

# Phenotypic diversity of USDA guayule germplasm collection grown under different irrigation conditions



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## ABSTRACT

*Parthenium argentatum* (guayule), originated from northern Mexico and southern Texas deserts, is a good candidate for arid and semi-arid sustainable agricultural systems to produce domestic natural rubber and other industrial byproducts. Exploring the genetic and phenotypic diversities of guayule germplasm collections is required for continuous genetic improvement of guayule characteristics to meet the growing demand of guayule for rubber, resin and latex industries. The current study phenotypically evaluated a larger guayule USDA germplasm collection than before, with 56 accessions for 10 important morphological and agronomic traits evaluated, which include plant biomass, rubber and resin content, rubber and resin yield. The accessions were grown under two different irrigation conditions: well-watered and water-stressed environments. Significant genotypic effects were found for all studied traits indicating the wide genetic variability of this collection. Significant Environmental effects were found in rubber content, resin content, rubber yield and dry weight biomass. No significant genotype-by-environment interactions (GEI) were observed indicating the similarity of those accessions across growing conditions. Moderate to high entry-mean heritability values were estimated for these traits, suggesting that selection is feasible to enhance the genetic gain. Significant inter-trait correlations were found between biomass-related traits and resin/rubber yield, as well as ploidy levels and resin/rubber content, indicating the possibility of improving multiple traits at one time. To conclude, this study explored the phenotypic variations of the USDA guayule germplasm under different irrigation conditions, which will provide recommendations for parent and off-spring selections to enhance the breeding values in guayule breeding programs.

## 1. Introduction

Natural rubber (NR) is a strategical industrial natural resource. Due to its outstanding industrial properties such as elasticity, resilience, heat dispersion and abrasion resistance, it cannot be completely replaced by petroleum-derived synthetic rubber (Suchat et al., 2013; van Beilen and Poirier, 2007). However, the current production of NR, mainly harvested from *Hevea brasiliensis*, is faced with many obstacles including the shortage of supply due to increased demands, the risks of fatal diseases, changing in economic and social behaviors in rubber-producing regions, and the allergic reactions triggered by *Hevea's* NR latex (Cornish et al., 2004; Suchat et al., 2013). Therefore, the exploration of alternative NR resources is highly encouraged and needed (Luo et al., 2018).

Guayule (*Parthenium argentatum* A. Gray), a woody perennial shrub native to the Chihuahuan desert of Central North Mexico and Southwest Texas, is considered as a source domestic for NR as well as

hypoallergenic latex (Cornish et al., 2001; Ray et al., 2005), and is suitable crop for semi-arid and arid sustainable agricultural systems. Beside rubber and latex, guayule could also be a rich source for resin and bagasse (85–90% of biomass) for biofuel application and pharmaceutical industries (Nakayama, 2005). By nature, guayule is a drought-tolerant desert shrub, which could survive on about 250–380 mm of annual rainfall in its native regions (Bekaardt et al., 2010). Sufficient irrigation condition is required to ensure the maximum production of rubber yield (Nakayama et al., 1991), where an increase in irrigation would increase overall rubber yield via improving plant biomass (Hunsaker and Elshikha, 2017).

In potential guayule growing regions such as Texas, New Mexico, Arizona and California (Hunter and Kelley, 1946), drought stress is a common abiotic stress facing guayule cultivation. In order to facilitate guayule breeding programs and genetic improvement, an accurate phenotyping procedure and catalog for present guayule collection under different irrigation management conditions is needed to

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accommodate different varietal responses among those regions. In addition, the exploration of relationships between ploidy levels and agronomic traits could provide some guidance in future polyploidy guayule breeding programs.

Genetic improvement of guayule occurred sporadically during four fluctuations of domestication and commercialization events (Coffelt and Ray, 2015). However, challenges still exist in guayule genetic improvement due to its reproduction mode and long lifecycle (Abdel-Haleem et al., 2018; Ray et al., 2005). The ploidy levels of guayule range from diploid ( $2n = 36$ ) to octaploid ( $8n = 144$ ) but commercialized guayule are primarily tetraploids ( $4n = 72$ ) (Ray et al., 1993). Guayule populations also contain aneuploid plants or plants with up to five accessory chromosomes (Ray et al., 1993). Diploid guayule reproduces sexually since they are self-incompatible and have to rely to outcrossing to realize reproduction. However, polyploid guayule reproduces predominantly asexually by facultative apomixis (Ray et al., 2007, 1993; Estilai et al., 1992), in which the seeds are produced from megaspore mother cells. This reproduction mode lead to little genetic variations between parents and off-springs, which could be a hurdle for guayule breeding since genetic variability is the basis for successful breeding programs (Ray et al., 1993). In addition, as a perennial shrub, guayule requires at least 2–3 years before the agronomic traits can be phenotyped in the field-grown plants, resulting in prolonged breeding cycles (Abdel-Haleem et al., 2018). To maximize guayule genetic gains through breeding cycles, exploring the genetic and phenotypic diversities of guayule germplasm collections is required. Ilut et al. (2017) genotyped USDA guayule germplasm, the largest and only documented guayule collection, with nearly 50,000 single-nucleotide polymorphism (SNP) genetic markers. However, no reports have been published yet for the phenotypic diversity of such collection.

The major objectives of current study are: 1) to evaluate and explore the phenotypic diversity among the USDA guayule collection under different field irrigation conditions; 2) to estimate the genetic and environment effects, genotype-by-environment interactions (GEI), and heritability for the traits of interests in this collection; 3) to explore the correlations between ploidy levels, plant biomass traits, and rubber and resin traits.

## 2. Materials and methods

### 2.1. Plant materials

A total of 56 guayule accessions from the USDA germplasm collection were transplanted under water-stressed and well-watered field conditions respectively for 2.5 years in an  $\alpha$ -lattice design with each accession replicated in three replications at Maricopa Agricultural Center (MAC), University of Arizona, Maricopa AZ, USA ( $33^{\circ} 04'16''N$ ,  $111^{\circ} 58'41''W$ , 361 m asl). The detailed information of origins and resource background for each accession was provided in Table 1. The soil series and textures were classified as Casa Grande series (fine-loamy, mixed, hyperthermic Typic Natrargids). Seeds of each accession were planted in the greenhouse then three months seedlings with no visual defect and free of pest damages were transplanted in the field in plots consists of four rows of 3 m length, 1 m among rows, and 0.30 m between transplants within rows to reach plant population at 27,000 plants  $ha^{-1}$ . Transplanting time was third week of November 2015. Water-stressed and well-watered trials were irrigated differentially to reach suitable stress levels following the soil water depletion model described by Hunsaker and Elshikha (2017). In general, the well-watered and water-stressed sites were surface irrigated with furrows using three-inches siphon tubes. The estimated flow rate of the siphon tubes was  $15.21 m^3/h$ . The well-watered site was scheduled to be fully-irrigated and received irrigations at  $\approx 55\%$  soil water depletion, while the water-stressed site received 50% of the irrigation applied to the well-watered site. At both sites, schedule irrigations were based on estimated soil water depletion using a neutron probe (Model 503, Campbell

Pacific Nuclear, CPN, Martinez, CA) and two-meters access tubes located in the center row of three plots across the field (three tubes at each site). Neutron probe measurements were made weekly at depths in 0.20 m increments and up to 2.0 m below the soil surface.

