Industrial Crops & Products 112 (2018) 691–697

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

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

Hussein Abdel-Haleem[a,*], Mike Foster[b], Dennis Ray[c], Terry Coffelt[a]

[a] USDA-ARS, US Arid-Land Agricultural Research Center, 21881 North Cardon Lane, Maricopa, AZ, 85138, USA
[b] Texas AgriLife Research, P.O. Box 1549, Pecos, TX, 79772, USA
[c] 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 gentypes 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 (H. Abdel-Haleem).

https://doi.org/10.1016/j.indcrop.2017.12.072
Received 30 September 2017; Received in revised form 14 December 2017; Accepted 31 December 2017
Available online 05 January 2018
0926-6690/ Published by Elsevier B.V.

Table 1
Mean squares for genotypes (MS_G), locations (MS_E), genotypes environment (MS_{GxE}) and heritability (h^2) for dry biomass (kg ha^{-1}), rubber content (%), total rubber production (kg ha^{-1}), resin content (%) and total resin production (kg ha^{-1}) 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^{-1})	%	(kg ha^{-1})	(%)	(kg ha^{-1})	
1-year old plants					
MS_E	34257999.5**	89.4**	75675**	34.0**	43195**
MS_G	5720853**	9.8**	8807**	8.1**	45110**
MS_{GXE}	2513328^{ns}	0.9**	6127^{ns}	0.7^{ns}	8285^{ns}
h^2	0.76	0.64	0.52	0.72	0.92
2-year old plants					
MS_E	316137453**	32.5**	378377**	11.6**	1482395**
MS_G	185752187**	13.4**	44037**	7.9**	947715**
MS_{GXE}	33180211**	0.8**	30635*	1.0**	143370**
h^2	0.80	0.94	0.60	0.86	0.83
3-year old plants					
MS_E	368409264**	11.0**	491058**	9.4**	1787935**
MS_G	63198293**	12.9**	76571**	10.6**	498220**
MS_{GXE}	26702220**	1.7**	35446 ns	1.2**	150000**
h^2	0.58	0.87	0.54	0.89	0.70

Table 2
Means of dry biomass (kg ha^{-1}) 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						
		MAC	MER	Pecos	YaMe	YaVa	CAC	Average
1-year plants		**5880**	–	**8964**	–	–	–	**7422**
	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**
	$LSD_{(0.05)}$	*2999*		*4540*				*2810*
2-year plants		**24069**	**23291**	**15603**	**12143**	**25217**	–	**20064**
	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**
	$LSD_{(0.05)}$	*10703*	*16081*	*9247*	*8589*	*13610*	–	*5205*
3-year plants		**11465**	**7343**	–	**16122**	**24040**	–	**14743**
	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**
	$LSD_{(0.05)}$	*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 it's 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 GxE 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

Table 3
Means of resin content (%) of nine improved guayule germplasm in individual locations.

	Variety	Location						
		MAC	MER	Pecos	YaMe	YaVa	CAC	*Average*
1-year plants		**7.42**	**5.92**	**6.11**	**4.54**	**4.66**	**5.48**	**5.69**
	N-565	5.50	5.68	4.95	3.98	4.20	4.77	**4.84**
	11591	8.78	6.53	4.53	3.53	3.53	4.77	**5.27**
	Az-1	5.30	5.35	6.40	4.55	4.78	5.80	**5.36**
	Az-2	7.55	6.20	6.78	4.63	4.85	5.57	**5.93**
	Az-3	7.98	5.40	6.60	4.45	4.80	5.60	**5.80**
	Az-4	8.05	6.13	6.28	4.85	5.15	5.80	**6.04**
	Az-5	7.70	6.38	6.40	4.80	4.88	5.60	**5.96**
	Az-6	8.25	5.98	6.48	5.10	4.65	5.87	**6.05**
	Az-101	7.68	5.63	6.58	4.98	5.13	5.53	**5.92**
	$LSD_{(0.05)}$	*1.36*	*1.07*	*0.90*	*0.76*	*0.68*	*0.73*	*0.37*
2-year plants		**7.01**	**6.26**	**5.97**	**5.76**	**5.76**	–	**6.15**
	N-565	5.25	5.65	5.75	5.39	5.38	–	**5.48**
	11591	5.68	5.15	5.40	4.47	4.45	–	**5.03**
	Az-1	8.48	6.63	5.73	6.12	6.13	–	**6.61**
	Az-2	7.90	6.73	6.63	5.69	5.68	–	**6.52**
	Az-3	6.93	6.45	6.23	5.52	5.50	–	**6.12**
	Az-4	8.35	6.43	6.35	6.18	6.15	–	**6.69**
	Az-5	5.90	6.55	–	6.09	6.10	–	**6.16**
	Az-6	7.22	6.40	5.58	6.33	6.35	–	**6.38**
	Az-101	7.35	6.35	6.08	6.10	6.10	–	**6.40**
	$LSD_{(0.05)}$	*1.41*	*0.78*	*0.99*	*0.72*	*0.70*		*0.39*
3-year plants		**7.91**	**6.88**	–	**7.51**	**6.85**	–	**7.28**
	N-565	6.15	7.28	–	6.10	5.10	–	**6.16**
	11591	6.08	5.85	–	5.68	4.95	–	**5.64**
	Az-1	8.30	6.75	–	8.10	7.63	–	**7.69**
	Az-2	8.58	7.08	–	7.93	7.35	–	**7.73**
	Az-3	8.65	6.85	–	7.95	6.85	–	**7.58**
	Az-4	9.45	7.18	–	8.03	7.30	–	**7.99**
	Az-5	7.77	7.43	–	7.55	7.65	–	**7.60**
	Az-6	8.48	7.13	–	8.30	7.25	–	**7.79**
	Az-101	7.70	6.38	–	7.93	7.55	–	**7.39**
	$LSD_{(0.05)}$	*1.27*	*0.66*		*0.82*	*0.89*		*0.35*

