Journal of Nuts

Journal homepage: ijnrs.damghaniau.ac.ir

ORIGINAL ARTICLE

Selection of Pecan Cultivars Aiming to Release Vigorous and Heat Stress Tolerant Rootstocks

Fereidoon Ajamgard

Safiabad Agricultural Research, Education and Natural Resources Center, Agricultural Research, Education and Extension Organization (AREEO), Dezful, Iran

A R T I C L E I N F O

Keywords:

Carya illinoinensis;
Seedlings;
Vegetative growth;
Warm weather stress

A B S T R A C T

With the development of pecan orchards in subtropical regions of Iran in recent years, it is very important to release vigorous and hot weather tolerant rootstocks for grafting. For this purpose, 15 pecan cultivars were evaluated in terms of seed germination rate, seedling growth rate, and summer heat stress damage in 2019-2021 at Safiabad Agricultural Research Center. The cultivars were included 'GraTex', 'Peruque', 'Comanche 4M', '10J', 'Wichita 6J', 'GraKing', 'Choctaw', 'Apache', 'Wichita 7J', 'Comanche 5M'. 'Stuart 2J', 'Stuart 4J', '6M', 'Mahan' and 'Mohawk'. The evaluated traits were seed germination rate, determination of seedling production index per kilogram of seed, seedling height and diameter, and amount of seedling damage due to heat stress. The results showed that 'Peruque', 'Apache', 'Mohawk', and 'Wichita 7J' were preferred with the possibility of producing 126, 86, 75, and 74 seedlings per kilogram of seed, respectively. The results of the height and diameter of the seedlings showed that 'Peruque', 'Stuart 2J', 'Comanche 5M', 'Mahan' and 'Apache' were vigorous. The heat stress was so intense in 2021 that even several decades old and large pecan trees were damaged. Meteorological information of Safiabad station showed that, the temperature was above 45 degrees Celsius for 435 hours in 2021. The results showed that the seedlings of 'Peruque' and 'Apache' were the most tolerant and 'GraTex' and 'Mohawk' were very sensitive. According to the evaluation of seedlings of pecan cultivars in the years, 2019 to 2021, 'Peruque' and 'Apache' for pecan rootstock production are recommended.

Introduction

Pecan is one of the nuts that can be grown in subtropical regions (Rajaram *et al*., 2001, Badyal and Upadhayay, 2004). The origin of pecan is North and Central America, especially the Mississippi River and Texas (Johnson, 1997). The name pecan is derived from the Latin word Pekin, meaning a nut broken by a stone. It has also had titles such as sweet pecan, Illinois nut, faux hickory, and pecan hickory (Arnold and Crocker, 1998).

In 1965, several pecan trees were imported from California and planted in Safiabad Agricultural Research Center. At present, pecan orchards are being developed in different provinces of the country, including Fars, Khuzestan, Kermanshah, Golestan, Kerman, Gilan, Mazandaran, and Tehran.

Budding and grafting are the most widely-used techniques for the propagation of fruit trees particularly walnut and pecan (Ebrahimi A and Vahdati, 2007; Rezaee *et al.*, 2008; Izadi *et al*, 2016; Valizadeh Kaji *et al*., 2020; Farsi *et al*., 2021). The most common method for pecan propagation is grafting (Ajamgard *et al.*, 2016) and the most

*Corresponding author: Email address: ajamgard.dezful@yahoo.com
Received: 22 October 2021; Received in revised form: 15 December 2021; Accepted: 18 January 2022
DOI: 10.22034/jon.2022.1942678.1139

commercially part used for the regeneration of pecan are seedlings (Reid, 2007). Micropropagation method has also been studied in this species, however it has not been commercialized (Vahdati et al., 2020). Many factors influence the quality of rootstock and grafting including, light quality (Sarikhani and Sarikhani, 2021), scion cultivar, rootstock age (Farsi *et al*., 2018) In nurseries, pecan seeds that are produced from open pollination are commonly used (Andersen and Crocker, 2004). For rootstock production, seeds of the same size and shape are selected from healthy trees. Seed size does not affect the germination rate (McEachern, 2010). 'Riverside', 'Mahan', 'Apache', 'More', 'Western', 'Major' and 'Peruque' cultivars are the most important cultivars commonly used to produce pecan rootstocks in the world (McEachern, 2000; Vahdati *et al.*, 2021)**.** The selection of pecan cultivars to produce strong, high-growth rootstocks and adapted to the climate of the region were important. In Texas, for example, the 'Riverside', and in the southern states, 'Elliott' and 'Curtis' are used to produce strong and healthy rootstock (Reid, 2010).