#### 2.1.1. Flow cytometry analysis

The ploidy level of each guayule accession was determined using flow cytometer (CyFlow® Ploidy Analyzer, Sysmex). About 100 mg of fresh leaf tissue growing at field was vigorously chopped with a sharp double-edged razor blade in a 55 mm petri dish containing 400  $\mu L$  Sysmex Cystain UV Precise P nuclei extraction buffer (with antioxidants added) over the tissue. About 1 mL of DNA UV Precise P staining buffer was then added to the cut-up sample in the petri dish. The entire contents in the petri dish was then transferred to a 3.5 mL Rohen tube after passing through the 30  $\mu m$  CellTrics filter. The use of the CyFlow® Ploidy Analyzer (Sysmex, Lincolnshire, IL) to determine the polyploidy level for each accession followed the guidance of manufacturer's manual. The cleaning procedure was performed after running each sample. The standard process of cleaning includes one cleaning run of 10% bleach, MilliQ water, green cleaning solution, purple decontamination solution, and followed by MilliQ water.

#### 2.2. Phenotypic observations

Morphological traits including plant height (cm), plant width (cm), plant height to width ratio, plant volume ( $cm^3$ ), and plant perimeter (cm) were estimated following the procedures described by Coffelt and Johnson (2011). Dry weight biomass ( $Kg ha^{-1}$ ), resin content (%), rubber content (%), resin yield ( $Kg ha^{-1}$ ) and rubber yield ( $Kg ha^{-1}$ ) were determined for ground samples. Two guarded plants were randomly harvested within the middle two rows of each plot at first week of April 2018. Plants were cut within 5 cm above the soil surface, plant material was chipped using the method described by Coffelt and Nakayama (2007). Harvested plants were dried in an open area then chipped using Troy-Bilt Model 47321 Chipper/Shredder (Garden Way, Inc., Troy, New York) with a 9.53-mm round-holed screen. After drying, the chipped samples were ground using a hammer mill with a 6-mm screen (Model W6H, Schutte-Buffalo Hammermill, LLC, Buffalo, NY). The samples were then fine-ground using a Model 4 Wiley mill to pass the material through two-mm sieves (Thomas Scientific, Swdesboro, NJ). The dried ground samples were stored in small sealed plastic bags at  $4^{\circ}C$  to limit risk of oxidation. Resin and rubber were extracted by acetone and cyclohexane sequentially using accelerated solvent extraction (ASE) on Dionex 200 (Dionex Corp., now ThermoFisher Scientific Inc., Waltham, MA), which was equipped with an auto-sampler carousel, a solvent controller that accommodated up to four different solvents, and a collection tray that allowed up to 24 samples to be sequentially extracted (Suchat et al., 2013; Ramirez-Cadavid et al., 2018). All ASE extraction cells were prepared uniformly. A cellulose micro-filter (20-mm diameter) was first placed at the bottom of each cell, which was then filled with dry ground samples mixed with diatomaceous earth (DE). Glass collecting vials (250 mL) were placed into the collection tray. The first cell, as a control, was only filled with DE. Extraction was performed under the following conditions. Each sample was first extracted with acetone at  $100^{\circ}C$  and 1500 psi of nitrogen, with a heating time of 5 min, static extraction time of 10 min, purge time of 60 s, and flush volume 100%, followed by cyclohexane extraction at  $140^{\circ}C$  under the pressure of 1500 psi of nitrogen, heating time of 7 min, static extraction for 20 min, purge time of 60 s, and a flush volume of 100%. Three static cycles were applied to each extraction stage. Following this, the extractant was transferred into a pre-weighed glass vial (250 mL). Evaporation of the solvent from the extract was done in a fume hood at room temperature (RT) for two weeks and dried in an oven at  $55^{\circ}C$  for 24 h before weighing again. Three samples were randomly selected from each ASE batch (11 samples) for moisture content estimation, which was determined by drying a 5-g sample at

**Table 1**  
The detailed information of origins and resource background of 56 USDA guayule accessions based on U.S. National Germplasm System (GRIN) databases.