series (sandy, alluvial fans and sand dunes), at YaVa is a Holtville series – Kofa series (clayey soils), at CAC is a sandy loam type, and Pecos is a Hoban silty clay loam series (fine-silty, mixed, thermic Ustollic Calciorthids).

At each location, seeds of each genotype 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 two rows 10 m long with one meter between two rows, and 0.30 m between plants within a row. Transplanting time was May 2, 2002 to May 30, 2002, based on the weather conditions at each location. The initial plant population was 27,000 seedlings ha^{-1}.

The experimental design was a randomized complete block design (RCBD) with four replications at each location. Seedlings were kept moist during plant establishment with sprinkler irrigation and then were irrigated every 2–3 weeks during the growing season at February to October, based on best agricultural recommendations at each location. Within each plot, randomly two guarded plants were harvested yearly at 1-year, 2-year, and 3-year old plants plots. Plants were cut within 5 cm of the soil surface and above ground plant material was chipped using the method described by Coffelt and Nakayama (2007). Harvested plants were analyzed for dry biomass weight, rubber and resin concentration (%), and yield (Kg ha^{-1}). Rubber and resin concentrations per plant were determined by a modification of the organic solvent based gravimetric method of Black et al. (1983) as described by Veatch-Blohm et al. (2006). Rubber and resin yields are derived from the rubber and resin concentration per plant multiplied by the total dry biomass harvested from plot.

2.2. Statistical analyses

For each harvesting time, 1-year, 2-year and 3-year, analysis of variance (ANOVA) of the data across locations was analyzed by SAS PROC GLM as randomized complete block experimental designs with locations, replicates and genotypes considered as random effects (Statistical Analysis System, SAS institute, 2003).

Variance components were estimated using the restricted maximum likelihood (REML) method of SAS PROC MIXED. Variance-component heritability estimates were calculated on an entry-mean basis (Holland et al., 2003; Nyquist and Baker, 1991) using the following equation: $h^2 = \sigma^2_G/[\sigma^2_G + (\sigma^2_{GXE}/e) + (\sigma^2_e/re)]$, where h^2 represents broad-sense heritability, $\sigma_G{}^2$ genotypic variance, σ^2_{GXE} genotype × locations variance, σ^2 error variance, r the number of replications, and e the number of locations. Because the data were unbalanced, the value for r was computed as the harmonic mean of the number of locations (Holland et al., 2003).

Pearson' phenotypic correlation coefficient analyses were performed for dry biomass weight, and rubber and resin concentrations and yields using means over the environments and PROC CORR in SAS (SAS, 2003).

3. Results and discussions

Guayule is gaining a wide attention as a supplemental source of natural rubber as well other bio-products such as hypoallergenic latex, resin, and bagasse. Since it is a desert shrub, guayule fits well as a commercial rubber crop in the arid and semi-arid regions of the Southwestern USA. That interest requires continuous improvement of guayule for these products through genetic enhancement, cultivar development and maximization of agronomic practices (Foster and Coffelt, 2005; Ray et al., 2005). As result and since 1970, new improved germplasm were released with higher rubber and resin concentrations, faster growth with high biomass and disease resistance (Estilai, 1985; Estilai, 1986; Ray et al., 1999; Tysdal et al., 1983).

