



# Phenotypic variations, heritability and correlations in dry biomass, rubber and resin production among guayule improved germplasm lines



Hussein Abdel-Haleem<sup>a,\*</sup>, Mike Foster<sup>b</sup>, Dennis Ray<sup>c</sup>, Terry Coffelt<sup>a</sup>

<sup>a</sup> USDA-ARS, US Arid-Land Agricultural Research Center, 21881 North Cardon Lane, Maricopa, AZ, 85138, USA

<sup>b</sup> Texas AgriLife Research, P.O. Box 1549, Pecos, TX, 79772, USA

<sup>c</sup> The School of Plant Sciences, The University of Arizona, 1140 E. South Campus Drive, Tucson, AZ, 85721, USA

## ARTICLE INFO

### Keywords:

Guayule  
Dry biomass  
Genetic variability  
Heritability  
Resin  
Rubber

## ABSTRACT

Gauyaule (*Parthenium argentatum* Gray) originated in the Southern Texas and Northern Mexico deserts, which suggests it as a good candidate for arid and semi-arid sustainable agricultural systems to produce natural rubber and other industrial byproducts. Continued improvement of guayule for higher biomass, rubber and resin production, and high resistance to environmental stresses, are required and necessary to meet the growing demand of guayule industry. The current study was conducted to evaluate the phenotypic variations in dry biomass, and rubber and resin content and production in nine improved guayule germplasm. The genotypes were grown in six environments and were harvested at one, two, and three years old. Results indicated that these germplasm has a good genetic variability at different growth stages in biomass rubber and resin production. The widest phenotypic variations were observed in plants harvested two year after transplanting. Significant genotypic by environmental interactions of these traits, suggest that evaluating guayule germplasm in multiple environments in order to select lines with the desired level of these traits is required. High heritability estimates of these traits suggest that selection is feasible in the first three years in general, and the highest after two years of transplanting, were plants reached maximum growth homogeneity with low competition among plants in the growing area. Positive correlation coefficients among these traits suggest the possibility of selection for more than one trait at a time, which could reduce guayule developing time and efforts to meet different industrial demands such as rubber and byproducts in the breeding programs.

## 1. Introduction

Guayule (*Parthenium argentatum* Gray), a woody desert shrub native to southern Texas and northern Mexico, is under re-development in the southwestern USA as a source of natural rubber, organic resins, latex and biomass for energy and fuel production (Boateng et al., 2016; Chow et al., 2008; Cornish et al., 1999; Nakayama, 2005; Ray et al., 2005; Siler and Cornish, 1994; Teetor et al., 2009). Besides the main products, high value rubber and resin, the bagasse (85–90% of the biomass) provides a potential feedstock for biofuel production (Nakayama, 2005). This crop provides an alternative and complimentary source to imported natural rubber from *Hevea*, the tree grown in Southeast Asia, and has potential to help stabilize the associated price volatility.

Rubber synthesis in guayule occurs primarily during the winter months when the plants enter a state of vegetative and reproductive dormancy (Appleton and Van Staden, 1989). Low night temperatures serves as important environmental factor that initiate rubber synthesis by shifting plants into the dormant stage and redirecting carbon from

vegetative and reproductive growth to storage in the stems and roots (Appleton and Van Staden, 1989, 1991; Benedict et al., 2008; Cornish and Backhaus, 2003; Cornish and Scott, 2005; Paterson-Jones et al., 1990; Veatch et al., 2005). During summer, higher day temperatures appear to increase the amount of rubber synthesized in vegetatively dormant plants (Blohm, 2005; Ponciano et al., 2012; Sundar and Reddy, 2001; Veatch-Blohm et al., 2007), suggesting that rubber synthesis might be carbon limited. Increasing temperatures and photoperiod increase the rate of photosynthesis and biomass accumulation, until leaf temperatures exceed a certain critical temperature, above which heat stress ensues (Appleton and Van Staden, 1989), which can affect plant growth. The effects of heat stress on photosynthesis and biomass production in guayule have not been characterized in detail, but will almost certainly be related to water availability, since water provides the driving force for heat avoidance via evapotranspiration.

As a native of the Chihuahua desert, guayule is considered a drought tolerant shrub (Foster and Coffelt, 2005; Ray et al., 2005), where guayule survives on about 250–380 mm of annual rainfall in its native

\* Corresponding Author.

E-mail address: [Hussein.abdel-haleem@ARS.USDA.GOV](mailto:Hussein.abdel-haleem@ARS.USDA.GOV) (H. Abdel-Haleem).

