Performance and Phytochemical Content of 22 Pomegranate (*Punica granatum*) Varieties

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***Abstract*. Pomegranate is a drought-tolerant and salt-tolerant crop. Its fruits contain high levels of phytochemicals that have many health benefits. Pomegranate has the potential to be an alternative crop in areas where water availability is limited, such as west Texas. However, more than 500 pomegranate varieties are estimated to exist worldwide, and little is known about which varieties are suitable for growing in the west Texas region. Therefore, the objective of this study was to evaluate the field performance of 22 pomegranate varieties, specifically based on phenology, resistance to sunburn, fruit split, fruit rot (resistance was calculated by subtracting the percent incidence by 100), yield, fruit phytochemicals, and Brix over the course of 3 years from 2016 to 2018. Cold damage, caused by below-freezing temperatures encountered from Nov. 2018 to Feb. 2019, was also evaluated in Apr. 2019. Our results showed significant varietal differences in nearly all response variables measured, indicating that varietal selection is important for pomegranate production for specific regions, such as west Texas. Leaf budding ranged from 47 to 62 days in 2016, 41 to 54 days in 2017, and 49 to 60 days in 2018. Anthesis ranged from 87 to 119 days in 2016, 80 to 94 days in 2017, and 92 to 114 days in 2018. Fruit resistance to split was broad and ranged from 7.3% to 79.1% in 2017 and from 14.2% to 99.7% in 2018. Fruit sunburn resistance ranged from 14.0% to 64.6% in 2017 and from 28.3% to 90.0% in 2018. Fruit heart rot incidence was nominal for all varieties. Total phenolic compound contents of the pomegranate fruit juice ranged from 0.81 to 1.52 mg GAE/mL, and the total antioxidant capacity ranged from 3.44 to 6.81 mg TE/mL. The yield per tree ranged from 1.00 to 7.96 kg in 2017 and from 0.81 to 10.26 kg in 2018. Brix ranged from 12.5% to 17.4% in 2017 and from 13.9% to 18.4% in 2018. Early winter below-freezing temperatures caused different degrees of cold damage; however, 5 of 22 varieties that originated from Russia did not show any cold damage. Results of a hierarchical cluster analysis based on the means of the key response variables of yield and Brix indicated that four varieties (Al-Sirin-Nar, Russian 8, Ben Ivey, and Salavatski) were notable for having both high yield and high Brix.**

Pomegranate (*Punica granatum*) is a small fruit-bearing deciduous tree and one of the oldest cultivated species in the world. It originated in Central Asia and is currently grown throughout the world (Khadivi et al., 2018). Pomegranate is adapted to arid and semi-arid regions worldwide and is considered drought-tolerant (Galindo et al., 2017; Rodríguez et al., 2012). In many southern states of the United States, pomegranate has been grown for decades as a common backyard tree. Its fruits have a long history of medicinal use and nutritional value (Teixeira da Silva et al., 2013) because they contain bioactive phytochemicals, which have health benefits such as reduced oxidative stress and inflammation (Gundogdu and Yilmaz, 2012; Masci et al., 2016; Shema-Didi et al., 2012). These attributes of pomegranate fruits have resulted in increased demand by the consumer (Kalaycioğlu and Erim, 2017). Recently, growers in southern states have responded to this increasing demand by growing pomegranate commercially. Moreover, due to climate change, unpredicted drought, and a limited supply of high-quality water, growers in the semi-arid west Texas region are looking for alternative new crops that can tolerate extended drought events and saline groundwater. Pomegranate, which is one of the most salt-tolerant fruit trees, is an excellent candidate crop for the semi-arid region (Sun et al., 2018).

There are more than 500 varieties of pomegranate that have been named (IPGRI, 2001; Stover and Mercure, 2007), and extensive genetic diversity exists within this species (Verma et al., 2010). In the United States, the pomegranate variety Wonderful is currently the industry standard variety and accounts for more than 90% of all commercial trees planted (Chater and Garner, 2018). However, 'Wonderful' pomegranate is susceptible to fruit splitting, which is a major production challenge that can cause significant fruit loss (Chater and Garner, 2018). Fruit split is thought to be caused by excessive or irregular irrigation, particularly during fruit maturation (Holland et al., 2009). This can lead to swelling of the arils that cause the rind to crack or split. Prasad et al. (2003) found reductions in fruit split when using drip irrigation. However, other factors, such as mineral nutrition, which affects the elasticity of the rind, are also thought to have a role. For example, Chater and Garner (2018) found that foliar nutrient applications, including zinc sulfate ($ZnSO_4$), magnesium sulfate ($MgSO_4$), and potassium nitrate (KNO_3), decreased the fruit split incidence in the variety Wonderful. There is some evidence that fruit splitting is variety-dependent (Holland et al., 2009); however, more research is needed to gain a better understanding of the genetic diversity that exists among pomegranate varieties.

Other production issues of pomegranate are fruit rot and sunburn. Fruit rot is often caused by fungal pathogens, such as *Coniella granati* (Levy et al., 2011) and *Alternaria alternata* (Munhuweyi et al., 2016), that enter the fruit during bloom, remain latent for 3 to 4 months, and then spread throughout the developing fruit; it is characterized as a black rot (Ezra et al., 2015). Fruit sunburn can occur in regions with intense solar radiation, such as Texas, and it is not known whether the color of the fruit is a factor (Yazici and Kaynak, 2009). Overall, there is evidence that these production challenges of pomegranate are variety-dependent based on anecdotal observation, but more research is needed.

Variety trials are essential to identify region-specific varieties that are resistant to biotic (diseases and pests) and abiotic (drought, salt, and heat) stresses. For pomegranate, the purpose of a variety trial is to identify alternative varieties with similar or superior quality to the industry standard, 'Wonderful'. In addition to the biotic and abiotic factors that affect variety selection, consumer acceptance also has a significant impact on marketing. Chater et al. (2018) conducted a sensory study of consumer acceptance using eight varieties of pomegranate. They found that 'Wonderful' was perceived as bitter by the untrained panelists. Furthermore, it was not the best variety based on its aril color, sweetness, tartness, seed hardness, bitterness, and overall desirability among the eight varieties. The production site also impacts the fruit quality, even for the same variety. For example, the variety

Wonderful grown on the west coast in Ventura County, CA, was less acceptable overall to consumers than Wonderful grown inland in Riverside, CA. This was attributed to the much higher acidity and less sweetness in the pomegranate grown on the west coast (Chater et al., 2018). These results indicate that significant potential exists to identify region-specific pomegranate varieties that are superior to Wonderful. Therefore, the objective of this study was to evaluate the field performance of 22 pomegranate varieties in west Texas based on phenology, yield, fruit phytochemicals, fruit sunburn, split, and rot over the course of 3 years, from 2016 to 2018.

