Polyploidy induced by colchicine in *Taraxacum kok-saghyz* and its effects on morphological and biochemical traits

Zinan Luo, Brian J. Iaffaldano, Katrina Cornish*

Contact Information

Zinan Luo, luo.356@osu.edu, The Ohio State University, Department of Horticulture and Crop Science, Wooster OH, 44691, USA

Brian J. Iaffaldano, iaffaldano.1@osu.edu, The Ohio State University, Department of Horticulture and Crop Science, Wooster OH, 44691, USA

Katrina Cornish*, cornish.19@osu.edu, The Ohio State University, Department of Horticulture and Crop Science, Wooster OH, 44691, USA

Abstract

Taraxacum kok-saghyz (TK), rubber dandelion, also known as Russian dandelion, is under development as a source of natural rubber but has not been fully domesticated; rubber yield is highly variable, and biomass is low compared to most crops. One strategy to accelerate breeding is polyploid induction, which could improve rubber concentration and plant size. Colchicine was used to treat seeds and induce tetraploid germinants. Flow cytometry was used to identify induced tetraploids. The optimal induction rate (formula) of 4.92% was obtained after 0.2% colchicine for 24h, followed by 0.1% for 48h with an induction rate of 3.77%. However, 0.1% colchicine for 48h resulted in a higher induction efficiency (formula) (56.6%) than in 0.2% for 24h (46.45%). An early-screening strategy successfully identified tetraploid 2-month old seedlings based on atypical leaf morphology. Comparisons were made between tetraploids and diploids in terms of leaf, stomata, root morphological traits as well as biochemical traits including rubber, resin and inulin/sugars content. An increase in rubber concentration but a decrease in inulin/sugars content was observed in tetraploid plants. However, there was no significant difference in the amount of rubber per root system among the greenhouse-grown plants in this research due to root stunting observed in tetraploids and treated diploids. Evaluation of progeny is needed to circumvent the confounding physiological impact of colchicine treatment.

Key words: tetraploids, *Taraxacum kok-saghyz*, flow cytometry, ASE, rubber content, inulin/sugars content.

1 Introduction

Natural rubber is a critical resource that is commercially produced by *Hevea brasiliensis* (*Hevea*), a tree that has narrow genetic diversity, long breeding cycles and is restricted to specific tropical countries (Lieberei, 2007). Changes in landscape usage towards the production of more labor-efficient crops such as palm oil trees in these countries have contributed to unstable rubber prices (Basiron, 2007). These geographical restrictions, and monoculture of the *Hevea* crop amplify the potential impact of diseases, such as South American Leaf Blight (SALB) (Rivano et al., 2013) and white root rot (Irogue, 2012). It is very unlikely that *Hevea* cultivation can expand sufficiently to meet accelerating natural rubber global demands due to economic and ecological constraints. *Taraxacum kok-saghyz* (TK), an alternative sources of high quality rubber, is an obligate outcrossing dandelion species which can be cultivated as an annual. Additionally, TK is adapted to grow in a wider range of temperate areas. However, TK has not been domesticated and competes poorly with weeds when grown in the field. Moreover, wild populations usually exhibits large variation in rubber production and accurate phenotyping is not time- and cost-efficient, impeding the breeding and domestication process needed to turn TK into a rubber-producing crop. In addition, its self-incompatibility makes it an obligate outcrossing species, in which dominant mutations are usually more readily selected but most of domestication traits confirm the predominance of recessive mutations. Moreover, its outcrossing features make it impossible to either fix traits or reproduce progenies with uniform genetic background for accurate multi-location phenotyping. Therefore, only limited breeding efforts (Kupzow, 1980; Tysdal et al., 1953; Warmke, 1944) have been carried out, and TK breeding will face numerous challenges.