To increase genetic gain of guayule rubber and resin and related traits, continuous selection under different production areas and environments is necessary. The phenotypic variations in dry biomass, and rubber and resin content and production were studied in nine guayule genotypes, that were planted at six environments and harvested after one, two and three years of transplanting. Combined analyses of variance (ANOVA) was conducted and the contribution of each source of variation was summarized in Table 1. In general, data indicated that GXE interactions had no significant effects during early plant growth compare to matured plants (Table 1), where the GXE interaction was non-significant for the dry biomass, resin and rubber production of 1-year old plants. The GXE interactions were significant at 2-year and 3-year old plants (Table 1). On other hand, there were significant GXE interactions in rubber and resin content in 1-year harvest as well subsequent harvests after two and three years (Table 1). Locations had significant effects on studied traits across plant ages (Table 1–6). For example the rubber contents for plants harvested after two years range from 2.69% at Yuma-mesa (YaMe) location to 5.06% at Maricopa (MAC) location (Table 5). The same results were noticed with biomass content (Table 2), resin content and production (Tables 3 and 4) and rubber production (Table 6). The present results are in agreement with previous reports that showed the significant effects of locations (environments) on guayule traits such as latex, rubber and biomass (Coffelt et al., 2009; Coffelt et al., 2005; Dierig et al., 2001). Harvesting time (plant age) had significant effect on the studied traits (Table 1), where rubber and resin contents increased with plant age (Tables 3 and 5). The rubber content from plants harvested after one, two and three years at MAC location were 5.56, 5.06 and 4.84%, respectively, while at MER location, the rubber contents were 3.51, 4.38 and 4.99%, respectively for the same harvests. The average biomass was greatly affected by plant age where it reached 5880 kg ha^{-1} after one year at MAC location, increased to 24069 kg ha^{-1} in 2-year plants, then declined to reach 11465 kg ha^{-1} in 3-year old plants (Table 2). Within genotypes, biomass was affected by plant age, for example the average dry biomass of AZ-2, a selection from 11591 (Ray et al., 1999), were 7887, 23288

Table 4
Means of total resin production (kg ha^{-1}) of nine improved guayule germplasm in individual locations.

	Variety	Location						
		MAC	MER	Pecos	YaMe	YaVa	CAC	*Average*
1-year plants		**439.9**	–	**549.4**	–	–	–	**494.6**
	N-565	169.5	–	511.7	–	–	–	**340.6**
	11591	201.9	–	244.2	–	–	–	**223.0**
	Az-1	627.9	–	911.8	–	–	–	**769.8**
	Az-2	475.4	–	627.3	–	–	–	**551.3**
	Az-3	662.0	–	548.8	–	–	–	**605.4**
	Az-4	355.9	–	358.9	–	–	–	**357.4**
	Az-5	442.5	–	441.5	–	–	–	**442.0**
	Az-6	501.8	–	637.8	–	–	–	**569.8**
	Az-101	522.0	–	663.0	–	–	–	**592.5**
	LSD$_{(0.05)}$	*210.3*		*277.5*				*85.6*
2-year plants		**1888.2**	**1497.1**	**935.7**	**716.5**	**1471.0**	–	**1301.7**
	N-565	724.9	778.0	705.5	347.3	501.3	–	**611.4**
	11591	781.2	548.5	534.8	251.5	619.3	–	**547.0**
	Az-1	2303.7	2454.0	1199.3	1169.3	2440.2	–	**1913.3**
	Az-2	1645.8	1832.5	956.3	791.5	2207.0	–	**1486.6**
	Az-3	3472.9	2065.5	1364.0	896.3	1537.0	–	**1867.1**
	Az-4	1023.0	1061.0	983.0	531.4	1451.9	–	**1010.1**
	Az-5	2332.5	1938.5	–	664.8	1423.0	–	**1589.7**
	Az-6	2728.0	1270.5	743.3	792.4	955.9	–	**1298.0**
	Az-101	1981.5	1525.5	999.3	1004.3	2103.8	–	**1522.9**
	LSD$_{(0.05)}$	*969.8*	*1098.3*	*573.1*	*550.8*	*835.0*		*338.2*
3-year plants		**914.8**	**512.0**	–	**1254.2**	**1685.4**	–	**1091.6**
	N-565	336.3	327.2	–	460.0	721.5	–	**461.2**
	11591	362.8	337.8	–	382.0	539.0	–	**405.4**
	Az-1	1328.2	685.3	–	1650.5	1886.0	–	**1387.5**
	Az-2	960.9	563.8	–	1346.5	2226.5	–	**1274.4**
	Az-3	1539.0	740.7	–	1219.0	2237.0	–	**1433.9**
	Az-4	588.7	444.4	–	2042.0	944.5	–	**1004.9**
	Az-5	1282.0	618.5	–	1542.5	1360.0	–	**1200.8**
	Az-6	887.8	382.5	–	1301.5	2960.0	–	**1383.0**
	Az-101	947.4	507.9	–	1344.0	2294.0	–	**1273.3**
	LSD$_{(0.05)}$	*520.0*	*351.5*		*1098.4*	*1261.3*		*311.6*