Materials and Methods

Plant material propagation. Hardwood cuttings (≈15 cm) of 22 varieties of pomegranate (Table 1) were obtained from a commercial nursery (Marcelino's Nursery, Tornillo, TX) in 2014. The cuttings were propagated in a greenhouse using RL98 Ray Leach Cone-tainers (Stuewe and Sons, Tangent, OR). Rooted cuttings were transplanted to 5-L treepots (width, 12.7 cm; height, 30.5 cm; CP512CH; Stuewe and Sons) filled with commercial substrate Metro-Mix 902 (Sun-Gro, Agawam, MA). All plants were grown in a greenhouse that was equipped with pad-and-fan cooling and natural gas heating systems to control the temperature. Plants were irrigated with a nutrient solution at an electrical conductivity (EC) of 1.2 $dS \cdot m^{-1}$, which was prepared by adding 15N–2.2P–12.5K (Peters 15–5–15 Cal-Mag Special; Scotts, Marysville, OH) to reverse osmosis (RO) water at a nitrogen concentration of 150 $mg \cdot L^{-1}$. Plants were pruned to ≈30 cm every 3 to 4 months to prevent overgrowth.

Field plot. The field plot is located at Texas A&M AgriLife Research Center in El Paso, TX (lat. 31°41′46.25″ N, long. 106°16′53.58″ W, elevation 1139 m). The dominant soil type of El Paso County is a sandy loam (USDA, 1971). A 20-m × 20-m field plot was tilled entirely, and fresh manure (WEBB FEED Enterprises, Clint, TX) was added at 5.5 wheelbarrows (≈33 kg each) per 18.6-m^2 plot; then, the area was tilled again. Then, the entire planting area was covered with a weed barrier (DeWitt Company, Sikeston, MO) to prevent weed growth. Between 21 and 23 Apr. 2015, 1-year-old pomegranate trees were planted in the field plot.

The plot was partitioned into four blocks to account for any soil and environmental variations. The field trial was arranged in a randomized complete block design (RCBD) with four blocks and a total of four plants per variety. In each block, 22 plants (one tree per variety) were planted in two rows with distances of 2.6 m between rows and 1.8 m between plants in the same row. Plants were watered through a drip irrigation system twice daily at 0830 HR and 1330 HR (≈1.3 L), except on rainy days. The irrigation time was increased each year as trees grew larger, with up to 11.0 L per day used during the final year. Each pomegranate tree received 12 g of Scotts Osmocote Lo-Start 14–16 Months 18–6–12 (The Scotts Company, Marysville, OH) to provide additional nitrogen during the first year. During subsequent years, Scotts Osmocote 5–6 Months 15–9–12 was applied up to a rate of 270 g per tree during the final year. Fertilizer was incorporated into the soil surface within an area of ≈1 m^2 under the canopy in March of each year.

A second field plot adjacent to the first plot with the same varieties and randomization of the pomegranate trees with six blocks with a total of six trees per variety was created in Spring 2017. The field preparation and management in the two plots were the same. That is, the trees in the second plot were 2 years younger than those in the first plot. The only data from the second plot included in this article were the cold damage ratings.

Data collection. Phenology data were collected from 2016 to 2018 to determine the time of onset of budding and anthesis. Trees were checked every other day from February to May. The onset of leaf budding and the onset of anthesis were recorded for each variety as the numerical day of the year (i.e., 1–365). Leaf budding was determined as the stage when the bud opens and the first leaves appear with a bright red color. Anthesis was determined when the calyx was completely open, with protruding petals, and the anther and stamen were visible for pollination to occur, as described by Melgarejo et al. (1997). Growing degree days (GDD) were calculated as the sum of the daily average temperature minus the base temperature of 10 °C, which is the temperature at which bud development is activated in pomegranate (Baldini, 1992).

Cold hardiness was evaluated in Apr. 2019 in both plots when cold damage was exhibited, as evidenced by dead or partial dead shoots that were caused by the below-freezing temperatures from Nov. 2018 to Feb. 2019. Cold damage of all trees was rated based on visual evaluation scores of 1 to 5: 1, dead; 2, mostly dead; 3, half dead; 4, partly dead; and 5, alive without damage.

All fruits were harvested in the beginning of October each year, except for the first year, when no fruits were harvested. Fruits were harvested by following the block order. The following data were collected: fruit count, fruit fresh weight (FW), yield, fruit soluble sugar content (Brix), and fruit resistance to split, sunburn, and black rot. Fruits were individually inspected for split, sunburn, and black rot, and the incidences were recorded as the percent of the fruit showing symptoms. Resistance was calculated by subtracting the percent incidence by 100. Fruit FW was recorded in grams and yield was recorded in kilograms per tree. Arils were collected from three representative fruits per block and variety, and the juice was extracted using a hand garlic press to measure soluble sugar as Brix (%) with a hand refractometer (Extech Instruments, Nashua, NH). In 2018, a portion of the harvested arils were stored immediately in a freezer at –80 °C (IU1786A; Thermo Fisher Scientific, Marietta, OH) for phytochemical analyses.

Phytochemical analyses. Total phenolic compounds were determined using a modified Folin-Ciocalteu reagent as described by Dou et al. (2018). Briefly, a 100-µL pomegranate juice sample was added to a mixture of 150 µL distilled water and 750 µL 1/10 dilution Folin–Ciocalteu reagent and mixed thoroughly. Then, 600 µL 7.5% Na_2CO_3 was added, and the mixture was incubated at 45 °C in a water bath for 10 min before the absorbance was measured at 765 nm using a Microplate Reader (ELx800; BioTek, Winooski, VT). Total phenolics were calculated as milligrams of gallic acid equivalents (GAE) in 1 mL of sample.

Total antioxidant capacity was determined using a Trolox equivalent antioxidant

Table 1. Description of the 22 pomegranate varieties evaluated in the field plots in west Texas.

Variety	Native region	Aril color
Al-Sirin-Nar	Russia	Red
Angel Red	United States	Red
Apseronski	Russia	Light pink
Arturo Ivey	Local	Red
Ben Ivey	Local	Red
Carolina Vernum	Local	Red
Chiva	Local	Red
DeAnda	Local	Red
Early Wonderful	United States	Red
Kandahar	Afghanistan	Light red
Kazake	Russia	Light pink
Kunduzski	Russia	Light pink
Larry Ceballos	Local	Red
Marcelino Lozano	Local	Red
Mollar de Elche	Spain	Light pink
Purple Heart	United States	Red
Russian 8	Russia	Light red
Salavatski	Russia	Light pink
Spanish Sweet	Spain	Red
Surh-Anor	Russia	Yellow
Utah Sweet	United States	Red
Wonderful	United States	Red

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Table 2. Mean leaf budding and anthesis onset in days of the 22 pomegranate varieties evaluated in a field plot in west Texas. Data were collected from 2016 to 2018, when trees were 2 to 4 years old, respectively.[z]