Rubber yield can be improved by increasing rubber concentration and/or root size. Polyploid plant genotypes are commonly used in agricultural and horticultural crops as they often

possess superior agronomic traits over their diploid counterparts (Liu et al., 2007). For example, polyploids may have larger leaves and flowers, thicker stems and roots, darker green leaves, an increased width-to-length ratio of the leaves, a more compact growth habit and a higher tolerance to environmental stress (Lavania et al., 2012; Liu et al., 2007; Shao et al., 2003). Furthermore, previous studies have demonstrated that genomic multiplication commonly increases the concentration of secondary metabolites (Dhawan et al., 1996) as in *Cymbopogon flexuosus* (cochin grass) (Ammal et al., 1966), *Hyoscyamus muticus* (Egyptian henbane) (Lavania, 1986), and *Papaver bracteatum* (Iranian poppy) (Milo et al., 1987). In addition, autotetraploids and triploids are frequently of low fertility, or may even be sterile, and this can be a desirable characteristic in clonally propagated plants as it reduces contamination of the environment by pollen or seed escapes (Dhawan et al., 1996).

In 1945, Warmke successfully induced tetraploids in TK through colchicine treatment and compared them to diploids in terms of rubber yield, fresh root weight, and reproductive pattern. He found a 3.3-fold increase in rubber yield in the field, but no significant change in greenhouses. Also, root size significantly increased among tetraploids. However, he detected tetraploids only by counting chromosomes, and comparing pollen size, seed size and leaf morphology, which could be subjective and prone to human error. As biotechnology developed, flow cytometry has become the most efficient and precise method for detecting changes in ploidy level (Dolezel et al., 1989). Samples are easily and rapidly prepared, there is no need to divide cells and only a few milligrams of tissue are needed, and it is a quick and reproducible method for determining the ploidy levels of large numbers of samples (Sakhanokho et al., 2009). Moreover, during the 1940's (and more recently), TK was often conflated with *Taraxacum brevicorniculatum* (TB) —a triploid

Taraxacum species—because it coexists with TK in nature, is phenotypically similar to TK, especially at early stages and can also produce small amounts of high-quality rubber.

With such situations in mind, we have developed and optimized a method of inducing TK tetraploid plants, and confirmed the ploidy levels using flow cytometry to ensure accurate ploidy determination. Moreover, we demonstrated that phenotypic screening can enrich for doubling events, allowing for the development of large populations requisite of an outbreeding plant species. Chemical components in roots including rubber, inulin and resin were compared between tetraploid and diploid plants.

2 Materials and Methods

2.1 Plant material and seed preparation

Seeds were harvested from open-pollinated progeny derived from high rubber parents in summer 2016 and stored under low humidity in a seed storage room (4°C) and then were treated with a colchicine concentration gradient for different durations.

2.2 Colchicine treatment and preliminary screening for putative tetraploids

Twenty-five 1.5ml Eppendorf centrifuge tubes were used, each one containing 60 seeds (0.02g). The seeds were immersed for 12, 24, 48, 72 or 96 h at room temperature in 0% (control), 0.05, 0.1, 0.2 or 0.5% aqueous solutions of colchicine dissolved in 1% DMSO. After treatment, seeds were washed with distilled water five times, with each wash step lasting 5 minutes. The seeds then were transferred directly to Promix soil and germinated in a growth chamber with 90% humidity, 20°C and a daylight period of 12h. All the treatments were replicated three times. One week after treatment, the germination rate was calculated from the number of germinated seedlings divided by the total number of treated seeds. Four weeks after treatment, the survivorship was measured by dividing the surviving plants by the total number of treated seeds. Since a large

population of seedlings (>1500 germinated seeds) was generated following the seed treatment, we carried out a preliminary morphological screening of the two-month-old seedlings to identify a smaller population of putative polyploids. Plants with atypical leaf morphologies, i.e. darker green leaves, thicker leaves, rougher leaves or leaves with abnormal edges and prominent veins or veinlets were selected as putative polyploids, which were then tested by flow cytometry. The same number of seedlings with normal leaf morphologies were also selected.