Accession Name	Plant ID	Origin	Breeding Status	Pedigree Resources	Ploidy Levels
593	PI 478639	California, United States	Improved breeding material		4x
11231	PI 478653	Mexico	Improved breeding material		3x
11591	PI 478640	California, United States	Improved breeding material	Selection from 4264-I, Power, McCallum and oleson collection, Mexico 1948	3x
11600	PI 478641	California, United States	Improved breeding material	Selection from 4264-I, Power, McCallum and oleson collection, Mexico 1949	4x
11604	PI 478642	California, United States	Improved breeding material	Selection from 4264-I, Power, McCallum and oleson collection, Mexico 1948	4x
11605	PI 478643	California, United States	Improved breeding material	Selection from 4264-I, Power, McCallum and oleson collection, Mexico 1948	4x
11609	PI 478644	California, United States	Improved breeding material		3x
11619	PI 478645	California, United States	Improved breeding material	Selection from 4264-I, Power, McCallum and oleson collection, Mexico 1948	Mixed
11633	PI 478646	Mexico	Improved breeding material		5x
11634	PI 478647	California, United States	Improved breeding material		Mixed
11635	PI 478648	California, United States	Improved breeding material		4x
11646	PI 478649	Mexico	Improved breeding material		3x
11693	PI 478650	California, United States	Improved breeding material	N563	4x
11701	PI 478651	California, United States	Improved breeding material		4x
12229	PI 478652	California, United States	Improved breeding material		Mixed
4265-XF	PI 478661	Mexico	Improved breeding material	Selection from PI 478660	4x
4265X	PI 478660	Mexico	Improved breeding material	Selection from A4265	4x
A48118	PI 478662	Mexico	Improved breeding material		4x
AZ1	PI 599674	Arizona, United States	Improved breeding material	Selected from PI 478660	4x
AZ2	PI 599675	Arizona, United States	Improved breeding material	Selection from PI 478640	4x
AZ3	PI 599676	Arizona, United States	Improved breeding material	Selection from PI 478640	4x
AZ4	PI 599677	Arizona, United States	Improved breeding material	Mass selected line selected from original accession received from NSSL	4x
AZ5	PI 599678	Arizona, United States	Improved breeding material	Mass selected line selected from original accession received from NSSL	4x
AZ6	PI 599679	Arizona, United States	Improved breeding material	Mass selected line selected from original accession received from NSSL	4x
CAL2		California, United States	Improved breeding material	<i>P. argentatum</i> / <i>P. fruticosum</i>	4x
CAL3	PI 478664	California, United States	Improved breeding material	Selection from diploid plants	2x
CAL4	PI 478665	California, United States	Improved breeding material		3x
CAL5		California, United States	Improved breeding material	<i>P. argentatum x tomentosum v. stramonium</i>	3x
CAL6	W6 549	California, United States	Improved breeding material	Selection from "Bulk: Arboreutum"	3x
CAL7	W6 7157	California, United States	Improved breeding material	Selection from "Mexican Bulk"	4x
CFS16	PARL 803	Texas, United States	Wild		4x
CFS17	PARL 804	Texas, United States	Wild		4x
CFS18	PARL 805	Texas, United States	Wild		3x
CFS21		Texas, United States	Wild		4x
CFS24		Texas, United States	Wild		Mixed
N396	PI 478654	Mexico	Improved breeding material		3x
N565	PI 478655	California, United States	Improved breeding material	Selection from 4264-I, Power, McCallum and oleson collection, Mexico 1948	3x
N565II	PI 478656	California, United States	Improved breeding material		3x
N566	PI 478657	California, United States	Improved breeding material		5x
N575	PI 478658	Mexico	Improved breeding material		4x
N576	PI 478659	Mexico	Improved breeding material	Selection from 4264-I, Hammond and Hinton collection, Mexico 1948	3x
R1037	W6 2189	Coahuila de Zaragoza, Mexico	Wild		4x
R1040	W6 2192	Coahuila de Zaragoza, Mexico	Wild		4x
R1092	W6 2244	Zacatecas, Mexico	Wild		4x
R1093	W6 2245	Zacatecas, Mexico	Wild		3x
R1095	W6 2247	Zacatecas, Mexico	Wild		3x
R1096	W6 2248	Zacatecas, Mexico	Wild		3x
R1097	W6 2249	Zacatecas, Mexico	Wild		5x
R1100	W6 2252	Coahuila de Zaragoza, Mexico	Wild		4x
R1101	W6 2253	Coahuila de Zaragoza, Mexico	Wild		4x
R1103	W6 2255	Durango, Mexico	Wild		4x
R1108	W6 2260	Texas, United States	Wild		Mixed
R1109	W6 2271	Coahuila de Zaragoza, Mexico	Wild	<i>P. argentatum</i> / <i>P. incanum</i>	4x
R1110		Arizona, United States	Wild	<i>P. argentatum</i> / <i>P. stramonium</i>	4x

(continued on next page)

Table 1 (continued)

Accession Name	Plant ID	Origin	Breeding Status	Pedigree Resources	Ploidy Levels
Richardson W6-429	W6-550 PI 478663	Arizona, United States California, United States	Improved breeding material Improved breeding material		3x Mixed

105 °C in an oven for 24 h, and then kept 8 h in a desiccator before weighing. The moisture content values of each batch were averaged and used to adjust rubber and resin content for further use with the following adjustment formulas:

$$\text{adjusted resin (\%)} = \text{dry resin content} \times (1 - \% \text{ moisture content})$$

$$\text{adjusted rubber (\%)} = \text{dry rubber content} \times (1 - \% \text{ moisture content})$$

### 2.3. Statistical analysis

The phenotypic data were tested for homogeneity assumptions, including Shapiro-Wilk’s test to check the normality of residual distribution (Alva and Estrada, 2009) and Levene’s test to analyze the homogeneity of variance (Schmetterer, 1964) in R (v3.5.3) statistical language (Team, 2014). Q-Q plots and the histogram of residuals were generated (not shown). Data was then transformed using a two-step data transformation method in IBM SPSS (Templeton, 2011). The transformed data was then used for subsequent analyses. Phenotypic variation across USDA guayule collection was analyzed using mixed linear models with the lme4 package in R and was partitioned into genetic effects, environment effects, genotype-by-environment interactions (GEI), replication effects, and block effects. The observed trait  $Y$  was analyzed as the response from  $i^{th}$  genotype,  $l^{th}$  block nested in  $k^{th}$  replicate over  $j^{th}$  environment using the model  $Y_{ijkl} = \mu + g_i + e_j + ge_{ij} + r(e)_{jk} + b(re)_{jkl} + error_{ijkl}$ , where  $\mu$  is the grand mean,  $g_i$  is the effect of  $i^{th}$  genotype,  $e_j$  is the effect of  $j^{th}$  environment,  $ge_{ij}$  is the interaction effect between the  $i^{th}$  genotype and  $j^{th}$  environment,  $r(e)_{jk}$  is the  $k^{th}$  replicate effect nested in the  $j^{th}$  environment, and  $b(re)_{jkl}$  is the  $l^{th}$  block effect nested in the  $k^{th}$  replicate within the  $j^{th}$  environment. For separated environment, the statistical model was calculated as:  $Y_{ijk} = \mu + g_i + r_j + b(r)_{jk} + error_{ijk}$ , where  $\mu$  is the grand mean,  $g_i$  is the effect of  $i^{th}$  genotype,  $r_j$  is the  $j^{th}$  replicate effect, and  $b(r)_{jk}$  is the  $k^{th}$  block effect nested in the  $j^{th}$  replicate.

Best Linear Unbiased Predictors (BLUPs) for each accession against each trait was predicated, which could provide plant breeders the potential breeding values for different guayule accessions in a germplasm collection and inform selection choices for breeding programs.