and 16734 kg ha^{-1} harvested from 1-year, 2-year and 3-year old plants, receptively, compared to 4640, 10777 and 8144 kg ha^{-1} dry biomass from 11591, a check genotype that was released during 1950s (Ray et al., 2005). Coffelt et al. (2005) indicated that improved guayule lines could reach their maximum growth after two years of transplanting then growth rates slowed-down thereafter, which could be related to planting distance and competition among plants (Dierig et al., 2001). Total rubber and resin production were higher in 2-year old plants compared to 1-year or 3-year old plants (Tables 4 and 6), suggesting that genetic selection for higher biomass, rubber, and/or resin production could be feasible during the first two years of guayule planting.

Within each plant age group, newer developed genotypes responded well for different environments and produced higher biomass, rubber and resin. Genotypes effects were significant ($P \leq 0.001$) for all studied traits and plant ages, suggesting that the impact of G × E detected in these traits appears to be minimally based on the relative variance components of these traits. Still the GXE interaction should not be ignored and warrants to evaluate guayule genotypes in multiple environments in order to select lines with desired traits. Genotypes were significantly different in dry biomass (Table 2), rubber and resin contents (Tables 3 and 5) and production (Tables 4 and 6) over the harvested plants ages and locations. There were genotypic differences among genotypes for these traits even in the presence of significant G × E interaction ($P \leq 0.001$) (Table 1). Dry biomass, rubber and resin contents and production in the recent improved germplasm (AZ-1,AZ-2, AZ-3, AZ-4, AZ-5, AZ-6 and AZ-101) responded better to environments (locations) compared to old released selections (N-565 and 11591). For example 2-yers old 11591 plants produced dry biomass ranged from 5643 kg ha^{-1} (YaVe location) to 14070 kg ha^{-1} (MAC location), while AZ-62 ranged from 13031 kg ha^{-1} (Pecos location) to 33610 kg ha^{-1} (MAC location) (Table 2). Resin content ranged from 4.45 (YaMe location) to 5.68% (MAC location) in 11591, while resin% in AZ-2 plants ranged from 5.68 (YaMe location) to 7.9% (MAC location), and from

Table 5
Means of rubber content (%) of nine improved guayule germplasm in individual locations.

	Variety	Location						
		MAC	MER	Pecos	YaMe	YaVa	CAC	avarage
1-year plants		**5.56**	**3.51**	**5.25**	**1.44**	**1.76**	**4.32**	**3.64**
	N-565	5.63	3.18	5.98	1.78	1.78	5.23	**3.93**
	11591	6.08	3.55	5.70	1.55	1.78	5.10	**3.96**
	Az-1	5.68	2.63	4.43	1.25	1.50	3.67	**3.19**
	Az-2	4.75	2.50	4.05	1.18	1.53	3.27	**2.88**
	Az-3	4.18	3.03	4.43	1.23	1.35	3.13	**2.89**
	Az-4	6.73	4.70	6.65	1.68	2.43	5.00	**4.53**
	Az-5	5.08	4.48	6.55	1.58	2.20	5.20	**4.18**
	Az-6	7.18	4.63	5.33	1.53	1.75	4.87	**4.21**
	Az-101	4.75	2.90	4.18	1.25	1.55	3.40	**3.00**
	LSD$_{(0.05)}$	*1.20*	*0.86*	*0.89*	*0.36*	*0.44*	*1.47*	*0.35*
2-year plants		**5.06**	**4.38**	**4.18**	–	**2.69**	–	**4.08**
	N-565	5.90	4.90	4.93	–	3.65	–	**4.84**
	11591	6.25	4.65	6.03	–	2.88	–	**4.95**
	Az-1	4.43	3.98	3.18	–	2.08	–	**3.41**
	Az-2	4.80	3.80	3.23	–	2.05	–	**3.47**
	Az-3	3.95	3.60	3.68	–	1.83	–	**3.26**
	Az-4	6.78	5.43	4.75	–	3.10	–	**5.01**
	Az-5	4.43	4.78	–	–	2.93	–	**4.04**
	Az-6	5.02	4.90	4.48	–	3.85	–	**4.56**
	Az-101	4.03	3.43	3.15	–	1.90	–	**3.13**
	LSD$_{(0.05)}$	*0.93*	*0.85*	*1.41*	–	*1.02*		*0.38*
3-year plants		**4.84**	**4.99**	–	**3.81**	**4.19**	–	**4.46**
	N-565	5.78	6.13	–	4.00	4.21	–	**5.03**
	11591	6.43	5.40	–	4.00	3.80	–	**4.91**
	Az-1	3.65	5.08	–	3.30	3.97	–	**4.00**
	Az-2	3.53	3.50	–	2.95	3.76	–	**3.43**
	Az-3	3.23	4.08	–	3.10	3.14	–	**3.39**
	Az-4	6.93	5.95	–	4.90	5.40	–	**5.79**
	Az-5	4.73	5.38	–	4.40	5.45	–	**4.99**
	Az-6	6.04	6.03	–	4.60	3.84	–	**5.13**
	Az-101	3.25	3.40	–	3.05	4.16	–	**3.46**
	LSD$_{(0.05)}$	*0.96*	*1.05*		*1.15*	*1.22*		*0.50*