2.3 Flow Cytometric Analysis

About 100 mg of fresh leaf tissue from each selected seedling was chopped with a razor blade in a 55 mm Petri dish containing 1.5 ml Galbraith's buffer (Loureiro et al., 2006) (45mM $MgCl_2$, 30mM sodium citrate, 20mM 4-morpholinepropane sulfonate (MOPS), 0.1% (v/v) Triton X-100, pH 7.0), a of a tomato seed (lot#: D21OH101NC) with an estimated genome size of 2.05 Gb (2n DNA content) (Michaelson et al., 1991; Sato et al., 2012) was included as an internal control for each sample. The solution was then filtered through a 30µm nylon mesh. The suspension of released nuclei was stained with 75µl of 4',6-diamidino-2-phenylindole (DAPI, 10 mg/ml) for 15 min. The fluorescent intensity peak of samples, which is proportional to DNA content, was adjusted such that the control peak and expected diploid TK peak appeared between channel 50 and 100. The stained DNA was then excited by a green laser as a narrow stream of nuclei passed by a red-light detector in the Cyflow® Ploidy Analyzer (Partec, Munich, Germany). The peaks of test samples were compared to peaks derived from control tomato seeds to determine relative ploidy levels as either diploid, with a diploid genome size of 2.4 GB, tetraploid, or chimeric (having both diploid and tetraploid peaks). If the ratio of genome size of sample DNA to the tomato seed (standard) was double that of diploid TK control to the standard, then that sample was counted as tetraploid. Tetraploid seedlings were maintained and retested 4 months later, as described.

Those seedlings that twice tested as tetraploid were grown for several months and evaluated for morphology and biochemical characteristics.

2.4 Morphological measurements

Leaf morphology traits, including number of leaves, leaf width (cm), leaf length (cm) and leaf shape index (length/width) were compared between treated diploids, control diploids and tetraploid plants at four months of age. Three leaves from 8 tetraploid individuals (a total of 8 tetraploids were obtained), 21 treated diploids and 25 control diploids were examined. In addition, the size and density of leaf stomata of five confirmed tetraploids, five randomly selected treated diploids and five control diploids were measured on leaves, in triplicate. To make these observations, a thin piece of abaxial epidermis was mounted in tap water on a slide and immediately examined using a graduated eyepiece. The visual field area of the ocular was 4 mm^2 and 1 mm^2 under magnification of 20× and 40×, respectively (Xing et al., 2011). The stomatal density (d), stomatal length (l), and width (w) were measured under magnifications of 20× and 40×. The stomatal area (As) was calculated as follows (Xing et al., 2011): $As = 1/4 \times \Pi \times l \times w$, and total stoma area (At) was calculated as follows: $At = As \times d \times 100\%$ (Xing et al., 2011). Digital images were manually analyzed with ImageJ 1.50i (Wayne Rasband, National Institutes of Health, USA).

2.5 Analysis of biochemical components

The concentrations of inulin (with some smaller sugars) resin and rubber were determined sequentially in diploid and polyploid plants at six to seven months of age using an Accelerated Solvent Extractor (ASE) (ASE-350, Dionex Corp., Thermo Fischer Scientific Inc., Waltham, MA). The inulin was extracted using ASE parameters of N_2 pressure at 1500 psi; temperature at 95°C; preheating time for 5 min; heat time for 5 min (automatic software default); static time for 20 min;

flush volume of 150%; purge time for 60 s and 2 static cycles. The resin and rubber settings were as previously described (Ramirez et al., 2017).