The broad-sense heritability based on the entry-mean basis ( $H_{entry-mean}$ ) was calculated as:  $H_{entry-mean} = \sigma_G^2 / (\sigma_G^2 + \sigma_{error/re}^2 + \sigma_{G \times E/e}^2)$ , where  $\sigma_G^2$  is genetic variance,  $\sigma_{G \times E}^2$  is genotype-by-environment interaction (GEI) variance,  $\sigma_{error}^2$  is residual error variance,  $r$  is the number of replicates, and  $e$  represents the number of environments (Piepho and Mohring, 2007); The entry-mean heritability was calculated on the basis of adjusted means for field design at several different environments or locations (Utz and Laidig, 1989).

Inter-trait Pearson’s correlations among studied traits were estimated by generating a Sums of Squares and Cross Products (SSCP) matrix using correlation option in Excel and the significance of pairwise correlations was generated by regression option in Excel.

Principle component analysis (PCA) was conducted to identify clusters corresponding to the variables due to ploidy levels and to discover the relationships among the accessions in the USDA guayule germplasm collection. The package ggplot2 implemented in R was used to perform PCA (Ginestet, 2011). A covariance matrix among variables was first generated and eigenvector (or principle component) decomposition was then completed. Entries of every eigenvector are also called loadings (Jolliffe and Cadima, 2016). The first two or three eigenvectors (or factors, axes or PCs) usually explain the most variations of variables among different accessions (Jolliffe and Cadima, 2016). Based on the loadings, factors or axes along which accessions vary most can be identified. In this case, accessions with different ploidy levels can be clustered into groups on a certain axis or PC, where the most variations among variables can be explained.

### 3. Results and discussion

In an effort to explore the phenotypic diversity of USDA guayule collection, the collection was transplanted and grown under two irrigation regimes. From the breeding goals perspective, an accurate estimation of phenotypic traits for a selected population can provide reliable guidance for plant breeders to make decisions on parent choice, crossing strategies, selection pressure and make reasonable expectations on genetic gain. The ploidy levels determined by flow cytometry for 56 guayule accessions were provided in Table 1, in which the BLUPs of each guayule accession for different phenotypic traits were also summarized. Detailed information of accession origins and resource background were provided in Table 1. A total of 17, 29, 3 and 6 guayule accessions were categorized into triploid, tetraploid, pentaploid and mixed ploids, respectively. These are in agreement with previous ploidy characterization results (Thompson and Ray, 1988; Ilut et al., 2017; Gore et al., 2011). CAL3 was determined as the only diploid. Combined with the information of origins and selection resources of these accessions (Table 1), BLUPs for guayule USDA germplasm explained the variations within the collection for plant morphological traits such as plant height, plant width, plant diameter, and plant biomass as well rubber and resin productions (Table 2). For example, the average values for plant height, plant width, plant volume and perimeter were 79.0 cm, 85.1 cm, 498594.8 cm<sup>3</sup> and 267.3 cm with the rough range of 47–116 cm, 69–108 cm, 142100–1031762 cm<sup>3</sup> and 217–341 cm, respectively (Table 2). Rubber (and resin) yield equals to rubber (and resin) content multiplied by dry weight biomass. Therefore, with the average dry weight biomass of 27259.710 Kg/ha, rubber and resin yield can reach up to 882.9 and 3455.0 Kg/ha with an average rubber and resin content of 3.35% and 12.70%, respectively (Table 2). In general, traits related to plant biomass (i.e. plant height, width, DWY, etc.) and secondary metabolites (i.e. rubber and resin contents) were improved across the past few decades when comparing wild accessions to improved accessions (Table 1). These improvements ranged from 3.2% in plant width and perimeter to 31% in rubber yield. For example, the improved germplasm such as AZ5, AZ2 and the hybrid accession R1109 were the highest in plant height while CF17 (wild accession collected from Texas) was the shortest one (Table 1 & 2). N369, N565 and 11591 are all improved germplasm were the highest in rubber yield while the lowest four were all wild accessions (Table 1 & 2) such as R1100, R1101, R1037 and R1103. Successful hybridization among *Parthenium* species played a role in determining the off-spring characteristics, for example, CAL5 (a hybrid between guayule and *P. tomentosum*) was low in rubber yield since *P. tomentosum* is a wild relative to guayule with non-significant amounts of rubber but high in plant biomass (Ray et al., 2010). Similar situation occurred in another hybrid, R1109, a hybrid between guayule and *P. incanum* (or mariola), the closest sister taxon of guayule, that was the highest in plant height, width, plant volume and plant perimeter (Powers and Rollins, 1945). Nine out of the 10 accessions with highest dry weight biomass were improved accessions, while 8 out of 10 accessions with lowest dry weight biomass were wild accessions (Table 1 & 2). Similar situations also occurred in rubber content and yield, where the highest 10 accessions for these traits were improved accessions (Table 1 & 2).

Analyses of variances (ANOVA) (Table 3) indicated moderate to high entry-mean heritability values for the studied traits ranging from 0.657 (dry weight biomass) to 0.933 (plant height) (Table 3). In correspondence with the current study, previous studies also estimated moderate to high entry-mean heritability values for plant height, plant width, and rubber- and resin-related traits (Dierig et al., 2001; Abdel-Haleem et al., 2018). However, these studies were either based on only 9 transplanted accessions (Abdel-Haleem et al., 2018) or individual clonally propagated plants (Dierig et al., 2001). High heritability estimates indicate the possibility to select guayule accessions at early generations of breeding cycles for the desirable traits. As a perennial shrub, guayule field trials based on multi-locations and multi-years are

more difficult than annual crops, the selection at early generations could ease the efforts for guayule breeders and reduce the number of breeding cycles to reach a specific genetic gain.

Significant genotypic effects were found for the 10 traits evaluated, only four of these traits had significant environmental effects, and there was no genotype-by-environment interaction (Table 3). Therefore, we further analyzed these four traits to compare the phenotypic effects of different irrigation conditions (Table 4). In general, the trends of phenotypic variations were similar under two different irrigation conditions for these traits (Fig. 1, Table 4), corresponding to the non-significant GEI in Table 3, suggests that the accessions in this germplasm collection performed similarly when grown under different irrigation conditions. Besides, our results indicated that guayule tended to grow shorter and smaller but can accumulate more resin and rubber under stressed condition compared to the well-watered one (Table 4, Table S2). For example, a 26.2% increase was indicated in dry weight biomass under well-watered condition compared to water-stressed condition. However, rubber content decreased from 3.9% for plants grown under water-stressed condition to 2.4% under well-watered condition (Table 4). Resin content was also affected by irrigation where a reduction of 13.92% to 11.8% was observed in plants grown under water-stressed compared to well-watered growing conditions (Table 4). Previous studies observed decreases in both plant biomass and rubber yield and an increase in rubber content under water-stressed conditions (Hunsaker and Elshikha, 2017; Allen et al., 1987; Benedict et al., 1947; Bucks et al., 1985; Benzioni et al., 1989). However, our results showed an increase in rubber yield under water-stressed condition (Table 4). This might be due to the fact that when averaging the rubber yield for a large population, the differences of genetic response for the individual accession were mitigated (Fig. 1D & Figure S1D) when compared to the previous studies where only a few improved guayule genotypes were used. It has been suggested that the increase in rubber content under water-stressed condition might be due to the increased stem proportions to the total biomass and increased bark to wood ratio of the stem, which were the two major effects of water stress (Blohm, 2005; Chow et al., 2008). Researchers also showed that water stress might allocate carbon fixation products (e.g. sucrose) to rubber synthesis rather than plant growth (Kelly and Vanstaden, 1993, 1991; Benzioni and Mills, 1991; Blohm, 2005).