**Table 1**

Mean squares for genotypes (MS<sub>G</sub>), locations (MS<sub>E</sub>), genotypes environment (MS<sub>G×E</sub>) and heritability (h<sup>2</sup>) for dry biomass (kg ha<sup>-1</sup>), rubber content (%), total rubber production (kg ha<sup>-1</sup>), resin content (%) and total resin production (kg ha<sup>-1</sup>) over combined locations for 1-year, 2-year and 3-year old plants. \*\* refers to significant at P < 0.001, and ns refers to non-significant.

Parameter	Dry Biomass	Rubber%	Total Rubber	Resin (%)	Total Resin
(kg ha <sup>-1</sup> )	%	(kg ha <sup>-1</sup> )	(%)	(kg ha <sup>-1</sup> )	
<b>1-year old plants</b>					
MS <sub>E</sub>	34257999.5**	89.4**	75675**	34.0**	43195**
MS <sub>G</sub>	5720853**	9.8**	8807**	8.1**	45110**
MS <sub>G×E</sub>	2513328 <sup>ns</sup>	0.9**	6127 <sup>ns</sup>	0.7 <sup>ns</sup>	8285 <sup>ns</sup>
h <sup>2</sup>	0.76	0.64	0.52	0.72	0.92
<b>2-year old plants</b>					
MS <sub>E</sub>	316137453**	32.5**	378377**	11.6**	1482395**
MS <sub>G</sub>	185752187**	13.4**	44037**	7.9**	947715**
MS <sub>G×E</sub>	33180211**	0.8**	30635*	1.0**	143370**
h <sup>2</sup>	0.80	0.94	0.60	0.86	0.83
<b>3-year old plants</b>					
MS <sub>E</sub>	368409264**	11.0**	491058**	9.4**	1787935**
MS <sub>G</sub>	63198293**	12.9**	76571**	10.6**	498220**
MS <sub>G×E</sub>	26702220**	1.7**	35446 ns	1.2**	150000**
h <sup>2</sup>	0.58	0.87	0.54	0.89	0.70

**Table 2**

Means of dry biomass (kg ha<sup>-1</sup>) for nine guayule genotypes for 1-year, 2-year and 3-year old plants. LSD refers to least significant differences at 0.05 level.

	Variety	Location						Average
		MAC	MER	Pecos	YaMe	YaVa	CAC	
<b>1-year plants</b>		<b>5880</b>	-	<b>8964</b>	-	-	-	<b>7422</b>
	N-565	3167	-	10101	-	-	-	6634
	11591	3860	-	5421	-	-	-	4640
	Az-1	7192	-	14359	-	-	-	10775
	Az-2	6406	-	9368	-	-	-	7887
	Az-3	8350	-	8399	-	-	-	8374
	Az-4	4618	-	5856	-	-	-	5237
	Az-5	5858	-	7098	-	-	-	6478
	Az-6	6114	-	9827	-	-	-	7970
	Az-101	7355	-	10253	-	-	-	8804
LSD <sub>(0.05)</sub>	2999	-	4540	-	-	-	2810	
<b>2-year plants</b>		<b>24069</b>	<b>23291</b>	<b>15603</b>	<b>12143</b>	<b>25217</b>	-	<b>20064</b>
N-565	13970	13860	12234	6429	9310	-	-	11161
11591	14070	10615	9827	5643	13730	-	-	10777
Az-1	27640	35700	20656	19039	39670	-	-	28541
Az-2	21870	27130	14677	13914	38850	-	-	23288
Az-3	42230	31460	21959	16143	28150	-	-	27988
Az-4	12530	16525	15740	8566	23430	-	-	15358
Az-5	25695	30450	-	10334	23920	-	-	22600
Az-6	33610	19785	13031	12597	15680	-	-	18940
Az-101	27210	24090	16706	16619	34215	-	-	23768
LSD <sub>(0.05)</sub>	10703	16081	9247	8589	13610	-	-	5205
<b>3-year plants</b>		<b>11465</b>	<b>7343</b>	-	<b>16122</b>	<b>24040</b>	-	<b>14743</b>
N-565	5519	4483	-	7435	14240	-	-	7919
11591	5961	5548	-	6665	10955	-	-	8144
Az-1	15617	9485	-	20395	24740	-	-	17559
Az-2	11888	7829	-	16860	30360	-	-	16734
Az-3	17848	10929	-	15350	32615	-	-	19186
Az-4	6328	6202	-	25395	12980	-	-	12726
Az-5	17208	8391	-	19980	17895	-	-	15868
Az-6	10369	5321	-	15685	42370	-	-	18436
Az-101	12446	7902	-	17335	30205	-	-	16972
LSD <sub>(0.05)</sub>	7135	4779	-	13693	17970	-	-	5850