	2016		2017		2018		Mean		GDD[y]
Variety	Leaf budding onset (d)								
Al-Sirin-Nar	52	bcdefg	45	cde	55	abcde	50	defg	92
Angel Red	52	bcdefg	41	e	49	f	47	fghi	76
Apseronski	53	bcdefg	46	bcde	58	ab	52	cde	101
Arturo Ivey	49	fg	42	e	53	cdef	48	fghi	81
Ben Ivey	47	g	41	e	51	cdef	46	i	73
Carolina Vernum	49	fg	43	e	51	ef	47	hi	76
Chiva	50	defg	43	e	51	def	48	fghi	81
DeAnda	49	efg	43	e	51	def	48	ghi	81
Early Wonderful	49	efg	45	cde	54	bcde	49	efghi	87
Kandahar	55	bcdef	44	de	51	def	50	efgh	92
Kazake	57	abc	53	a	58	ab	56	ab	112
Kunduzski	59	ab	54	a	60	a	58	a	117
Larry Ceballos	50	defg	43	e	51	ef	48	ghi	81
Marcelino Lozano	49	efg	41	e	52	cdef	47	hi	76
Mollar de Elche	62	a	51	abc	58	ab	57	ab	114
Purple Heart	48	fg	42	e	52	cdef	47	hi	76
Russian 8	56	abcd	52	ab	59	ab	55	abc	110
Salavatski	56	abcde	51	abc	55	bcd	54	bcd	110
Spanish Sweet	52	cdefg	46	bcde	56	abc	51	def	97
Surh-Anor	58	abc	50	abcd	58	ab	55	abc	110
Utah Sweet	49	fg	43	e	51	def	48	ghi	81
Wonderful	48	fg	42	e	51	ef	47	hi	76
Mean	52	B	45	C	54	A			
CV	8.2		9.4		6.1				
	Anthesis onset (d)								
Al-Sirin-Nar	94		82		97	cde	90	bc	355
Angel Red	101		88		109	abc	97	abc	418
Apseronski	98		85		101	abcde	95	abc	393
Arturo Ivey	109		88		104	abcde	100	abc	452
Ben Ivey	107		86		103	abcde	97	abc	418
Carolina Vernum	105		89		108	abcd	100	abc	452
Chiva	112		92		98	cde	100	abc	452
DeAnda	95		87		102	abcde	94	abc	383
Early Wonderful	110		82		102	abcde	96	abc	405
Kandahar	97		81		94	de	91	c	362
Kazake	100		82		94	de	92	abc	370
Kunduzski	97		85		95	cde	92	abc	370
Larry Ceballos	103		94		114	a	103	a	482
Marcelino Lozano	112		86		113	ab	103	a	482
Mollar de Elche	113		93		104	abcde	103	a	482
Purple Heart	119		83		107	abcd	103	ab	482
Russian 8	98		85		93	e	92	abc	370
Salavatski	100		80		92	e	91	c	362
Spanish Sweet	99		82		98	cde	93	abc	376
Surh-Anor	87		87		100	bcde	91	bc	362
Utah Sweet	97		89		98	cde	95	abc	393
Wonderful	101		90		100	abcde	96	abc	405
Mean	102	A	86	B	101	A			
CV	7.5		4.6		6.1				

[z]Means followed by different letters indicate significant variety differences according to Tukey's honestly significant difference test ($P < 0.05$). Lowercase letters indicate differences among varieties. Uppercase letters indicate differences among years.

[y]Growing degree days calculated as the sum of the average daily temperature minus the base temperature of 10 °C averaged across all 3 years.

capacity (TEAC) method using 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^+$) described by Ozgen et al. (2006). Briefly, $ABTS^+$ was prepared by mixing with 2.45 mM potassium persulfate and allowing the mixture to stand overnight at room temperature in the dark. Then, the $ABTS^+$ solution was diluted with phosphate-buffered saline (PBS) to an absorbance of 0.700 at 734 nm using a spectrophotometer (Genesys 10S ultraviolet-VIS; Thermo Scientific). Then, 3 mL of the $ABTS^+$ solution and 20 μL of the pomegranate juice sample were mixed and the absorbance was determined at 734 nm. Total antioxidant capacity was calculated as milligrams Trolox equivalents (TE) in 1 mL of sample.

Statistical analyses. An analysis of variance was conducted to test the responses of the different varieties. Mean separation among varieties was conducted using Tukey's honestly significant difference test. All statistical analyses were performed using JMP 14.2 (SAS Institute, Cary, NC). Multivariate correlations were performed using the restricted maximum likelihood (REML) method. Hierarchical cluster analyses of means were performed using the Ward method.

Results

Phenology. Regarding leaf budding onset, there were significant ($P < 0.0001$) variety differences during each of the 3 years and when the data were pooled across all 3 years (Table 2). For each variety, the coefficient of variation (CV) ranged from 6% to 14%, with average CV of 8.2%, 9.4%, and 6.1% in 2016, 2017, and 2018, respectively, indicating that leaf budding was relatively consistent across the 3 years. Based on the mean of the 3 years, leaf budding was earliest for 'Ben Ivey' (46 d) and latest for 'Kunduzski' (58 d). Overall, the varieties Angel Red, Arturo Ivey, Ben Ivey, Carolina Vernum, Chiva, DeAnda, Early Wonderful, Kandahar, Larry Ceballos, Marcelino Lozano, Purple Heart, Utah Sweet, and Wonderful had significantly earlier leaf budding than Kazake, Kunduzski, Mollar de Elche, Russian 8, Salavatski, and

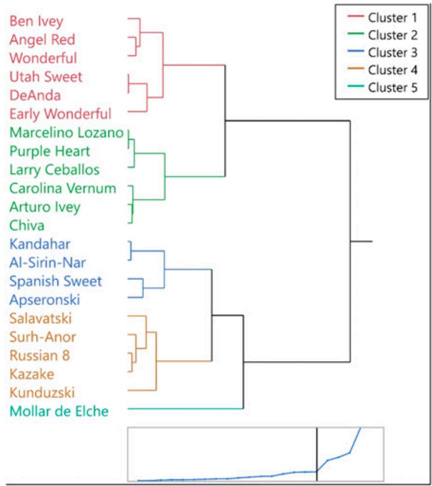

Fig. 1. Hierarchical cluster analysis of the means of leaf budding and anthesis onset of the 22 pomegranate varieties evaluated in a field plot in west Texas from 2016 to 2018. The distance plot at the bottom of the figure was used to determine the number of clusters based on the distance between clusters. Good separation was indicated. A total of five clusters were separated. For a detailed description of each cluster, refer to the text.