2.6 Statistical Analysis

Data were combined from three independent biological replicates, and subjected to two-way analysis of variance (ANOVA) to analyze the effects of colchicine concentration and exposure time on induction rate, survival rate and tetraploid induction efficiency. Tetraploid induction efficiency was measured as described (Bouvier et al., 1994), where % efficiency = % seedling survival × % polyploid induction. Efficiency can range from 0 to 100, in which 100 indicates that all surviving seedlings were polyploid, whereas zero indicates that all treated seedlings either died or no polyploidy induction occurred. The Least Significant Difference (LSD) test was used for the mean separation with significant differences claimed at the different significance levels of 0.05, 0.01, and 0.001 using R software. Differences between mean values from diploid and tetraploid plants for the morphological and biochemical traits were evaluated using t tests. All data were assessed for normality (Alva et al., 2009) and homogeneity of variances prior to analysis (Schmetterer, 1964).

3 Results

3.1 Survival rate and early screening of potential polyploids

The germination rates of the colchicine-treated seeds, especially at higher concentration levels, were much lower than controls, and germination was delayed. Moreover, the survivorship of seedlings, ranging from 0 to 42%, was greatly inhibited by colchicine treatment, especially at concentrations above 0.2%, decreasing as the concentration and duration time increased. The highest lethality was observed in 0.5% 96h (Table 1). Two months after colchicine treatment, some seedlings had atypical leaf morphology (i.e. wrinkled leaves, rough leaf edges, thick leaves (Figure

1). Flow cytometry confirmed that 8 of 10 pre-selected seedlings with atypical leaf morphology, were tetraploid. This morphological screen allowed the screening of over 3,000 seedlings germinated from colchicine treated seed to be sorted with a much smaller number being analyzed for ploidy level.

3.2 Flow cytometric analysis

The flow cytometry analysis indicated three different kinds of ploidy level, diploid, tetraploid and chimeric (Figure 2). The number of induced tetraploids differed among the colchicine treatments (Table 1). The highest induction rate was in 0.2% colchicine for 48h followed by 0.1% colchicine for 48h. The 0.1% colchicine for 48h treatment also gave the highest induction efficiency. No tetraploid was obtained from the 0.5% dose at any treatment duration. Eight tetraploids were identified among seedlings of all seed treatments.

3.3 Leaf and stomata morphological characteristics

The effects of polyploidy were evaluated morphologically, using traits known to be affected by ploidy level (Liu et al., 2007; Ye et al., 2010). Young tetraploids (2-month old) grew more slowly than the diploid plants of similar age. Six months after the colchicine treatment, the tetraploid plants had fewer leaves than the diploids, but their leaves were wider and thereby had a reduced leaf index (length/width) (Figure 3A-3D; Table 2). Tetraploid leaves were also thicker and darker green in color than diploid leaves (Figure 3A-3D). Stomatal length (l), width (w), stomata area (As), and total stomatal area (At) were significantly greater in tetraploid plants than in diploids (Table 2; Figure 3E-3F). However, fewer stomata per unit leaf area were observed in tetraploid than diploid plants (Table 2; Figure 3E-3F. The average stomatal frequency in control diploids was 56.9/mm^2, in treated diploids was 43.0/mm^2 and in tetraploids was 35.7/mm^2 (Table 2). The tetraploid seeds were also larger than diploids (Figure 3G).

3.4 Comparisons of root morphology and biochemical components between diploid and tetraploid plants

Significant differences were observed among tetraploids, treated diploids and control diploids for fresh plant weight, fresh root weight, dry root weight, rubber concentration inulin/sugar concentration, and resin and inulin/sugar per root (Table 3). However, there were no significant differences in rubber per plant root system among these greenhouse-grown plants.

4 Discussion

Colchicine is highly toxic and high concentrations cause plant cell death because this antimitotic agent blocks spindle fiber development and modifies the differentiation process (Trojak-Goluch et al., 2013). Even though the treatment of seeds with various colchicine concentrations permitted a moderate germination (emergence) frequency (up to 43%) (Table 1), many of these seedlings grew very poorly, especially from the higher concentration treatments or the longer treatment times. The elongation of the radicle was inhibited and root hairs were rarely formed, thereby severely compromising the ability of many seedlings to develop further. This was also observed by other researchers (Liu et al., 2007), who developed tetraploids from seeds of *Platanus acerifolia*; none of their tetraploid seedlings successfully grew into tetraploid plants due to poor root development.

