kim MY. Bae SW. Ryu SB. *Ind Crops Prod* 2024; 213: 117714.
14. Cornish K. Xie W. Natural rubber biosynthesis in plants: Rubber transferase, in: Hopwood, D.A. (Eds.), *Methods in Enzymology*. Elsevier, Netherlands; 2012.
15. Cherian S. Ryu SB. Cornish K. *Plant Biotechnol J.* 2019; 17: 2041–2061.
16. Benedict CR. Madhavan S. Greenblatt GA. Venkatachalam KV. Foster MA. *Plant Physiol.* 1990; 92: 816–21.
17. Cornish K. Castillón J. Scott D. *BioMacromolecules*. 2000; 1: 632–641.
18. Cornish K. *Natural Products Report*. 2001a;18: 182–189.
19. Cornish K. *Phytochemistry*. 2001b; 57: 1123–1134.
20. Cornish K. Scott DJ. *Ind. Crop Prod.* 2005; 22: 49–58.
21. Da Costa BMT. Keasling JD. Cornish K. *Biomacromolecules*. 2005; 6: 279–289.
22. Da Costa BMT. Keasling JD. McMahan CM. Cornish K. *Phytochemistry*. 2006; 67: 1621–1628.

23. Scott DJ. da Costa BMT. Espy SC. Keasling JD. Cornish K. *Phytochemistry*. 2003; 64: 121–132.
24. Bushman BS. Scholte AA. Cornish K. Scott DJ. Brichta JL. Vederas JC. Ochoa, O. et al. *Phytochemistry*. 2006; 67: 2590–2596.
25. Cornish K. Blakeslee JJ. *The Lipid Library*. 2011.
26. Collins-Silva J. Nural AS. Skaggs A. Scott DJ. Hathwai U. Woolsey R. Schegg K et al. *Phytochemistry*. 2012; 79: 46–56.
27. Lakusta A. Kwon M. Kwon EJG. Stonebloom S. Scheller H. Ro DK. *Front. Plant Sci*. 2019; 10: 165.
28. Dong C. Ponciano G. Huo N. Gu Y. Ilut D. McMahan C. *Scientific Reports*. 2021; 11: 21610.
29. Salehi M. Bahmankar M. Naghavi MR. Cornish K. *Ind Crops Prod*. 2022; 178: 114562.
30. Abdul Ghaffar MA. Cornish K. *Rubber Chem Technol*. 2024; 97: 133–144.
31. King-Smith N. Molnar K. Blakeslee JJ. McMahan CM. Pillai AS. Mutalkhanov M. Puskas JE. Cornish K. *Ind. Crops Prod*. 2023; 206, 117698.
32. Laibach N. Schmidl S. Muller B. Bergmann M. Prufer D. Gronover CS. *Plant J*. 2018; 93: 1045–1061.
33. Cornish K. Backhaus RA. *Ind Crops Prod*. 2003; 17: 83–92.
34. Cornish, K. Bartlett D. L. *Phytochemistry*. 1997; 8: 130–134.
35. Cornish K. Schloman Jr WW. *Encyclopedia of polymer Science and Technology*. 2002
<https://onlinelibrary.wiley.com/page/book/10.1002/0471440264/homepage/editorscontributors.html>.
36. Cornish K. Backhaus RA. *Phytochemistry*. 1990; 29: 3809–3813.
37. Epping J. Deenen N. Niephauss E. Stolze A. Fricke J. Huber C. Eisenreich W. Twyman RM. Prufer D. Gronover CS. *Nature Plants*. 2015; 1: 15048.
38. Placido DF. Dong N. Dong C. Cruz VMV. Dierig DA. Cahoom RE. Kang BG. Huynh T. Whalen M. Ponciano G. McMahan C. *Front. Plant Sci*. 2019;10. <https://doi.org/10.3389/fpls.2019.00760>.
39. Kovuttikulrangsie S. Sakdapipanich JT.. *J Sci Technol*. 2005; 27: 337–342
40. McMahan CM. Cornish K. Coffelt TA. Nakayama FS. McCoy RG. Brichta JL. Ray D T. *Ind Crops Prod*. 2006; 24: 321–328.