Table 3. Mean fruit resistance (percent of no incidence) to split and sunburn of the 22 pomegranate varieties evaluated in a field plot in west Texas. Data were collected in 2017 and 2018, when trees were 3 and 4 years old, respectively.[z]

	2017		2018		Mean	
Variety			Fruit split resistance (%)			
Al-Sirin-Nar	45.5	abc	58.7	ab	52.1	abc
Angel Red	7.3	c	14.2	b	10.1	c
Apseronski	48.5	abc	66.2	ab	57.3	abc
Arturo Ivey	62.5	ab	35.8	b	49.1	abc
Ben Ivey	41.2	abc	42.7	ab	42.0	bc
Carolina Vernum	65.2	ab	49.0	ab	58.2	abc
Chiva	55.7	ab	21.6	b	38.6	bc
DeAnda	55.2	ab	48.7	ab	51.9	abc
Early Wonderful	36.3	abc	23.6	b	29.9	bc
Kandahar	67.5	ab	27.1	b	47.3	abc
Kazake	79.1	a	99.7	a	89.4	a
Kunduzski	73.2	ab	71.0	ab	72.1	ab
Larry Ceballos	43.0	abc	85.0	ab	61.0	abc
Marcelino Lozano	30.8	bc	66.1	ab	48.4	abc
Mollar de Elche	58.8	ab	48.6	ab	53.7	abc
Purple Heart	67.6	ab	36.1	b	51.8	abc
Russian 8	62.0	ab	64.0	ab	63.0	ab
Salavatski	64.9	ab	81.3	ab	73.1	ab
Spanish Sweet	50.1	abc	63.4	ab	55.8	abc
Surh-Anor	59.1	ab	75.4	ab	67.3	ab
Utah Sweet	55.1	ab	43.3	ab	49.2	abc
Wonderful	51.2	abc	49.2	ab	50.2	abc
Mean	54.2		53.7			
CV	29.2		41.3			
			Fruit sunburn resistance (%)			
Al-Sirin-Nar	44.5		66.1	ab	55.3	ab
Angel Red	14.0		90.0	a	44.4	ab
Apseronski	55.7		60.6	ab	58.1	ab
Arturo Ivey	58.3		83.9	a	71.1	a
Ben Ivey	53.4		78.3	ab	65.8	a
Carolina Vernum	63.1		67.0	ab	64.8	a
Chiva	53.4		87.5	a	70.4	a
DeAnda	46.9		77.7	ab	62.3	ab
Early Wonderful	57.5		86.8	a	72.2	a
Kandahar	29.4		75.5	ab	52.5	ab
Kazake	43.0		50.6	ab	46.8	ab
Kunduzski	55.1		61.0	ab	58.0	ab
Larry Ceballos	64.6		28.3	b	49.0	ab
Marcelino Lozano	40.5		79.6	ab	60.1	ab
Mollar de Elche	46.5		71.7	ab	59.1	ab
Purple Heart	53.0		61.2	ab	57.1	ab
Russian 8	28.0		59.5	ab	43.7	ab
Salavatski	30.8		60.2	ab	45.5	ab
Spanish Sweet	38.9		49.1	ab	43.3	ab
Surh-Anor	22.6		29.8	b	26.2	b
Utah Sweet	38.8		79.1	ab	58.9	ab
Wonderful	46.4		60.8	ab	53.6	ab
Mean	44.7	B	66.7	A		
CV	29.6		28.4			

[z]Means followed by different letters indicate significant variety differences according to Tukey's honestly significant difference test ($P < 0.05$). Lowercase letters indicate differences among varieties. Uppercase letters indicate differences among years.

Surh-Anor based on the mean of the 3 years. The average GDD corresponding to the mean onset of leaf budding ranged from 73 to 117 GDD and showed highly significant correlations ($r = 0.99$, data not shown). Overall, the mean onset of leaf budding across all varieties was earliest in 2017 (45 d) and latest in 2018 (54 d).

Regarding flower anthesis, there were significant ($P < 0.0001$) variety differences during 2018 and when the data were pooled across the 3 years (Table 2). For each variety, the CV ranged from 6% to 19%, and the average CV was 7.5%, 4.6%, and 6.1% in 2016, 2017, and 2018, respectively, indicating similar phenological variations in anthesis to leaf budding. Based on the means of the 3 years, anthesis occurred earliest in 'Al-Sirin-Nar' (90 d) and latest in 'Larry Ceballos', 'Marcelino Lozano', 'Mollar de Elche', and 'Purple Heart' (103 d). The varieties Al-Sirin-Nar, Kandahar, Salavatski, and Surh-Anor had significantly earlier anthesis than Larry Ceballos, Marcelino Lozano, Mollar de Elche, and Purple Heart based on the mean of the 3 years. The average GDD corresponding to the mean onset of anthesis ranged from 355 to 482 GDD and showed highly significant correlations ($r = 1.00$, data not presented), similar to leaf budding. The mean onset of anthesis across all varieties was earliest in 2017 (86 d), and the latest occurred in 2016 and 2018 (102 and 101 d, respectively).

A multivariate cluster analysis was performed for the 22 pomegranate varieties based on the means of leaf budding and anthesis, and five distinct clusters were identified (Fig. 1). Cluster 1 included six varieties that had early leaf budding but late anthesis (47 and 101 d, respectively); Cluster 2 included six varieties with early leaf budding and moderately early anthesis (47 and 96 d, respectively); Cluster 3 included four varieties with moderately early leaf budding and early anthesis (51 and 92 d, respectively); Cluster 4 included five varieties with late leaf budding and early anthesis (56 and 92 d, respectively); and Cluster 5 included a single variety with both late leaf budding and anthesis (47 and 101 d, respectively).

Fruit resistance to split, sunburn, and heart rot. There were significant ($P = 0.0002$) variety differences in fruit split resistance in 2017 and 2018, and when the data were pooled from both years (Table 3). Split resistance was very broad among the 22 pomegranate varieties, ranging from 7.3% to 79.1% in 2017 and from 14.2% to 99.7% in 2018. The mean split resistance did not significantly differ between the 2 years. In 2017, the variety Kazake had significantly higher split resistance than Angel Red and Marcelino Lozano. Similarly, in 2018, Kazake had significantly higher split resistance than the varieties Angel Red, Arturo Ivey, Chiva, Early Wonderful, Kandahar, and Purple Heart. Based on the average of 2 years, 'Kazake' had the highest split resistance and 'Angel Red' had the lowest. The CV for fruit split resistance among varieties were 29.2% in 2017 and 41.3% in 2018 (Table 3); these were much greater than those for phenology.