Colchicine-induced chromosome doubling has a low induction rate, as seen in our research, in which the highest induction rate of 4.92% was obtained by treatment with 0.2% colchicine for 24h, followed by a 3.77% induction rate from treatment with 0.1% colchicine for 48h. The optimal treatment also was 0.2% 24h for seeds of *Catharanthus roseus (L.)* (Xing et al., 2011). Low overall induction rate and poor growth rates have previously been reported (Lam et al., 2014; Liu et al., 2007) and may be due to residual colchicine in the seed coat inhibiting later seedling growth.

Higher polyploid induction efficiencies, recovering near-normal development, have been first reported in *in vitro* cultured tobacco (Murashig et al., 1966), and more recently, in guayule (Hashemi et al., 1989), *Alocasia* (Thao et al., 2003), and *Lagerstroemia indica L.*(crape myrtle) (Ye et al., 2010). Therefore, other *in vitro* or *in vivo* induction strategies may improve the efficiency of TK tetraploid induction even though *in vitro* induction strategies may be more appropriate for limited starting materials.

Given the low efficiency of the seed induction technique, it was imperative to develop a high throughput screening method in order to rapidly eliminate as many of the unsuccessfully induced diploid seedlings as possible before embarking on time-consuming and costly cytological analyses. Abnormal leaf morphological traits such as dark green and thicker leaves (Amma et al., 1984; Liu et al., 2007), prominent veins or veinlets (Amma et al., 1984) and wrinkled leaf edges (Liu et al., 2007) usually found in polyploid plants were used to screen young seedlings. TK seedlings with abnormal leaf morphology were confirmed as tetraploids at an 80% rate using flow cytometry. This method is much faster than using stomatal length and density as indicators of ploidy for early population screening, without impairing the selection efficiency (Liu et al., 2007; Ye et al., 2010).

Tetraploid TK were confirmed when the ratio of the nuclei mean for tomato seed (G1 peak)/TK sample (G2 peak) was approximately 2.44, which is twice the ratio of tomato/TK diploid controls. This ratio was not always exactly 2.44 probably due to a combination of the following reasons. The DNA-protein (chromatin) packing may be tighter and more condensed in tetraploids than diploids (Rabinovitch), or the staining time may be too short, as polyploids have more DNA which needs to be stained. As tetraploids have greater DNA mass, it is expected that they will exhibit wider fluorescence distributions relative to diploids; however, our method was able readily

to detect doubling events, as these are large-scale changes. In some scans, peaks were poorly defined requiring manual selection of peak range, leading to variable S phase distribution, thereby affecting the means ratio, as has been previously noted (Yan et al., 2016).

Polyploid crops are deployed in agriculture and horticulture as they often possess superior agronomic traits over their diploid counterparts, such as larger leaves and flowers, thicker stems and roots, and a higher tolerance to environmental stress (Liu et al., 2007; Shao et al., 2003; Xing et al., 2011; Ye et al., 2010). Wider leaves and an increased leaf index were apparent in our TK tetraploids. In addition, stomatal length and density have proved to be reliable indicators of ploidy in a number of species (Dhawan et al., 1996), and the measurement is simple, largely non-destructive, and does not require specialized equipment. Our results were in agreement with previous studies of stomatal characteristics at different ploidy levels in *Hevea* (Amma et al., 1984), *Stevia rebaudiana* (stevia) (de Oliveira et al., 2004) and crape myrtle (Ye et al., 2010), where authors noted that diploid species had the highest stomatal density and the smallest stomatal size. All of them concluded that stomatal parameters represent a rapid and efficient method for screening putative polyploid plants and confirming ploidy levels. However, precautions should be taken since age (the older the leaf, the more indistinguishable stomatal parameters are from control diploid plants) (Liu et al., 2007) and position on the leaf (fewer stomata form near leaf veins than elsewhere) (Liu et al., 2007) could influence size and density data, resulting in unreliable identification of polyploidy (Sakhanokho et al., 2009). Moreover, natural variation in stomatal size also may lead to inaccurate ploidy determination, especially in a highly diverse outcrossing species such as TK. We also observed that stomatal density in colchicine-treated diploid TK plants was greatly reduced compared to untreated control diploids, and were near to the densities in confirmed tetraploids. This is presumably a residual toxic effect of the colchicine but could certainly lead to