Figures

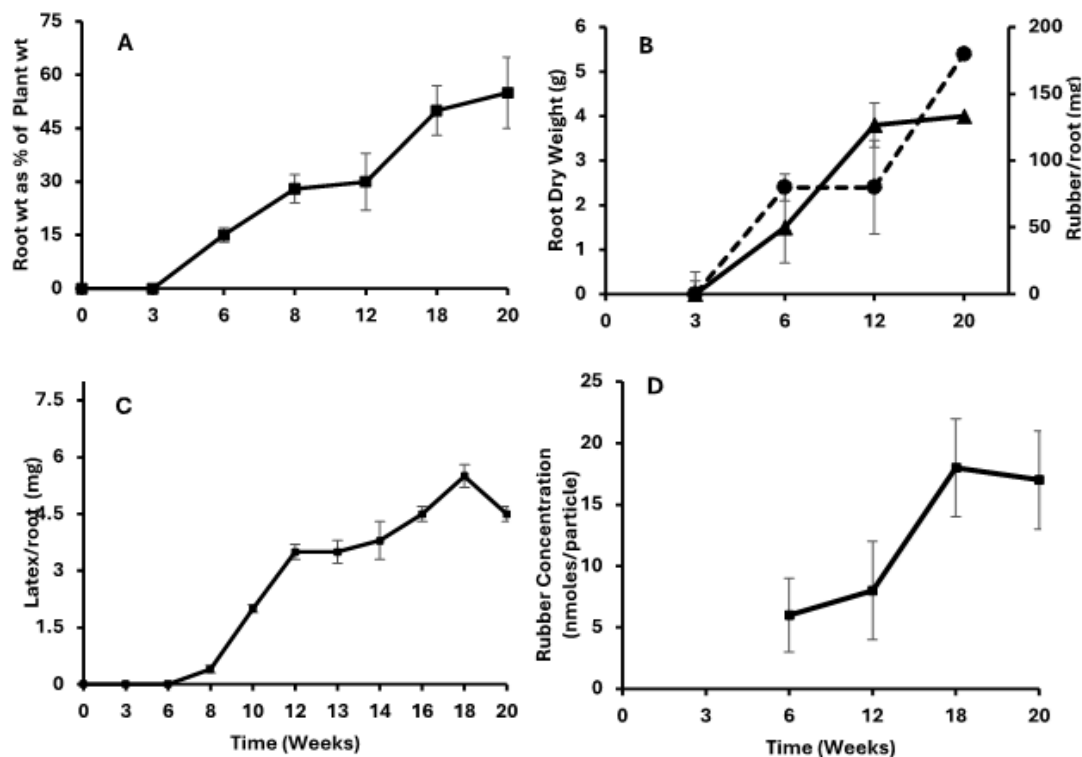


Figure 1: Rubber accumulation in plants. A) Root weight as % of plant weight, B) Weight of roots (—) and Weight of Rubber (---), C) % of plant weight as roots, D) Rubber concentration per particle

* Dots are content determined as average of at least three mls for 20 to 40 plants harvested at certain time points, error bars are standard error.

Figure 1

See image above for figure legend.