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Table 4. Mean total phenolics in mg of gallic acid equivalents (GAE) and total antioxidant capacity in mg Trolox equivalents (TE) of the 22 pomegranate varieties evaluated in a field plot in west Texas. Data were collected in 2018, when trees were 4 years old. The juice was extracted and analyzed from three representative fruit per variety, per block.[z]

Variety	Total phenolics (mg GAE/mL)		Total antioxidant capacity (mg TE/mL)	
Al-Sirin-Nar	1.17	abc	4.50	ab
Angel Red	1.52	ab	6.81	ab
Apseronski	1.00	bc	4.45	ab
Arturo Ivey	1.26	abc	4.92	ab
Ben Ivey	1.25	abc	5.26	ab
Carolina Vernum	1.08	abc	4.35	ab
Chiva	1.32	ab	5.40	ab
DeAnda	1.11	abc	5.11	ab
Early Wonderful	1.44	ab	6.17	ab
Kandahar	1.31	abc	6.26	ab
Kazake	0.97	bc	5.01	ab
Kunduzski	1.11	abc	5.31	ab
Larry Ceballos	1.12	abc	4.87	ab
Marcelino Lozano	1.49	a	6.59	a
Mollar de Elche	0.81	c	3.44	b
Purple Heart	1.25	abc	5.66	ab
Russian 8	1.19	abc	5.17	ab
Salavatski	1.26	abc	5.66	ab
Spanish Sweet	1.22	abc	5.70	ab
Surh-Anor	1.16	abc	5.30	ab
Utah Sweet	1.23	abc	3.99	ab
Wonderful	1.29	abc	5.84	ab
Mean	1.21		5.26	
CV	13.7		15.6	

[z]Means followed by different letters are significantly different according to Tukey's honestly significant difference test ($P < 0.05$).

Table 5. Mean yield in kg per tree of the 22 pomegranate varieties evaluated in a field plot in west Texas. Data were collected in 2017 and 2018, when trees were 3 and 4 years old, respectively.[z]

	2017		2018		Mean	
Variety	Yield (kg)					
Al-Sirin-Nar	7.02	ab	6.34	abcd	6.68	ab
Angel Red	1.00	cd	1.49	bcd	1.19	de
Apseronski	3.80	abcd	3.92	abcd	3.86	bcde
Arturo Ivey	2.82	bcd	5.13	abcd	3.97	bcde
Ben Ivey	5.60	abc	6.23	abcd	5.91	abc
Carolina Vernum	3.40	bcd	5.90	abcd	4.47	bcde
Chiva	3.30	bcd	4.02	abcd	3.66	bcde
DeAnda	3.61	abcd	4.64	abcd	4.13	bcde
Early Wonderful	2.54	cd	4.08	abcd	3.31	bcde
Kandahar	2.11	cd	5.53	abcd	3.82	bcde
Kazake	3.13	bcd	8.02	abcd	5.22	abcd
Kunduzski	2.92	bcd	8.58	abc	5.75	abcd
Larry Ceballos	1.19	d	0.81	d	1.02	e
Marcelino Lozano	3.08	bcd	5.07	abcd	4.07	bcde
Mollar de Elche	2.27	cd	1.98	cd	2.13	cde
Purple Heart	4.49	abcd	3.65	abcd	4.07	bcde
Russian 8	5.11	abcd	8.87	ab	6.99	ab
Salavatski	7.96	a	10.26	a	9.11	a
Spanish Sweet	4.07	abcd	8.38	abcd	5.91	abc
Surh-Anor	4.35	abcd	7.35	abcd	5.85	abcd
Utah Sweet	3.96	abcd	4.40	abcd	4.18	bcde
Wonderful	4.23	abcd	3.46	abcd	3.84	bcde
Mean	3.76	B	5.44	A		
CV	44.3		45.7			

[z]Means followed by different letters indicate significant variety differences according to Tukey's honestly significant difference test ($P < 0.05$). Lowercase letters indicate differences among varieties. Uppercase letters indicate differences among years.

For fruit sunburn resistance, there were significant ($P = 0.0007$) variety differences in 2018, but not in 2017 or when the data were pooled from both years ($P = 0.054$) (Table 3). Sunburn resistance among the 22 pomegranate varieties ranged from 14.0% to 64.6% in 2017 and from 28.3% to 90.0% in 2018. Overall, the mean sunburn resistance significantly increased by 21.6% from 2017 to 2018. In 2018, the varieties Angel Red, Arturo Ivey, Chiva, and Early Wonderful had significantly higher sunburn resistance than Larry Ceballos and Surh-Anor. The CV for fruit sunburn resistance among varieties in 2017 and 2018 were similar, 29.6% and 28.4%; however, they were still greater than those for phenology (Table 3).

Regarding fruit heart rot, the incidence was nominal and there were no significant ($P = 0.19$) variety differences in 2017 and 2018 (data not shown). In fact, no incidence of heart rot was found in many of the varieties and the mean resistance to heart rot during both years ranged from 91.8% to 100.0%. The lowest resistance was found in the Al-Sirin-Nar variety (91.8%).

Phytochemicals. There were significant ($P = 0.0005$) variety differences in the total phenolics of the pomegranate fruit juice, ranging from 0.81 to 1.52 mg GAE/mL (Table 4). The varieties Angel Red, Early Wonderful, and Marcelino Lozano had significantly higher contents of total phenolics than Apseronski, Kazake, Kunduzski, and Mollar de Elche. Regarding total antioxidant capacity, there were significant ($P = 0.01$) variety differences, ranging from 3.44 to 6.81 mg TE/mL (Table 4). The variety Marcelino Lozano had a significantly higher total antioxidant capacity than Mollar de Elche. Variations in the total phenolics and total antioxidant capacities were greater than those in phenology but smaller than those in fruit split and sunburn resistance.

Yield. There were significant ($P = 0.0003$) variety differences in yield in 2017 and 2018, and when the data were pooled from both years (Table 5). Yield ranged from 1.00 to 7.96 kg in 2017 and from 0.81 to 10.26 kg in 2018. The mean yield increased significantly by 44% from 2017 to 2018; however, the relative performance of the varieties was consistent in both years. The variety Salavatski had the highest yield in both years, whereas the variety Larry Ceballos had the lowest yield. Based on the average of 2 years, the varieties Al-Sirin-Nar, Russian 8, and Salavatski had significantly higher yields than the varieties Angel Red, Larry Ceballos, and Mollar de Elche. The variations in yield among varieties are the largest among all response variables except for cold hardiness.

Brix. There were significant ($P = 0.005$) variety differences in Brix in 2017 and 2018,

Table 6. Mean soluble sugar content (Brix) in percent of the 22 pomegranate varieties evaluated in a field plot in west Texas. Data were collected in 2017 and 2018, when trees were 3 and 4 years old, respectively.[z]

	2017		2018		Mean	
Variety	Brix (%)					
Al-Sirin-Nar	15.8	ab	15.6	ab	15.7	abcde
Angel Red	16.1	ab	18.4	a	17.0	abc
Apseronski	12.5	b	15.5	ab	14.0	de
Arturo Ivey	16.0	ab	18.1	a	17.0	ab
Ben Ivey	16.4	ab	15.9	ab	16.1	abcde
Carolina Vernum	16.0	ab	16.0	ab	16.0	abcde
Chiva	15.8	ab	17.4	ab	16.6	abc
DeAnda	16.9	a	16.1	ab	16.5	abc
Early Wonderful	17.4	a	17.1	ab	17.2	a
Kandahar	15.3	ab	17.3	ab	16.3	abcde
Kazake	14.3	ab	15.9	ab	15.0	abcde
Kunduzski	14.6	ab	14.7	b	14.7	bcde
Larry Ceballos	15.5	ab	16.2	ab	15.8	abcde
Marcelino Lozano	15.5	ab	16.9	ab	16.2	abcde
Mollar de Elche	13.9	ab	14.9	ab	14.3	cde
Purple Heart	16.0	ab	16.7	ab	16.3	abcd
Russian 8	15.9	ab	15.9	ab	15.9	abcde
Salavatski	16.2	ab	14.6	b	15.4	abcde
Spanish Sweet	13.5	ab	13.9	b	13.6	e
Surh-Anor	14.7	ab	14.9	b	14.8	bcde
Utah Sweet	15.8	ab	17.4	ab	16.5	abc
Wonderful	16.1	ab	16.5	ab	16.3	abcde
Mean	15.4	B	16.1	A		
CV	7.3		7.2			

[z]Means followed by different letters indicate significant variety differences according to Tukey's honestly significant difference test ($P < 0.05$). Lowercase letters indicate differences among varieties. Uppercase letters indicate differences among years.