The performance of USDA guayule collection accessions under different irrigation conditions was observed (Fig. 1). These can be used by guayule breeders to integrate these accessions as parents for specific environments. For example, CAL4 has highest dry weight biomass under water stressed condition and narrow differences on this trait under the two studied environments (Fig. 1A), suggesting that CAL4 can be grown under water-stressed condition without significant drop in biomass production. However, its low rubber content (Fig. 1C) led to lower rubber yield (Fig. 1D), suggesting that CAL4 might not be a good parental candidate for the breeding program aiming to breed for stable and high rubber yielding. Four out of the highest five accessions in rubber content, 11591, N565II, 12231, and R1095 accessions, under water-stressed condition were also the highest under well-watered condition (Fig. 1C), indicating the similarity of these accessions across studied environments, thus these accessions can be used as parental candidates for high rubber content. Besides, it is difficult to conclude that one environment was always better than the other for rubber yield across the entire germplasm (Fig. 1D). This might be because rubber yield depends on both rubber content and dry biomass, and the accessions with highest rubber content were not always the highest in dry biomass, but rather, were the ones with low biomass as shown in Figure S1 (i.e. R1095, 11591, 12231).

Principle component analysis (PCA) aims to explain most variations among a population by reducing the dimensionality of datasets without losing the interpretability of information. This study is the first one applying PCA to analyze the effects of ploidy levels on genotypic responses in guayule. In our study, the first three PCs accounted for

**Table 2**  
Summary of Best Linear Unbiased Predictions (BLUPs) of 56 the accessions of USDA guayule collection for different phenotypic traits.

Accession Name	Dry weight biomass (Kg ha <sup>-1</sup> )	Plant Height (cm)	Width (cm)	Plant Volume (cm <sup>3</sup> )	Plant Perimeter (cm)	Ratio	Resin Conc. (%)	Resin Yield (Kg ha <sup>-1</sup> )	Rubber Conc. (%)	Rubber Yield (Kg ha <sup>-1</sup> )
593	25528.5	71.0	80.8	393087.0	254.0	1.1	9.2	2117.2	4.2	973.2
11231	22920.1	64.8	83.6	416433.8	262.6	1.2	10.3	2089.6	5.0	1089.3
11591	22435.7	91.2	82.1	511321.6	258.0	1.0	10.4	2015.0	5.4	1185.8
11600	25973.4	65.2	78.9	316012.1	247.9	1.1	15.5	3942.5	3.0	752.7
11604	32090.8	84.8	84.7	527191.2	266.0	1.0	14.3	4634.1	3.4	1111.4
11605	31961.6	56.7	81.5	328440.1	256.1	1.2	10.0	3345.3	3.8	1149.0
11609	33262.4	63.8	81.9	367521.6	257.4	1.2	13.5	4629.0	2.9	962.7
11619	31034.1	82.4	95.2	681762.0	299.1	1.2	11.1	3645.3	3.3	1069.0
11633	29896.3	77.6	84.0	468668.5	263.8	1.1	13.2	3898.4	2.6	751.5
11634	29357.4	78.9	90.6	597863.0	284.7	1.2	13.3	3918.2	3.0	849.5
11635	27453.6	84.8	85.6	541570.8	268.8	1.1	16.3	4310.4	3.0	797.6
11646	24754.7	80.1	78.8	406983.7	247.5	1.0	12.9	3142.2	4.0	927.8
11693	29461.3	88.0	88.0	604349.9	276.6	1.1	15.8	4399.6	3.8	1021.4
11701	28484.2	74.8	83.6	446839.1	262.6	1.1	13.7	3909.3	3.2	889.5
12229	23602.6	62.5	71.3	200278.9	224.0	1.1	14.8	3505.6	3.0	674.4
4265-XF	32550.8	82.8	87.9	572356.3	276.2	1.1	14.1	4577.4	3.0	948.9
4265X	29448.7	72.1	74.9	289078.2	235.4	1.0	14.4	4266.6	4.4	1182.2
A48118	32407.3	88.8	82.3	503577.9	258.5	1.0	14.5	4575.7	3.6	1101.1
AZ1	27396.8	85.6	87.6	578359.5	275.1	1.1	15.3	4206.0	3.5	932.7
AZ2	31432.4	104.4	95.6	792751.1	300.5	1.0	14.4	4499.5	2.9	892.1
AZ3	33660.6	108.8	98.3	869098.4	308.8	1.0	15.1	4885.9	3.4	1096.5
AZ4	29825.1	86.1	86.2	558761.0	270.8	1.1	16.3	4621.8	3.5	997.0
AZ5	33363.5	111.2	102.6	927598.2	322.5	1.0	14.6	4782.9	2.5	847.4
AZ6	27152.4	86.8	81.7	481597.1	256.5	1.0	16.2	4355.7	4.0	1037.2
CAL2	35202.8	115.5	96.8	833368.9	304.3	1.0	9.8	4057.5	1.4	602.7
CAL3	23945.6	54.4	76.9	247859.7	241.5	1.2	12.7	3058.2	2.4	574.6
CAL4	40387.1	102.6	102.6	867824.9	322.2	1.1	10.8	4482.3	1.9	1010.5
CAL5	25085.4	98.5	90.1	666304.6	283.2	1.0	8.9	1943.5	1.8	501.1
CAL6	26526.6	88.0	87.5	586853.6	275.0	1.1	9.8	2381.9	4.3	1072.3
CAL7	26013.7	81.4	83.5	576466.1	262.4	1.0	11.6	3324.1	3.1	831.2
CFS16	18306.9	57.5	81.8	335176.1	257.0	1.3	12.2	2783.0	2.5	572.1
CFS17	20535.0	47.2	76.2	197969.3	239.3	1.3	13.0	2682.4	3.5	717.5
CFS18	26310.5	86.4	81.9	488672.5	257.4	1.0	12.0	3026.7	4.2	1016.9
CFS21	24977.7	54.4	72.5	160437.1	227.8	1.2	13.1	3301.9	2.7	645.3
CFS24	26362.2	71.8	81.2	394753.7	255.1	1.1	12.9	3342.7	3.6	866.3
N396	26892.1	64.4	91.5	542506.2	287.6	1.3	10.4	2624.1	5.3	1292.6
N565	28636.3	92.7	83.1	528587.0	261.2	1.0	10.9	3051.7	4.9	1263.9
N565II	24852.1	84.0	80.7	447683.5	253.4	1.0	10.5	2393.7	5.0	1127.8
N566	30083.0	76.0	85.9	504095.0	269.7	1.2	12.9	3845.3	2.9	821.9
N575	25708.2	75.4	85.4	486894.1	268.3	1.1	14.0	3836.6	4.1	928.0
N576	26983.5	84.3	82.5	469626.9	259.2	1.0	10.0	2482.3	4.1	981.7
R1037	24455.8	96.9	82.4	548091.6	258.8	0.9	10.4	2356.3	1.5	376.8
R1040	26560.4	79.0	80.5	418775.8	252.8	1.0	13.4	4425.8	3.4	969.1
R1092	22726.1	62.9	77.8	290973.2	244.6	1.1	14.0	3265.1	2.3	542.4
R1093	18730.5	59.4	69.3	142100.2	217.7	1.1	14.0	2747.4	2.8	553.5
R1095	21406.1	56.5	89.5	463355.9	281.1	1.4	10.8	2087.8	5.0	1050.3
R1096	20287.1	76.9	81.6	420328.1	256.2	1.1	11.8	2057.0	4.4	850.8
R1097	26806.3	63.8	87.2	463338.9	274.1	1.2	12.2	3183.1	4.1	984.8
R1100	17713.9	69.0	86.0	458744.7	270.2	1.2	10.8	1500.2	1.3	231.5
R1101	21666.2	66.5	81.5	378638.7	256.2	1.2	12.7	2751.8	1.4	327.2
R1103	27368.0	75.2	86.9	513189.1	273.0	1.2	12.1	3264.1	1.8	495.7
R1108	30863.6	77.6	83.1	461253.6	261.2	1.1	13.8	4227.7	3.6	1022.7
R1109	34750.0	116.0	108.7	1031762.2	341.4	1.1	14.1	4949.7	2.6	944.6
R1110	27413.7	77.9	90.9	586638.4	285.7	1.2	13.6	3816.3	3.3	959.5
W6-429	26909.5	68.0	84.5	438756.9	265.5	1.2	12.2	3204.4	3.8	948.2
W6-550	26623.6	91.2	87.1	588780.0	273.5	1.0	10.9	2754.5	4.7	1116.5
Mean	27259.7 ± 4516.6	79.0 ± 15.9	85.1 ± 7.5	498594.8 ± 182614.4	267.3 ± 23.5	1.1 ± 0.1	12.7 ± 1.9	3455.0 ± 913.6	3.4 ± 1.0	882.9 ± 237.5
Range	17713.8 - 40387.1	47.2-116.0	69.3-108.6	142100.1 - 1031762.1	217.7-341.4	0.9 - 1.4	8.9 - 16.3	1500.2- 4949.7	1.3 - 5.4	231.5 - 1292.6