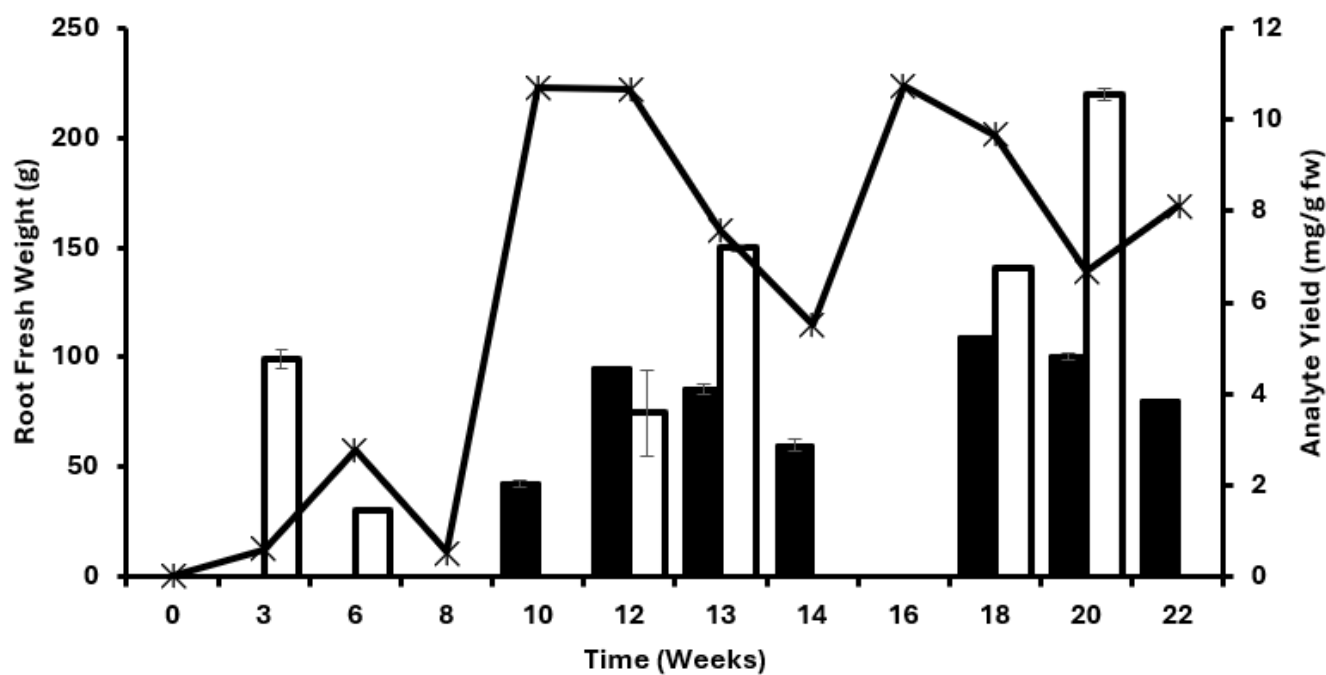


Figure 2

Root fresh weight and analyte concentration in *Taraxacum kok-saghyz* roots: resin, black bars; rubber, white bars; and root weight, black line with crosses.

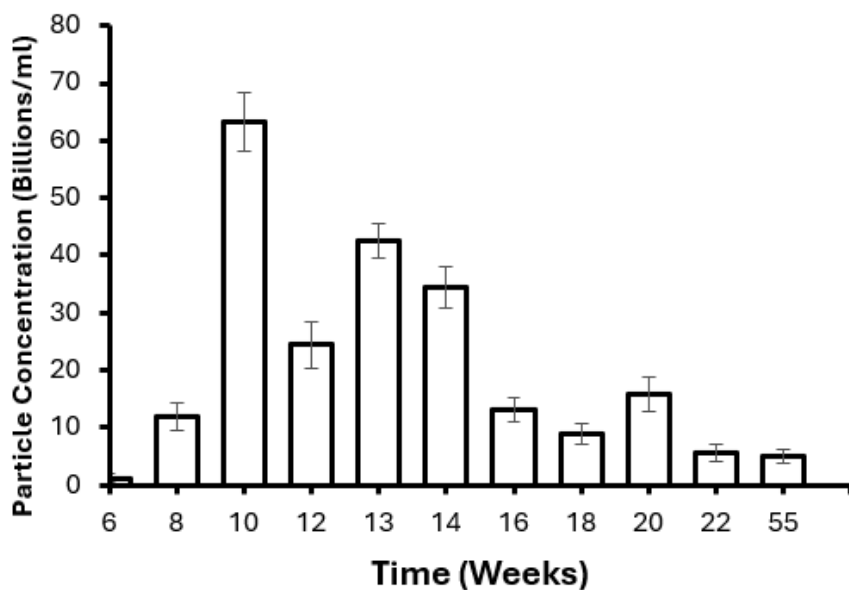


Figure 3

Concentration of rubber particles in purified latex.