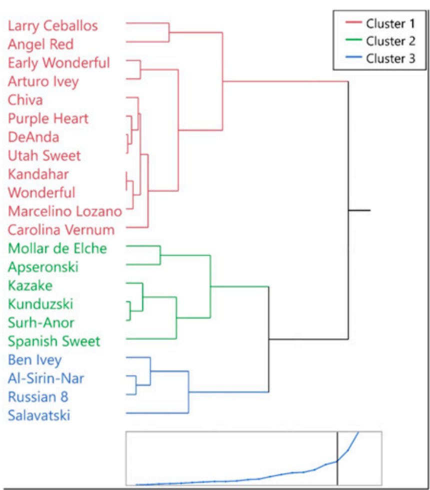

Fig. 2. Hierarchical cluster analysis of the means of yield and Brix of the 22 pomegranate varieties evaluated in a field plot in west Texas. The distance plot at the bottom of the figure was used to determine the number of clusters based on the distance between clusters. Good separation was indicated. A total of three clusters were separated. For a detailed description of each cluster, refer to the text.

and when the data were pooled from both years (Table 6). Brix ranged from 12.5% to 17.4% in 2017 and from 13.9% to 18.4% in 2018. The mean Brix increased significantly by 4% from 2017 to 2018. In 2017, the varieties DeAnda and Early Wonderful had the highest Brix and Apseronski had the lowest. In 2018, the varieties Arturo Ivey and Angel Red had the highest Brix while Kunduzski, Salavatski, Spanish Sweet, and Surh-Anor varieties had the lowest. Based on the means of both years, the varieties Arturo Ivey, Angel Red, and Early Wonderful had significantly higher Brix than the varieties Apseronski and Spanish Sweet.

A hierarchical cluster analysis was performed for the 22 pomegranate varieties-Three distinct clusters were found based on the means of the key response variables of yield and Brix, which are directly related to productivity (profit) and sweetness of the fruit (Fig. 2). Cluster 1 included 12 varieties that had a low yield but a high Brix (3.48 kg and 16.5%, respectively). Cluster 2 included four varieties that had a high yield and a high Brix (7.17 kg and 15.8%, respectively). Cluster 3 included six varieties that had a moderate yield and a low Brix (4.79 kg and 14.4%, respectively).

Cold hardiness. There were significant variety ($P < 0.0001$) and plot (tree age) ($P = 0.0004$) differences in the cold damage ratings. Seven of 22 varieties did not show any damage (visual score of 5) (Table 7), and these varieties were originally from Russia. All other varieties showed different degrees of cold damage in the aboveground shoots. When the two plots were compared, the older trees had less cold damage (average visual score of 3.2) than the younger trees (average score of 2.3). Also, the younger trees had large variations among varieties as evidenced by the large CV. These results indicated that the younger trees are less cold-hardy. In one of the local nurseries with 10-year-old trees, minimal cold damage was observed; however, other growers with younger trees (younger than 4 years) all reported significant cold damage, as shown in our research field plots (personal communication with Mr. Lozano, owner of Marcelino Nursery). The cold damage was mostly caused by early winter below-freezing temperatures when the trees were still actively growing (Fig. 3). Short periods of below-freezing temperatures (a few hours during night and early morning) are frequently observed in January and February in west Texas, when pomegranate trees are already at dormant stage, and no visible cold damage was exhibited from 2015 to 2018.

Discussion

Phenology. Our results indicate that there is substantial phenological diversity among these 22 pomegranate varieties. Regarding both leaf budding and anthesis, there was an average difference of 12 d between the earliest and latest varieties, which corresponded to an average difference of 44 GDD for leaf budding and 120 GDD for anthesis. This broad difference, particularly in the GDD, indicates that these pomegranate varieties have adapted to a wide range of geographical and environmental regions.

The use of GDD allows for comparisons to be made across different years and geographical areas (Melgarejo et al., 1997). Ikinci et al. (2014) reported the sprouting of first leaves and anthesis for three pomegranate varieties, Hicaznar, Suruc, and Karirbasi, at 127 and 351, 146 and 359, and 142 and 362 GDD, respectively. These results were higher than the values we reported in the present study for leaf budding, and were somewhat lower for anthesis (Table 2). This supports our findings and further indicates that significant genetic diversity exists in pomegranate that controls some aspects of the phenology of leaf budding and anthesis.

In addition to genetics, pomegranate is a temperate fruit species, and it is known to have both chilling and heating requirements to break dormancy and flower (Ikinci et al., 2014). The high correlation between phenology and GDD that was found during our study indicates that air temperature warmer than 10 °C influences the onset of leaf budding and anthesis in pomegranate. Air temperature was recorded on-site throughout the experiment (Fig. 4). During the months of January to April, warmer temperatures were recorded in 2017 that corresponded to earlier leaf budding and anthesis, based on the mean of all varieties (Table 2).

There was a significant correlation ($P = 0.02$, Table 8) between leaf budding and anthesis, indicating that the ontogenetic stages were correlated for all varieties. Rajaei and Yazdanpanah (2015) also reported consistent phenology between leaf budding and anthesis over 2 years in the pomegranate variety Rabbab-e-Neyriz. In the present study, varieties with similar phenology were identified during the cluster analysis based on the means of leaf budding and anthesis (Fig. 1). Notably, clusters 1 and 2 included local varieties and important U.S. varieties, such as Wonderful, that tended to have early leaf budding but late flowering. These varieties were determined to be the most suitable for the warm climate of west Texas. In contrast, clusters 3 and 4 included varieties from Russia and Afghanistan that tended to have late leaf budding but early flowering. These varieties may be good for reducing late freeze damage and early fruit maturity in cooler regions. Finally, cluster 5 included a single variety from Spain, Mollar de Elche, which had late leaf budding and late flowering.