Note: ratio = plant height/width.

82.77% of phenotypic variations. PCA based on various ploidy levels among all the guayule accessions showed two clustered groups, one mainly comprising triploid accessions and another one consisting of tetraploid accessions (Fig. 2). Since the sample size of diploids and pentaploids were too small, no obvious clustering of those accessions was observed. Mixed ploids dispersed within the first three principle components as shown in Fig. 2. The inter-trait correlations showed that traits related to plant biomass such as plant height, plant width, plant

volume, plant perimeter and height to width ratio, were found to be significantly correlated with each other (Table 5), suggesting the possibility of selecting multiple traits at once in order to improve plant biomass. Resin and rubber content were not significantly correlated to biomass-related traits (Table 5). However, resin/rubber yield was significantly correlated with dry biomass and resin/rubber content, indicating that resin or rubber yield can be improved via either improving dry weight biomass or resin/rubber content or both. No correlations

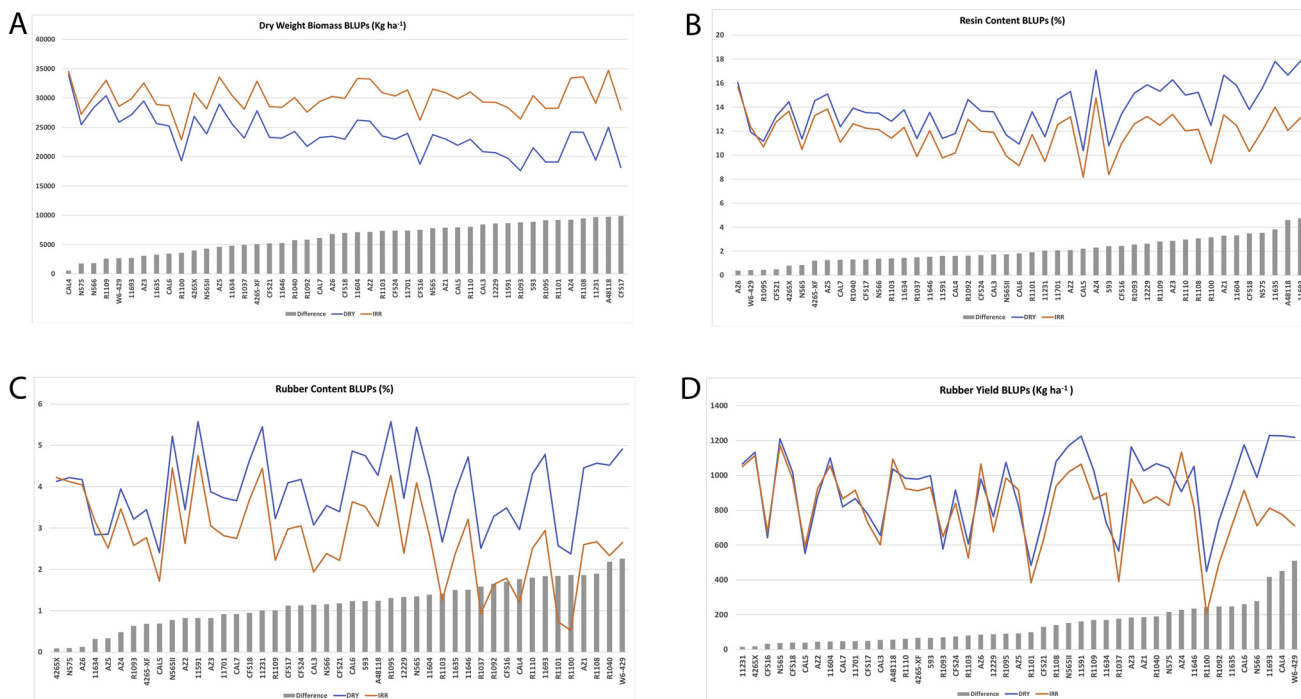
**Table 3**  
Variance components and heritability estimates for 10 phenotypic traits of USDA guayule collection based on transformed data.