setting (Bekaardt et al., 2010). Agronomic studies have shown that increasing irrigation decreases rubber concentration per plant, but increases overall rubber yields as a result of increasing plant biomass (Hunsaker and Elshikha, 2017). Drought-stressed plants had a greater contribution of stem biomass to overall biomass and a reduced stem

diameter with higher bark to wood ratio, which could account for the higher rubber concentration per plant (Angulo-Sánchez et al., 2002; Chow et al., 2008; Veatch-Blohm and Ray, 2005).

As a perennial crop, one of the major challenges for applying and improving guayule through molecular breeding and biotechnology strategies is to shorten the time required to assess new phenotypes and germplasm. With the new germplasm, assessment of rubber production in field-grown plants requires 2–3 years, which can be complicated by the strong influence of environmental effects (Coffelt and Ray, 2010; Coffelt et al., 2009; Dierig et al., 2001; Foster and Coffelt, 2005). Another hurdle for guayule genetic improvement is its asexual reproduction nature (apomixis) assuming low or zero genetic variation from generation to generation. Guayule is facultative apomictic plant where both asexual and sexual reproductive system are present (Ray et al., 1990). Together, the facultative nature and the presence of high amount of heterozygosity in individual plants could result in considerable genetic variation whenever sexual reproduction succeed (Powers and Rollins, 1945; Ray et al., 1990; Ray et al., 1993; Rollins, 1945, 1949).

The target of guayule breeding programs is to increase genetic gain of rubber, resin and related traits by continuously selecting superior genotypes under different production areas and environments. Heritability estimate is used to predict the genetic gain (Holland et al., 2003), where high heritability estimates indicate the feasibility of selection for a trait of interest during the early generations of breeding programs. Broad-sense heritability estimates were calculated for plant height, width, rubber and resin traits on data collected from individual open pollinated and clonally propagated two years old plants planted in a single environment (Dierig et al., 2001). For more efficient non-biased heritability estimates, data should be taken from multiple replications and environments to assess the environmental effects, as well G×E interactions. The objectives of current study were to evaluate nine improved guayule germplasm in a diverse set of environments, including possible guayule economic production regions in Arizona and Texas, USA, for dry biomass, rubber and resin production traits, explore the correlations among these traits and their roles in potential selection procedure, estimate the environmental effects and GXE interactions of these traits, and estimate the heritability estimates for these traits and compare between these estimates at different guayule harvests (age).

**2. Materials and methods**

**2.1. Plant materials**

Nine guayule germplasm were used in the current study. The lines AZ-1, AZ-2, AZ-3, AZ-4, AZ-5 and AZ-6 are improved guayule germplasm for high rubber and resin concentration and biomass (Ray et al., 1999). The AZ-101 line is a selection of a naturally occurring cross between guayule line 11591 and a *Parthenium tomentosum*, a related species with high biomass (Ray et al., 2005). The lines N-565 and 11591 are USDA lines used as a standard check in guayule genetic studies (Ray et al., 2005). The trials were conducted at six locations across Arizona and Texas, USA as follow: Marana Agricultural Center (MAR), Univ. of Arizona at Marana (32° 27'40"N, 111° 14'00"W, 601 m above sea level (asl)), Maricopa Agricultural Center (MAC), Univ. of Arizona at Maricopa, Arizona (33° 04'07"N, 111° 58'18"W, 361 m asl), Yuma Agricultural Center at Yuma where experiments were conducted at Yuma-Mesa (YaMe) (32° 36'43"N, 114° 38'02"W, 58 m asl) and Yuma-Valley (YaVa) (32° 42'45"N, 114° 42'18"W, 32 m asl), Campus Agricultural Center (CAC), Univ. of Arizona, at Tucson, AZ (32° 16' 49" N, 110° 56' 45' W, 713 m asl) and the Texas Agricultural Experiment Station at Pecos, TX (31°22'45.6"N 103°37'43.6"W, elevation 830 m a.s.l). These locations has different soil series ant textures, where soil at MAC is Casa Grande series (fine-loamy, mixed, hyperthermic Typic Natrargids), at MAR is an Agua series (dominantly loam to gravelly, sandy loam, on flood plains and alluvial fans), at YaMe is Superstition

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