Table 6
Means of rubber production (kg ha^{-1}) of nine improved guayule germplasm in individual locations.

	Variety	Location						
		MAC	MER	Pecos	YaMe	YaVa	CAC	Average
1-year plants		**307.9**	–	**452.9**	–	–	–	**380.4**
	N-565	172.3	–	604.7	–	–	–	**388.5**
	11591	232.7	–	306.8	–	–	–	**269.7**
	Az-1	400.5	–	638.0	–	–	–	**519.3**
	Az-2	291.0	–	383.8	–	–	–	**337.4**
	Az-3	335.8	–	370.5	–	–	–	**353.1**
	Az-4	305.9	–	386.6	–	–	–	**346.3**
	Az-5	284.0	–	458.9	–	–	–	**371.5**
	Az-6	432.2	–	511.8	–	–	–	**472.0**
	Az-101	316.7	–	414.8	–	–	–	**365.7**
	$LSD_{(0.05)}$	*126.8*		*208.4*				*60.8*
2-year plants		**1255.8**	**967.4**	**612.2**	–	**608.3**	–	**861.0**
	N-565	819.4	683.5	597.3	–	339.5	–	**609.9**
	11591	873.7	497.2	597.9	–	399.2	–	**592.0**
	Az-1	1212.7	1386.9	660.0	–	860.2	–	**1029.9**
	Az-2	1011.9	1012.4	462.5	–	830.0	–	**829.2**
	Az-3	2005.7	1156.9	843.4	–	500.7	–	**1126.7**
	Az-4	830.2	911.5	715.5	–	721.0	–	**794.5**
	Az-5	1621.5	1290.8	–	–	629.7	–	**1180.6**
	Az-6	1855.4	952.9	531.5	–	542.3	–	**970.5**
	Az-101	1072.3	814.8	489.8	–	652.8	–	**757.4**
	$LSD_{(0.05)}$	*563.4*	*546.4*	*383.7*	–	*360.9*		*183.0*
3-year plants		**492.5**	**342.7**	–	**618.5**	**956.3**	–	**602.5**
	N-565	311.7	275.9	–	301.3	634.8	–	**380.9**
	11591	379.8	257.3	–	266.9	414.8	–	**329.7**
	Az-1	571.2	436.0	–	678.0	989.2	–	**668.6**
	Az-2	398.9	275.0	–	499.9	1153.6	–	**581.9**
	Az-3	557.9	438.3	–	475.2	1075.7	–	**636.8**
	Az-4	421.5	363.8	–	1202.2	696.2	–	**670.9**
	Az-5	749.5	436.9	–	928.3	895.6	–	**752.6**
	Az-6	641.8	326.2	–	696.3	1486.1	–	**787.6**
	Az-101	400.0	275.4	–	518.3	1260.6	–	**613.6**
	$LSD_{(0.05)}$	*266.3*	*190.4*		*712.5*	*695.2*		*174.5*

6.08 (Pecos location) to 7.35% (MAC location) for AZ-101 plants (Table 3). The same trend was observed in these genotypes for resin production (Table 4), where 11591 plants accounted for lower resin production compare to newer released genotypes (AZ-2 and AZ-101). There was a trend for greater rubber production in 11591 than its off-spring (AZ-2), and other improved genotypes (Table 5), but was lower than its off-spring, AZ-101. In other hand, higher rubber production in newer guayule lines compare to 11591 (Table 6), could be direct relation of higher plant dry weight as well faster growth potential (Ray et a., 1999). The phenotypic variations within the studied guayule genotype group suggests the possibility of different genetic make-up of these genotypes (Blohm, 2005; Coffelt et al., 2005; Ray et al., 1999; Ray et al., 2005) where AZ-2 and AZ-3 were developed from individual plant selections from 11591 (Ray et al., 1999), and AZ-101 is a selection of a naturally occurring cross between 11591 and *Parthenium tomentosum* plants (Ray et al., 2005). In the present study, these genotypes out yielded their progenitor (11591) suggesting that there is chances for considerable genetic variations to capitalize on the new recombinants resulting from either or both reduction and fertilization of the embryo Ray (Dierig et al., 2001; Ray et al., 2005, 1993) even though with the presence of facultative apomictic nature, and that could help developing new breeding lines. Pervious report indicated the possibility of inducing new recombinants due to the presence of high amount of heterozygosity in individual plants (Dierig, 1987; Powers and Rollins, 1945; Rollins, 1949).