Source	Plant height	Width	Plant volume	Plant perimeter	Ratio	Resin Content	Rubber Content	DWY	Resin Yield	Rubber Yield
Genotype	274.200***	88.370***	4.389E+10***	872.700***	0.015***	4.403***	1.182***	32671323***	1063848***	74071.930***
Environment (env)	1.927	0.000	0.000	0.000	0.001	1.904***	0.7589***	17286745***	6036.000	6593.93*
GEI	0.000	0.844	770.400	8.229	0.002	0.000	0.000	0.000	0.000	0.000
Rep (env)	26.630	0.000	1.039E+11	0.008	0.009	3.037	1.877	15555710.000	52794.000	20539.430
Block (rep*env)	82.250	16.190	1.954E+11	116.500	0.028	2.509	1.297	38342674.000	208573.000	44.970
Entry-mean heritability	0.933	0.660	0.784	0.660	0.692	0.884	0.892	0.657	0.810	0.788

Note: GEI = genotype-by-environment interactions; Rep = replication. Rep (env) means replicates nested in environments; Block (rep\*env) means blocks nested in replicates across environments.

**Table 4**  
Simple statistics summary of 10 different guayule phenotypic traits under water-stressed (DRY) and well-watered (IRR) conditions.

Phenotypic Trait	Mean		Range		Kurtosis		Skewness	
	Water-stressed (DRY)	Well-watered (IRR)	Water-stressed (DRY)	Well-watered (IRR)	Water-stressed (DRY)	Well-watered (IRR)	Water-stressed (DRY)	Well-watered (IRR)
Plant height (cm)	80.6 ± 2.09	78.1 ± 2.50	57.2 - 117.4	40.7 - 111.1	0.12	-0.48	0.38	0.04
Width (cm)	84.4 ± 0.78	85.5 ± 1.31	71.1 - 98.2	67.9 - 108.2	1.11	0.72	0.10	0.78
Plant Volume (cm <sup>3</sup> )	504412.4 ± 22608.37	493140.7 ± 29148.21	163587.0 - 907908.7	192583.0 - 966906.9	0.76	0.31	0.16	0.68
Plant Perimeter (cm)	265.0 ± 2.46	268.5 ± 4.12	223.4 - 308.7	213.3 - 339.8	1.11	0.72	0.10	0.78
Height/width ratio	1.1 ± 0.01	1.1 ± 0.02	0.94 - 1.2	0.9 - 1.4	-0.37	-0.42	0.17	0.32
Resin Conc. (%)	13.9 ± 0.30	11.9 ± 0.25	10.4 - 17.9	8.2 - 15.7	-0.81	-0.07	0.07	-0.32
Resin Yield (Kg ha <sup>-1</sup> )	3373.9 ± 124.62	3564.4 ± 122.61	1831.9 - 4956.0	966.8-4968.1	-0.98	0.75	-0.05	-0.64
Rubber Conc. (%)	3.9 ± 0.13	2.8 ± 0.15	2.4 - 5.6	0.5 - 4.8	-0.77	-0.21	0.05	-0.15
Rubber Yield (Kg ha <sup>-1</sup> )	927.7 ± 32.69	826.4 ± 32.01	448.5 - 1229.2	204.7 - 1173.2	-0.68	0.52	-0.55	-0.80
Dry weight biomass (Kg ha <sup>-1</sup> )	23829.3 ± 515.32	30065.6 ± 360.27	17591.8 - 33966.8	22902.7 - 34761.9	0.57	0.57	0.47	-0.18



**Fig. 1.** Line plots showing Best Linear Unbiased Predictions (BLUPs) of guayule accessions for different phenotypic traits under water-stressed (DRY) and well-watered (IRR) conditions. A) BLUPs of guayule accessions for dry weight biomass under DRY and IRR conditions; B) BLUPs of guayule accessions for resin content under DRY and IRR conditions; C) BLUPs of guayule accessions for rubber content under DRY and IRR conditions; D) BLUPs of guayule accessions for rubber yield under DRY and IRR conditions. *Difference informs the difference between two different environments for each accession.*