Fruit resistance to split, sunburn, and heart rot. The significant varietal differences that were found in our study indicate that at least some aspects of fruit splitting and sunburn are genetically controlled in pomegranate. For fruit splitting, even though drip irrigation was used in our study to provide consistent irrigation, significant differences in fruit split were still observed among varieties. However, fruit splitting is also known to be a problem in regions where fruit maturation overlaps a rainy season (Holland et al., 2009). In west Texas, El Paso receives an average of 22 cm of precipitation annually, mostly during the monsoon season from July to September, which corresponds with the time of fruit maturation of pomegranate. Therefore, this could explain the high percentages of fruit splitting that we recorded for some varieties. Overall, varietal selection is important for growers depending on their geographical location and irrigation method to help minimize fruit splitting. For example, a highly resistant variety to fruit split, such as Kazake, would be ideal in regions with frequent rain during fruit maturation and if drip irrigation is not used.

Regarding fruit sunburn, even though there was no apparent effect on the quality of the arils, it caused the skin of the pomegranate fruit to appear brown and black, which negatively affects fruit appearance and marketability. However, Weerakkody et al. (2010) showed that sunburn damage in the pomegranate variety Wonderful reduced the total phenolic content and total antioxidant capacity, indicating that sunburn damage impacts not only the exterior quality of the fruit but also the interior quality. There is some evidence that Kaolin-based sunscreens effectively reduce sunburn damage of pomegranate fruit (Melgarejo et al., 2004); however, more research in this area is recommended. Nevertheless, varietal selection for sunburn resistance in pomegranate could be critical for successful production, especially in regions with high solar radiation, such as Texas.

The low incidence of fruit heart rot that we recorded may be attributed to the warm and dry climate of west Texas, especially during spring and early summer (Fig. 3). Arid regions may be more suitable for pomegranate

Table 7. Cold damage rating of all pomegranate trees in both field plots. Trees in plot 1 were planted in 2015, and those in plot 2 were planted in 2017.[z]

Varieties	2015 Planting		2017 Planting	
Arturo Ivey	2.0	cd	1.0	c
Al-Sirin-Nar	5.0	a	2.5	bc
Apseronski	5.0	a	5.0	a
Angel Red	1.0	d	1.0	c
Ben Ivey	1.5	cd	1.3	c
Chiva	3.8	abc	1.7	c
Carolina Vernum	2.0	cd	1.0	c
DeAnda	2.5	bcd	1.0	c
Early Wonderful	2.5	bcd	1.0	c
Kandahar	3.0	abcd	1.0	c
Kunduzski	5.0	a	5.0	a
Kazake	5.0	a	5.0	a
Larry Ceballos	1.0	d	1.0	c
Marcelino Lozano	1.5	cd	1.2	c
Mollar de Elche	3.5	abc	1.3	c
Purple Heart	1.5	cd	1.0	c
Russian 8	5.0	a	5.0	a
Salavatski	5.0	a	4.3	a
Spanish Sweet	4.5	ab	3.5	ab
Surh-Anor	5.0	a	4.7	a
Utah Sweet	3.0	abcd	1.5	c
Wonderful	1.5	cd	1.2	c
Mean	3.2	A	2.3	B
CV	48.2		73.1	

[z]Means followed by different letters indicate significant variety differences according to Tukey's honestly significant difference test ($P < 0.05$). Lowercase letters indicate differences among varieties. Uppercase letters indicate differences among plots (tree age).

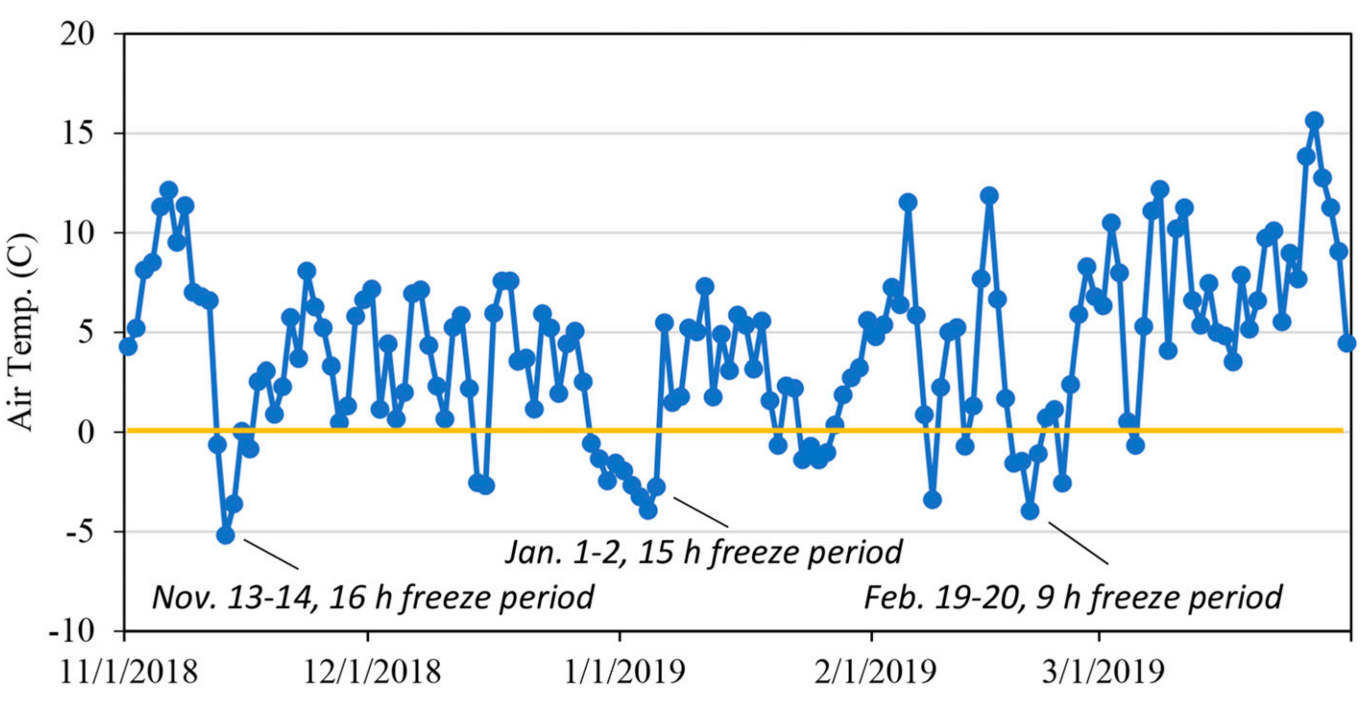

Fig. 3. Minimum air temperatures from Nov. 2018 to Feb. 2019, in the field plot of pomegranate trees. Data were recorded by a weather station located on-site at the Texas A&M AgriLife Research Center in El Paso, TX.

production if the aim is to avoid fungal pathogen diseases, such as heart rot. However, it remains unclear whether the disease is transmitted by abiotic or biotic methods (Kahramanoglu et al., 2014). Management strategies for preventing heart rot in pomegranate are undergoing investigation, and more research is needed to combat this disease that can cause up to 50% fruit loss in commercial orchards (Ezra et al., 2015).