Heritability is an estimate of the variation in a phenotypic trait and the contribution of genetic component of variance, where high heritability estimates indicates the high genetic contribution in trait and the feasibility of selection for that trait during the early generations. In current study, heritability estimates were high to moderate for studied traits (Table 1). The heritability estimates were affected by harvested plant ages, where higher heritability estimates were calculated for the traits at 2-year old, compare to that were calculated form 1-year and 3-year old plants. Dierig et al. (2001) concluded that after third year of guayule growth, the estimated heritability of plant height and width were abolished due to masking the genetic effects by environment, due to plant competition. In current study, heritability estimate for dry biomass for plants harvested after one year was 0.76, and increased to be 0.80 for 2-years old plants and declined to reach 0.58 after 3 years of growth (Table 1). This finding suggests that selecting for higher biomass will be more effective during the first two years of guayule's growth. Our data suggest that the selection for economically important guayule traits, such as rubber and resin content and production, is effective during the second year of growth. For example, the heritability for rubber content was 0.94 compared to 0.64 and 0.87 for 1-year and 3-year old plants, respectively. For resin, heritability estimate for 2-year plants was 0.86 compare to 0.72 and 0.89 for 1-year and 3-yers plant old, respectively. Since biomass is correlated with resin and rubber production (Table 7), heritability estimated of those traits were moderate ($h^2 = 0.60$) to high ($h^2 = 0.94$). Dierig et al. (2001) estimated the heritability for rubber ($h^2 = 0.74$) and resin ($h^2 = 0.53$) for 2 years plants only. The different heritability estimates in the two studies could be explained by different sample size, number of locations and estimation methods used. Dierig et al. (2001) estimated heritability from data collected from individual clonally propagated plants planted in a single environment, while current estimates are based on the data collected on total plot values of multiple-plot and several environments, which include the GXE component.

In guayule, as well other plants, plant breeding programs are targeting more than one trait to improve at one time. To explore this possibility, we conducted Pearson's phenotypic correlation coefficients analyses among studied traits as summarized in Table 7. Our data showed that rubber and resin production were positively and highly correlated with dry biomass, (r = 0.85 and r = 0.97, respectively) after

Table 7
Pearson' correlation coefficients (r) of dry biomass (kg ha^{-1}), rubber content (%), total rubber production (kg ha^{-1}), resin content (%) and total resin production (kg ha^{-1}) over combined locations for 1-year, 2-year and 3-year old plants, r values are above the horizontal axis, corresponding *p*-values are below the horizontal axis.

	Year 1					Year 2					Year 3				
	Dry Biomass	Resin	Rubber	Total Resin	Total Rubber	Dry Biomass	Resin	Rubber	Total Resin	Total Rubber	Dry Biomass	Resin	Rubber	Total Resin	Total Rubber
Dry Biomass		**−0.153**	**−0.492**	**0.929**	**0.86**		**0.139**	**−0.199**	**0.978**	**0.851**		**0.132**	**−0.372**	**0.983**	**0.934**
Resin content	0.198		**0.612**	**0.192**	**−0.138**	*0.05*		**0.268**	**0.306**	**0.286**	*0.11*		**0.1**	**0.278**	**0.18**
Rubber content	*< .0001*	*< .0001*		**−0.464**	**−0.026**	*0.006*	*0.0002*		**−0.121**	**0.271**	*< .0001*	*0.24*		**−0.348**	**−0.09**
Resin production	*< .0001*	*0.11*	*< .0001*		**0.787**	*< .0001*	*< .0001*	*0.094*		**0.889**	*< .0001*	*0.001*	*< .0001*		**0.935**
Rubber production	*< .0001*	*0.25*	*0.83*	*< .0001*		*< .0001*	*< .0001*	*< .0001*	*< .0001*		*< .0001*	*0.03*	*0.29*	*< .0001*	

two years of growth for rubber and resin production (Table 1). The trade-off of that decision will be the reduction in rubber content. Rubber content is negatively correlated with dry biomass (r = −0.20). In other words, selection for higher biomass during the second year of growth may be linked with selection for high rubber and/or resin production, but will reduce the rubber content as well. Correlation between rubber and resin content was the highest after two years of growth (r = 0.27; P < 0.0001) then the correlation declines to r = 0.1 (*P = 0.29*) at third year, suggesting that selection for both traits is feasible at 2-year old plants.