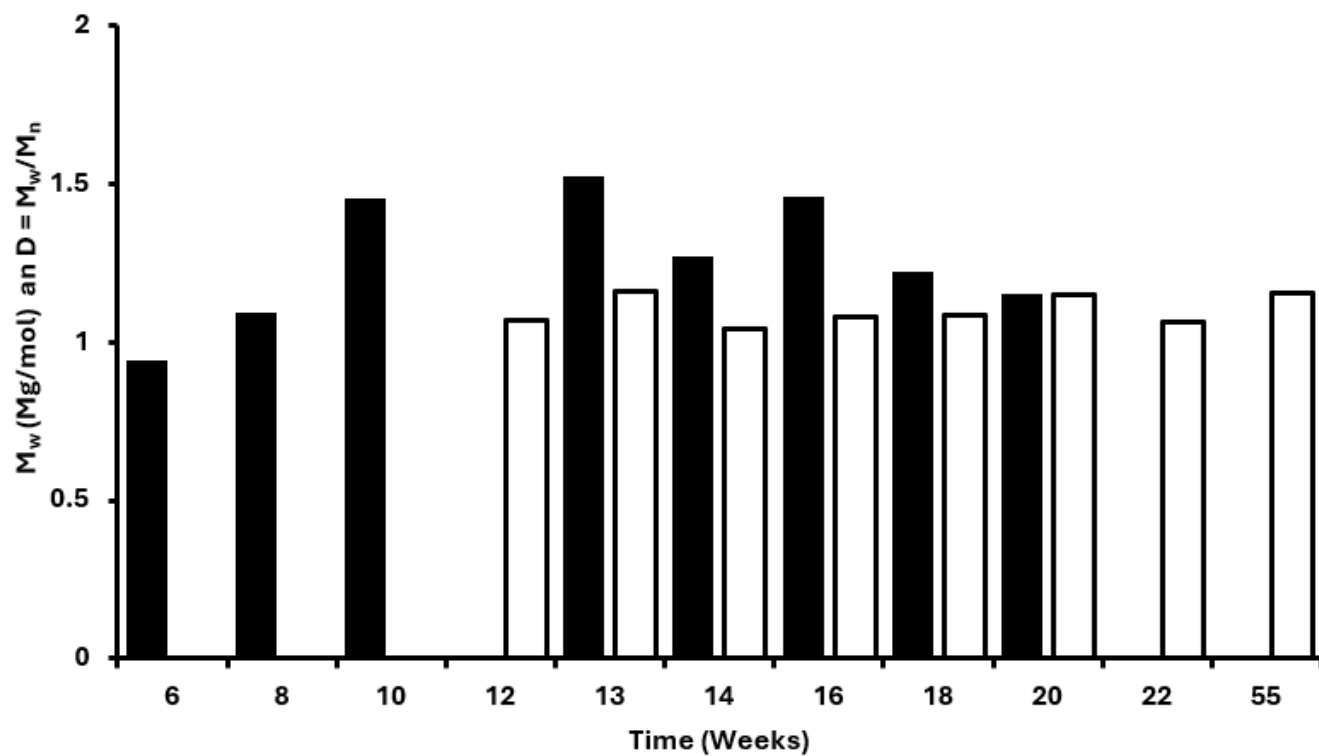


Figure 4

Molecular characteristics of latex rubber: A) Molecular weight (M_w), solid bars; B) Polydispersity ($D = M_w/M_n$) empty bars.