incorrect ploidy assignment. Therefore, a later reconfirmation six months or one year after treatment may be necessary to confirm ploidy level.

It should be noted that the data in this paper are gathered from generation zero (G0) tetraploids. It is clear that residual effects of colchicine persist when the data from treated and untreated diploids are compared (Table 3), which showed a significant decrease in root biomass of treated diploids as well as a reduction in stomatal density. The growth of TK tetraploid roots also appeared to be abnormally stunted, likely inhibited by residual colchicine, or indirect persistent adverse effects of treatment. In addition, we found higher rubber concentration but lower inulin/sugars concentration in tetraploid plants than in diploid plants (Table 3) but the G0 stunted roots meant that rubber yield per root system did not significantly differ from untreated diploids. There was no significant difference in resin concentration among the three groups, and so resin per root system was determined by root size. In contrast to our tetraploids, earlier colchicine-induced, greenhouse-grown, G0 tetraploids were reported to have significantly larger roots but with lower rubber concentration (Warmke, 1945).

In general, due to the self-incompatibility of TK, the genetic background of TK seeds is heterozygous and complex. This complexity may confound effects caused by chromosome doubling. Therefore, less diverse seed lots may be useful for future research. Also, comparisons can be made between progenies derived from treated diploids and tetraploids to eliminate lingering toxic effects of colchicine or by interaction between toxicity and ploidy. This may be challenging because tetraploid TK may have high levels of sterility and low seed set rate (Warmke, 1945). However, this may be overcome by acquiring larger sample sizes using the methods we have described. Furthermore, environmental conditions may require optimization to improve pollen production and seed.

5 Conclusion

In conclusion, tetraploids were produced in TK which may lead to enhanced rubber yield in the field. Atypical leaf morphology was proven effective as a leaf morphology early-screening strategy to preliminarily identify rare tetraploid seedlings among thousands of diploids, leading to an 80% confirmation rated by flow cytometry screening. Root rubber concentration increased in tetraploids while inulin/sugar concentration decreased. Tetraploid progeny will need to be evaluated to determine the impact of tetraploidy on root growth and rubber yield in the absence of residual toxic colchicine effects.

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Figure Legends

Figure 1. Pre-screening of two-month old seedlings for evaluation of putative tetraploids. The 2-month-old seedlings with atypical leaf morphology on the upper two photographs were selected as putative tetraploid plants and were later confirmed by flow cytometry analysis; The bottom two figures showed seedlings at 4-month-old stage. 2x: diploids; 4x: tetraploids.

Figure 2. Flow Cytometric displays from young leaves. (A) control (peak1), 2x (peak 2); (B) 4x (peak 3); and (C) 2x + 4x chimeric plantlets. Plantlets with (B) traces are desired tetraploids.

Figure 3. **Morphological comparisons of tetraploid and diploid plants.** (A-C) 4- month-old plants in three groups; (D) leaf morphology comparison; (E-F) stomata size comparison; (G) seed comparison.