Phytochemicals. There has been great interest in phenolic compounds and their antioxidant activity among consumers and the scientific community during the past decade because of the epidemiological studies linking the consumption of diets rich in antioxidants with decreased risks of cancer and cardiovascular disease (Pourreza, 2013). Pomegranate is known to be a good source of phenolic compounds that are rich in antioxidants, which are linked to potential health benefits (Mphahlele et al., 2014). Our data showed a strong correlation between the phenolic compound content and total antioxidant capacity and supported the aforementioned statement (Table 8). Caliskan and Bayazit (2012) reported that the phenolic contents in the juice of 76 pomegranate accessions from Turkey ranged from 1.08 to 9.44 mg GAE/g (mean, 2.74 mg GAE/g). These values were slightly higher than those reported in this study, probably because of genetic and environmental differences, and indicate substantial variations in phytonutrients among different pomegranate varieties. Çam et al. (2009) reported antioxidant capacities in the juice of eight pomegranate varieties using a method (TEAC) similar to the one used during this study. The values ranged from 2.21 to 4.18 mg TE/mL, similar to the results reported in this study. Therefore, the 22 pomegranate varieties evaluated during this study were determined to have excellent phytochemical contents and to be suitable for consumption as a juice or fresh fruit.

Yield. Our results showed that yields among the 22 pomegranate varieties ranged widely, possibly because these trees were not mature yet (planted in 2015; yield data were recorded in 2017 and 2018); therefore, the yields were expected to increase as the trees matured. According to MacLean et al. (2017), commercial pomegranate trees require 5 to 6 years to reach their full production potential. Nevertheless, in our study, the predominant variety in the United States, Wonderful, had only a moderate yield compared with the other varieties. This indicates that other varieties may be better suited for commercial pomegranate production in arid regions such as west Texas.

Brix. A higher Brix indicates a higher soluble sugar content and sweeter juice. The degree of Brix is one of the most important criterions for fruit juice, and it has been reported to range from 12% to 18% (Türkmen and Ekşi, 2011). Alcaraz-Mármol et al. (2017) reported Brix values ranging from 15% to 17% in 20 pomegranate varieties grown in Spain, which is one of the major producers of pomegranate worldwide. These Brix values are within the range of values reported by this study, indicating that marketable sweet pomegranates can be produced in arid regions such as west Texas. The cluster analysis based on the means of the yield and Brix identified several varieties with a high yield and Brix (Fig. 2). Notably, cluster 1 was the largest cluster, and it included the major U.S. varieties, such as Wonderful and Early Wonderful, as well as several local varieties and the Afghanistan variety, Kandahar. These varieties had a moderate yield but a high Brix. Cluster 2 included three Russian varieties (Al-Sirin-Nar, Russian 8, and Salavatski) and a local variety, Ben Ivey. These varieties had both a high yield and a high Brix and may be ideal candidates for the production of marketable pomegranates in arid regions such as west Texas. Cluster 3 included both Russian and European varieties that had a low yield and a low Brix.

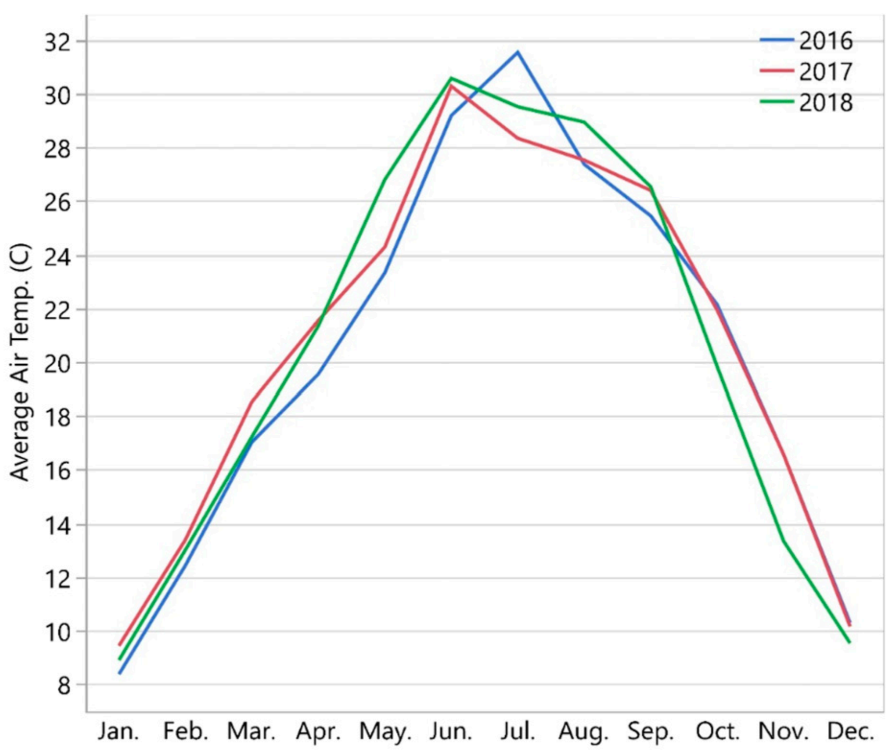

Fig. 4. Average daily air temperature from 2016 to 2018 of the pomegranate field plot in west Texas. Data were recorded by a weather station located on-site at the Texas A&M AgriLife Research in El Paso, TX.

Conclusions

This study evaluated the performance of 22 pomegranate varieties in a field plot in

Table 8. Correlation probability between response variables.

	Budding	Anthesis	Fruit rot res.	Fruit split res.	Sunburn res.	Fruit count	Fruit FW	Yield	Brix	Antioxidant	Phenolics
Budding	—	0.0221	NS	NS	NS	<0.0001	<0.0001	0.0055	NS	NS	0.0019
Anthesis		—	NS	NS	NS	NS	0.0086	NS	NS	NS	NS
Fruit rot res.			—	NS	NS	NS	NS	NS	NS	NS	NS
Fruit split res.				—	0.0338	0.0265	NS	0.0325	NS	0.0493	0.0379
Sunburn res.					—	NS	NS	0.6397	0.0103	NS	NS
Fruit count						—	0.0003	<0.0001	NS	NS	NS
Fruit FW							—	NS	NS	0.0045	NS
Yield								—	NS	NS	NS
Brix									—	NS	NS
Antioxidants										—	<0.0001
Phenolics											—

res. = resistance; FW = fresh weight; NS = not significant.

west Texas over the course of 3 years. Considerable varietal differences were found in nearly all response variables, including phenology, resistance to fruit split, sunburn and rot, cold hardiness, phytonutrient content, yield, and Brix. Our results indicated good diversity and potential among these 22 varieties of pomegranates. Regarding the key response variables of yield and Brix, the varieties, Al-Sirin-Nar, Russian 8, Ben Ivey, and Salavatski were determined to have the most potential for production and marketability in arid regions similar to west Texas. These findings aim to establish pomegranate as a viable and alternative fruit tree crop for production in arid regions, like west Texas.

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