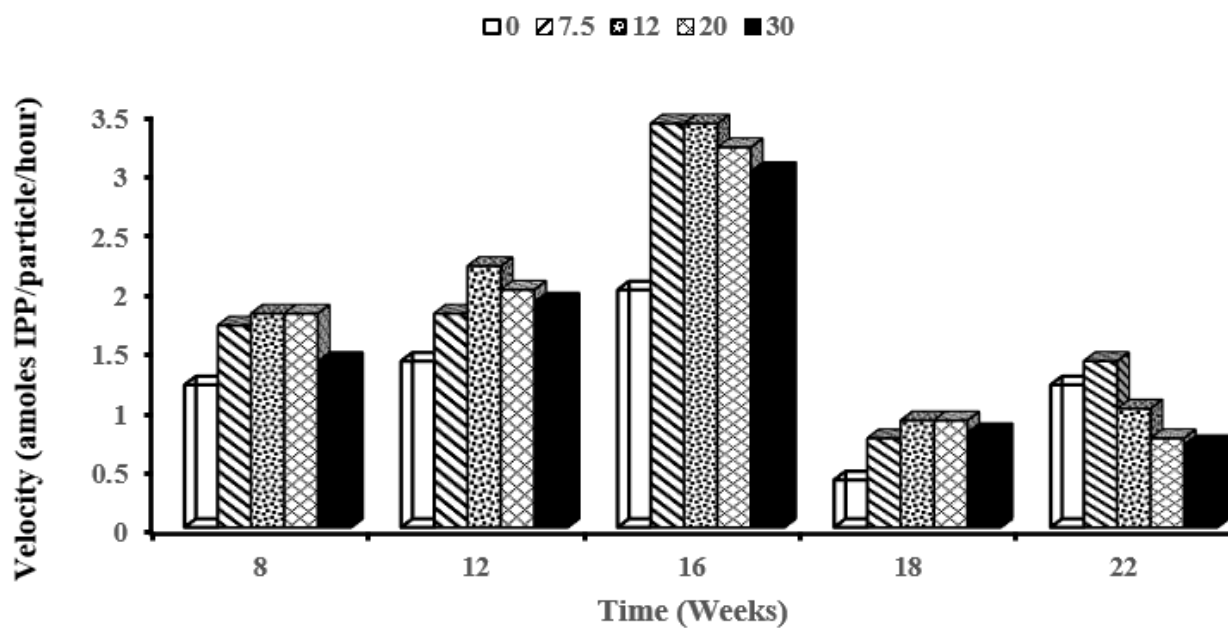


Figure 5

The velocity of rubber transferase activity (represented as incorporation of ^{14}C IPP amol/particle/hour) in WRP collected from *Taraxacum kok-saghyz* plants at various time points in the presence of 5 mM IPP and 0, 7.5, 12, 20 and 30 [FPP] (mM)

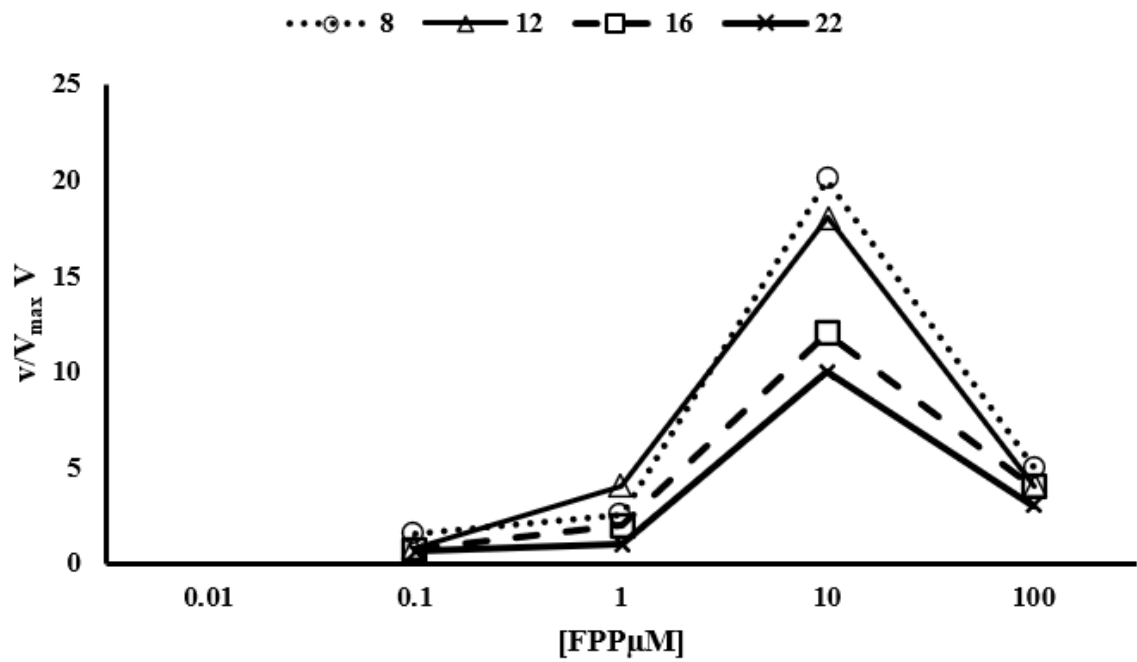


Figure 6

Hill plot of RTase activity in rubber particles harvested from roots of different age (8-22 weeks old). The K_m^{FPP} is indicated by the FPP concentration where the curves intercept $y = 1$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GA.png](#)