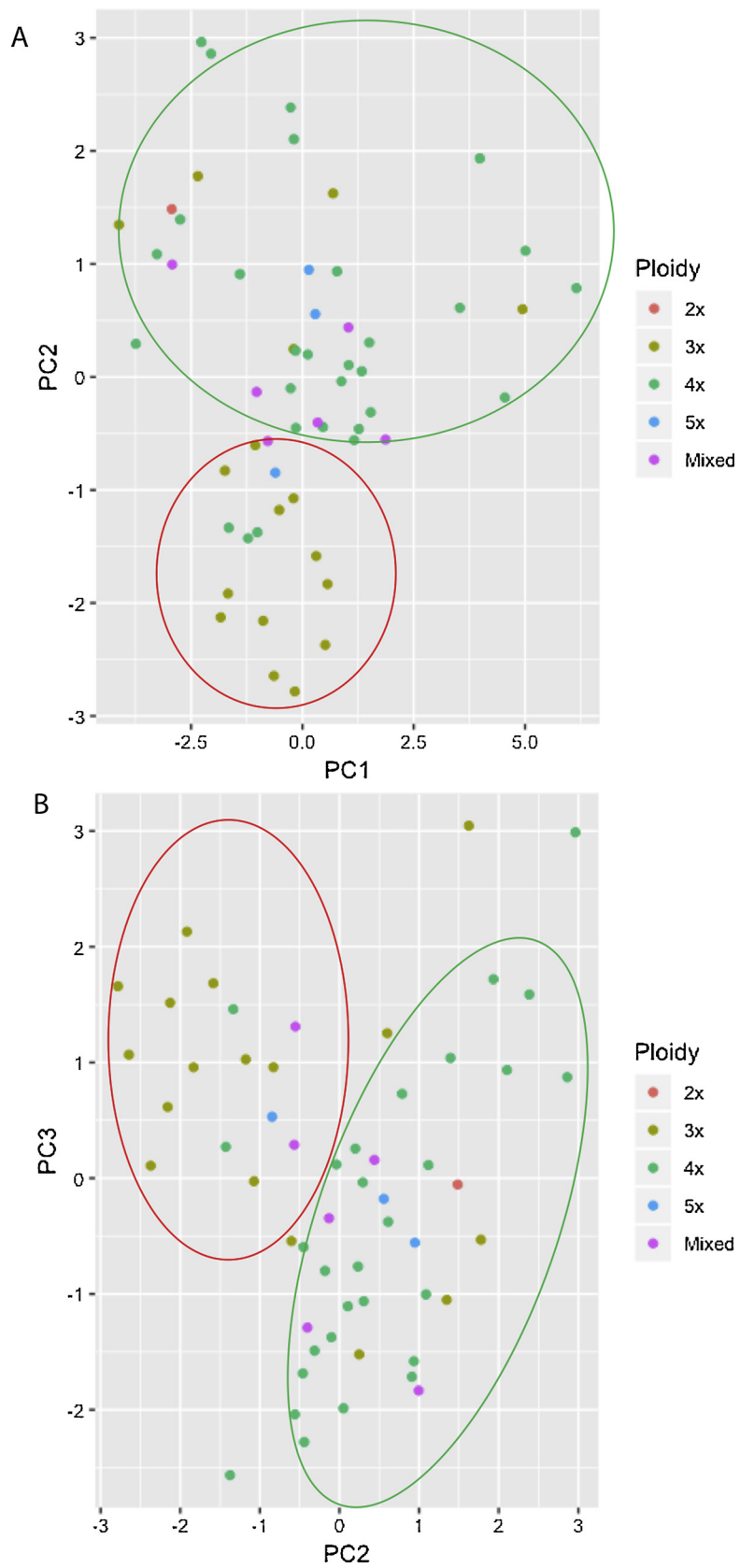


Fig. 2. Principle component analysis (PCA) based on phenotypic traits relative to ploidy levels showed two clustered groups within studied guayule accessions.



**Table 5**  
Pearson's inter-trait correlation coefficients among 10 different phenotypic traits in USDA guayule collection.

	Dry weight biomass	Plant height	Width	Plant volume	Plant perimeter	Ratio	Resin Content	Resin yield	Rubber Content	Rubber yield	Ploidy
Dry weight biomass	1.000	<0.0001***	<0.0001****	<0.0001****	<0.0001****	<0.05*	ns	<0.0001****	ns	<0.001**	ns
Plant height	0.605	1.000	<0.0001****	<0.0001****	<0.0001****	<0.0001****	ns	<0.01**	ns	ns	ns
Plant width	0.626	0.711	1.000	<0.0001****	<0.0001****	ns	ns	<0.01**	ns	ns	ns
Plant volume	0.658	0.884	0.951	1.000	<0.0001****	<0.05*	ns	<0.01**	ns	ns	ns
Plant perimeter	0.626	0.711	1.000	0.951	1.000	ns	ns	<0.01**	ns	ns	ns
Ratio	-0.268	-0.716	-0.039	-0.335	-0.039	1.000	ns	ns	ns	ns	ns
Resin Cont.	0.194	0.024	-0.063	-0.027	-0.063	-0.135	1.000	<0.0001****	ns	ns	<0.01**
Resin Yield	0.769	0.390	0.364	0.401	0.364	-0.239	0.742	1.000	ns	ns	<0.01**
Rubber Cont.	-0.119	-0.131	-0.161	-0.145	-0.161	0.015	-0.127	-0.204	1.000	<0.0001****	<0.05*
Rubber Yield	0.430	0.194	0.199	0.224	0.199	-0.109	0.003	0.252	0.828	1.000	0.33
Ploidy	0.244	0.070	0.152	0.139	0.152	-0.010	0.384	0.431	-0.289	-0.140	1.000

Note: p-values were provided on the top half of the diagonal division line. Correlation coefficient values were provided on the bottom half of the diagonal division line.

have been observed between resin-related traits and rubber-related traits, indicating that the selection for resin and rubber improvements is independent. In addition, resin content and resin yield were positively correlated with ploidy level while rubber content was negatively correlated with ploidy level (Table 5). Combined with the identified clusters (triploids and tetraploids) in PCA (Fig. 2), the correlation coefficients suggested that polyploidy breeding can be considered in future guayule breeding when specific breeding purposes are identified. Moreover, an increase was observed in dry weight biomass when increasing ploidy levels, even if the differences between these ploidy levels are not statistically significant (Table S3). A variation between triploid and tetraploid plants in rubber content and yield (Table S3) might be due to the unbalanced sample size between the two ploidy levels. In our study, CAL3, a diploid germplasm in the collection, had the lowest dry biomass, rubber content and yield (Table S3). This makes sense since most commercial guayule are polyploids. However, since the limitation of sample size in our study, whether the increase of ploidy levels will always lead to positive results is not known. More sample size within each ploidy level should be considered in future experiment.

#### 4. Conclusion

In conclusion, our study is the first to explore phenotypic diversity in important agronomic traits in 56 USDA guayule accessions that include improved germplasm as well as wild accessions collected from natural habitats at Mexico and United States. The results summarized their responses when grown under different irrigation regimes, in which water-stressed condition increased resin and rubber accumulation while well-watered condition increased dry weight biomass. The study also estimated moderate to high entry-mean heritability for the studied traits, suggesting the feasibility of selection to improve genetic gain and reasonable expectations for breeding targets at early generations of breeding cycles. Significant correlations between biomass-related traits and resin (or rubber) yield might suggest the possibility of selection for multiple traits at one time. The PCA results and significant correlations between ploidy level and resin (or rubber) content indicated the possibility of polyploidy breeding to achieve specific breeding goals. This study lays the foundation for guayule breeding efforts to select parental candidates in breeding programs to grow guayule under different growing conditions and to achieve different production goals.

#### Declaration of Competing Interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment,

consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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#### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.indcrop.2019.111867>.

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