4. Conclusion

Among more than 2000 plant species known to synthesize rubber, guayule and *Hevea* are the two species that produced commercial grade rubber and latex (Ray et al., 2005). Based on its origin in Southern Texas and Northern Mexico, guayule is a good candidate for the arid and semi-arid sustainable agricultural systems. Continue genetic improvement of guayule for higher biomass, rubber and resin production, and high resistance to environmental stresses, are necessary. Our results indicated that improved guayule germplasm had good phenotypic variation as indication of genetic variability. Harvesting at certain plant age could increase biomass, rubber and resin production that were highest when plants were harvested after two years of transplanting. High heritability estimates for these traits suggest that selection is feasible in the first three years in general, and highest at two years old plant, where plants reach maximum growth uniformity with low competition among plants. Positive correlation coefficients among these traits suggest the possibility of selection for more than one trait at the same time, which could reduce the guayule breeding time.

References

Angulo-Sánchez, J.L., De Rodríguez, D.J., Rodriguez-García, R., 2002. Relationship between guayule biomass production, rubber synthesis, and climatic conditions. Trends in new crops and new uses. Am. Soc. Hort. Sci. Alexandria VA 234–237.

Appleton, M., Van Staden, J., 1989. The relationship between season, growth and isoprenoid biosynthesis in parthentum argentatum. J. Plant Physiol. 134, 524–532.

Appleton, M., Van Staden, J., 1991. Influence of temperature and daylength on growth and isoprenoid biosynthesis in guayule under controlled environmental conditions. Bioresour. Technol. 35, 147–152.

Bekaardt, C.R., Coffelt, T.A., Fenwick, J.R., Wiesner, L.E., 2010. Environmental, irrigation and fertilization impacts on the seed quality of guayule (Parthenium argentatum Gray). Ind. Crops Prod. 31, 427–436.

Benedict, C., Greer, P.J., Foster, M., 2008. The physiological and biochemical responses of guayule to the low temperature of the Chihuahuan Desert in the biosynthesis of rubber. Ind. Crops Prod. 27, 225–235.

Black, L., Hamerstrand, G., Nakayama, F., Rasnik, B., 1983. Gravimetric analysis for determining the resin and rubber content of guayule. Rubber Chem. Technol. 56, 367–371.

Blohm, M.E.V., 2005. Genetic and Environmental Effects on Growth, Resin and Rubber Production in Guayule (Parthenium Argentatum, Gray), School of Plant Sciences. University of Arizona, Tuscon, AZ.

Boateng, A.A., Elkasabi, Y., Mullen, C.A., 2016. Guayule (Parthenium argentatum) pyrolysis biorefining: fuels and chemicals contributed from guayule leaves via tail gas reactive pyrolysis. Fuel 163, 240–247.

Chow, P., Nakayama, F.S., Blahnik, B., Youngquist, J.A., Coffelt, T.A., 2008. Chemical constituents and physical properties of guayule wood and bark. Ind. Crops Prod. 28, 303–308.

Coffelt, T., Nakayama, F., 2007. A Six Step Harvesting Procedure for Guayule Small Plots for Laboratory Analyses. Issues in New Crops and New Uses. ASHS Press, Alexandia, VA, pp. 66–71.

Coffelt, T., Ray, D., 2010. Cutting height effects on guayule latex, rubber, and resin yields. Ind. Crops Prod. 32, 264–268.

Coffelt, T.A., Ray, D.T., Nakayama, F.S., Dierig, D.A., 2005. Genotypic and environmental effects on guayule (Parthenium argentatum) latex and growth. Ind. Crops Prod. 22, 95–99.

Coffelt, T.A., Nakayama, F.S., Ray, D.T., Cornish, K., McMahan, C.M., Williams, C.F., 2009. Plant population, planting date, and germplasm effects on guayule latex, rubber, and resin yields. Ind. Crops Prod. 29, 255–260.

Cornish, K., Backhaus, R.A., 2003. Induction of rubber transferase activity in guayule (Parthenium argentatum Gray) by low temperatures. Ind. Crops Prod. 17, 83–92.

Cornish, K., Scott, D.J., 2005. Biochemical regulation of rubber biosynthesis in guayule (Parthenium argentatum Gray). Ind. Crops Prod. 22, 49–58.