Table 1. Germination, survival and induction rates obtained after treating diploid TK seeds with a range of colchicine concentrations for different times

Colchicine Conc. (%)	Treatment duration (h)	No. of germinated seedlings	% germination rate	No. of survival seedlings	% surviving rate	No. of induced seedlings	% induction rate	% Induction Efficiency
0.05	**12**	68	37.78	77	42.78	0	0.00	0.00
	24	86	47.78	85	47.22	0	0.00	0.00
	48	63	35.00	43	23.89	1	1.59	37.92
	72	43	23.89	40	22.22	0	0.00	0.00
	96	58	32.22	44	24.44	0	0.00	0.00
0.10	**12**	67	37.22	55	30.56	0	0.00	0.00
	24	67	37.22	37	20.56	1	1.49	30.68
	48	53	29.44	27	15.00	2	3.77	56.60
	72	56	31.11	17	9.44	0	0.00	0.00
	96	62	34.44	16	8.89	1	1.61	14.34
0.20	**12**	68	37.78	31	17.22	0	0.00	0.00
	24	61	33.89	17	9.44	3	4.92	46.45
	48	43	23.89	4	2.22	0	0.00	0.00
	72	29	16.11	6	3.33	0	0.00	0.00
	96	63	35.00	2	1.11	0	0.00	0.00
0.50	**12**	48	26.67	32	17.78	0	0.00	0.00
	24	36	20.00	3	1.67	0	0.00	0.00
	48	10	5.56	5	2.78	0	0.00	0.00

	72	74	41.11	2	1.11	0	0.00	0.00
	96	30	16.67	1	0.56	0	0.00	0.00
0	**12**	80	44.44	96	53.33	0	0.00	0.00
	24	125	69.44	133	73.89	0	0.00	0.00
	48	100	55.56	104	57.78	0	0.00	0.00
	72	91	50.56	145	80.56	0	0.00	0.00
	96	112	62.22	122	67.78	0	0.00	0.00

% germination rate = germinated seedlings/treated seeds (a week after treatment);

% surviving rate = living seedlings/treated seeds (a month after treatment);

% induction rate= induced polyploid seedling/germinated seedlings (2 months after treatment);

% induction efficiency= % seedling survival * % polyploid induction (2 months after treatment).

Table 2 Comparisons of nine morphological traits among three groups.

Morphological traits	**4x**	**Treated 2x**	**Control 2x**
Stomata density ($n*mm^{-2}$)	6.8±4.2298A	12.9333±5.2023B	12.1333±3.9231B
Stomatal length (mm)	0.0315±0.0038A	0.0219±0.0021B	0.0225±0.0031B
Stomatal width (mm)	0.0166±0.004A	0.0106±0.0025B	0.0106±0.0015B
Stomatal area (As, mm^2)	0.0004±0.0001A	0.0002B	0.0002B
Total stomata area (At, mm^2)	0.2873±0.183a	0.2347±0.108a	0.2272±0.0795a
Leaf index (length/width)	2.553±0.314A	4.065±0.226B	4.323±0.251B
Number of leaves	9.571±1.112A	14.571±0.84B	15.48±0.956B
Leaf width (cm)	2.187±0.135A	1.531±0.097B	1.373±0.107B
Leaf length (cm)	5.719±0.288a	5.269±0.384a	5.959±0.266a

Table 3 Comparisons of plant growth traits and biochemical components among three groups

Morphological and biochemical traits	4x	Treated 2x	Control 2x
Fresh plant weight (g)	10.683±2.194a	17.869±1.490b	27.968±1.075c
Fresh root weight (g)	6.467±0.877a	10.376±0.638b	14.540±0.526c
Dry root weight (g)	1.367±0.261a	2.988±0.190b	4.276±0.156c
Rubber content (mg/g)	57.575±7.013a	34.582±4.591b	30.132±3.662b
Rubber yield (mg)	78.807±26.025a	100.062±17.037a	129.667±13.591a
Resin content (mg/g)	27.453±7.320a	33.478±4.629a	32.374±4.113a
Resin yield (mg)	38.020±23.535a	90.233±14.885ab	125.754±13.226b
Inulin content (mg/g)	33.407±4.418a	47.322±2.794b	37.716±2.362b
Inulin yield (g)	54.200±33.095a	133.132±20.931ab	173.550±17.690b

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