Cornish, K., Chapman, M.H., Nakayama, F.S., Vinyard, S.H., Whitehand, L.C., 1999. Latex

quantification in guayule shrub and homogenate. Ind. Crops Prod. 10, 121–136.
Dierig, D.A., Ray, D.T., Coffelt, T.A., Nakayama, F.S., Leake, G.S., Lorenz, G., 2001. Heritability of height, width, resin, rubber, and latex in guayule (Parthenium argentatum). Ind. Crops Prod. 13, 229–238.
Dierig, D., 1987. Contribution of Yield Compoeonents to Rubber Production in Guayule. The University of Arizona.
Estilai, A., 1985. Registration of CAL-5 guayule germplasm. Crop Sci. 25, 369–370.
Estilai, A., 1986. Registration of CAL-6 and CAL-7 guayule germplasm. Crop Sci. 26, 1261–1262.
Foster, M.A., Coffelt, T.A., 2005. Guayule agronomics: establishment, irrigated production, and weed control. Ind. Crops Prod. 22, 27–40.
Holland, J.B., Nyquist, W.E., Cervantes-Martínez, C.T., 2003. Estimating and interpreting heritability for plant breeding: an update. Plant Breed. Rev. 22, 9–112.
Hunsaker, D.J., Elshikha, D.M., 2017. Surface irrigation management for guayule rubber production in the US desert Southwest. Agric. Water Manage. 185, 43–57.
Nakayama, F., 2005. Guayule future development. Ind. Crops Prod. 22, 3–13.
Nyquist, W.E., Baker, R., 1991. Estimation of heritability and prediction of selection response in plant populations. Crit. Rev. Plant Sci. 10, 235–322.
Paterson-Jones, J., Gilliland, M., Van Staden, J., 1990. The biosynthesis of natural rubber. J. Plant Physiol. 136, 257–263.
Ponciano, G., McMahan, C.M., Xie, W., Lazo, G.R., Coffelt, T.A., Collins-Silva, J., Nural-Taban, A., Gollery, M., Shintani, D.K., Whalen, M.C., 2012. Transcriptome and gene expression analysis in cold-acclimated guayule (Parthenium argentatum) rubber-producing tissue. Phytochemistry 79, 57–66.
Powers, L., Rollins, R.C., 1945. Reproduction and pollination studies on guayule, Parthenium argentatum Gray and P. incanum HBK. Agron. J. 37, 96–112.
Ray, D.T., Dierig, D.A., Thompson, A.E., 1990. Facultative apomixis in guayule as a source of genetic diversity. In: Janick, J., Simon, J.E. (Eds.), Advances in New Crops. Timber Press, Portland, OR, pp. 245–247.
Ray, D.T., Dierig, D.A., Thompson, A.E., Diallo, M.M., 1993. Parent-offspring relationships in apomictic guayule. J. Am. Oil Chem. Soc. 70, 1235–1237.
Ray, D.T., Dierig, D.A., Thompson, A.E., Coffelt, T.A., 1999. Registration of six guayule germplasms with high yielding ability. Crop Sci. 39, 1.
Ray, D.T., Coffelt, T.A., Dierig, D.A., 2005. Breeding guayule for commercial production. Ind. Crops Prod. 22, 15–25.
Rollins, R.C., 1945. Evidence for genetic variation among apomictically produced plants of several F1 progenies of Guayule (Parthenium argentatum) and Mariola (P. incanum). Am. J. Bot. 554–560.
Rollins, R.C., 1949. Sources of genetic variation in Parthenium argentatum Gray (Compositae). Evolution 3, 358–368.
SAS, 2003. SAS/STAT User's Guide, Version 9.1.
Siler, D.J., Cornish, K., 1994. Hypoallergenicity of guayule rubber particle proteins compared to Hevea latex proteins. Ind. Crops Prod. 2, 307–313.
Sundar, D., Reddy, A.R., 2001. Low night temperature-induced changes in photosynthesis and rubber accumulation in guayule (Parthenium argentatum Gray). Photosynthetica 38, 421–427.
Teetor, V.H., Ray, D.T., Schloman, W.W., 2009. Evaluating chemical indices of guayule rubber content: guayulins A and B. Ind. Crops Prod. 29, 590–598.
Tysdal, H., Estilai, A., Siddiqui, I., Knowles, P., 1983. Registration of four guayule germplasms1 (Reg. No. GP1 to GP4). Crop Sci. 23 189–189.
Veatch, M.E., Ray, D.T., Mau, C.J.D., Cornish, K., 2005. Growth, rubber, and resin evaluation of two-year-old transgenic guayule. Ind. Crops Prod. 22, 65–74.
Veatch-Blohm, M.E., Ray, D.T., 2005. (453) effect of drought stress on growth and secondary compound production in greenhouse-grown guayule. HortScience 40 1036–1036.
Veatch-Blohm, M.E., Ray, D.T., McCloskey, W.B., 2006. Water-stress-induced changes in resin and rubber concentration and distribution in greenhouse-grown guayule. Agron. J. 98, 766–773.
Veatch-Blohm, M., Ray, D.T., Gehrels, A., 2007. Night temperature, rubber production, and carbon exchange in guayule. Ind. Crops Prod. 25, 34–43.