Seed of pecan needs to stratification at temperature 1-4°C for 4 weeks (McEachern, 2010). Pecan seeds usually begin to germinate 4 to 6 weeks after planting. Seedlings produced by cross-pollination have high-growth vigor than self-pollination (Grauke, 2010; Ajamgard *et al.*, 2017). The roots of one year- old pecan seedlings are 90 to 120 cm in height, while the total length of the stem at this age may reach 12 - 30cm (Arnold and Crocker, 1998). Pecan seedlings slowly grow in the first year (Badyal and Upadhayay, 2004). After one year, the seedlings reach the height of 15 to 18cm (McEachern, 2000). The use of strong and high-growing seedlings is very important in reducing the time to prepare the rootstock for grafting and more importantly, reducing the losses of the grafted plants (McEachern, 2010).

Most pecan cultivars seedlings are often sensitive to high summer temperatures and their leaves are damaged by warm weather stress. Therefore, this research was implemented to select high-growth and warm climate-tolerant rootstocks for the production of grafted pecan cultivars.

Materials and Methods

In order to the selection of pecan cultivars to produce vigor and warm weather tolerant rootstocks, 15 pecan cultivars were evaluated in terms of seed germination rate, seedling growth rate, and summer heat stress damage in 2019-2021 at Safiabad Agricultural Research Center. The cultivars were 'GraTex', 'Peruque', 'Comanche 4M', '10J', 'Wichita 6J', 'GraKing', 'Choctaw', 'Apache', 'Wichita 7J', 'Comanche 5M'. 'Stuart 2J', 'Stuart 4J', '6M'', 'Mahan' and 'Mohawk'.

Meteorological specifications of Safiabad Agricultural Research Center, Dezful:

-The latitude: longitude - 32.38°N, 48.42°E
-Elevation above sea level: 143m
-The average annual temperature: 23.9°C
- Maximum temperature: 51.8°C
- Minimum temperature: -2.2°C
-The annual evaporation rate: 2800mm
- The annual rainfall: 320mm

Determining the germination rate of seeds

When the nuts ripened, the seeds of different pecan cultivars were harvested and kept in storage until planting. In mid-February, the seeds were stored in cloth bags in a refrigerator at 4°C for 4 weeks. In mid-March, the seeds were planted in small pots. The composition of the potting soil consisted of one-third clay, one-third sand, and one-third compost (Fig. 1). The moisture of the pots was maintained at the field capacity and the seed germination rate of pecan cultivars was recorded in mid-May. The pots were maintenance in the greenhouse until the end of April. Seedlings were transferred to large pots with a depth of 50cm in the 2 to 4 leaf stage (Fig. 2). Each experimental unit was consisting of 15 seedlings of each cultivar. After ensuring the establishment of

seedlings, the pots were transferred to the nursery and kept under a canopy net (Fig. 3).

Amount of seedlings that can be produced from one kilogram of seeds

The seedling production index for every cultivar was determined by multiplying the seed germination rate and the number of seeds per kilogram.

$$\text{Index} = 1000 / \text{Average seed weight (gr)} \times \text{seed germination rate}$$

Determining seedling diameter pecan cultivars

Every year, pecan seedling diameter was measured at the end of December by a caliper from 5 cm above the soil surface (Fig. 4).

Fig. 1. Planting seeds of pecan cultivars in small pots

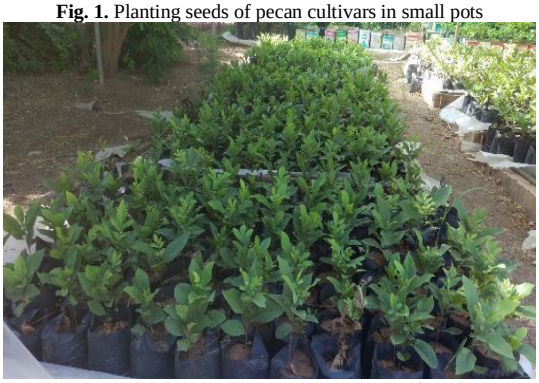

Fig. 3. Seedlings of pecan cultivars in the spring of the first year

In three consecutive years, 2019, 2020, and 2021 vegetative traits including increasing the height and diameter of seedlings were recorded (Fig. 4) and the severity of summer warm weather damage and leaf drying was recorded. The results were statistically

Height of seedlings of pecan cultivars

Pecan seedling height from the soil surface to terminal bud was recorded in late December of each year. Also in three consecutive years, 2019, 2020, and 2021 vegetative traits including increasing the height and diameter of seedlings were recorded.

Evaluation of pecan cultivars seedling tolerance against warm weather stress

To assess the damage of seedlings from summer warm weather, hourly temperature data were collected daily from May to the end of September and the damage to seedlings was analyzed. At the end of September of each year, the number of leaves and branches of seedlings damaged by summer heat stress compared to the total number of branches and leaves was determined.

Fig. 2. The stage when the pecan seedlings were transferred to large pots

Fig. 4. Record the trunk diameter and stem height of pecan cultivars

analyzed using SAS software. To normalize the obtained results, the data were converted to sine arc and the means were compared using Duncan's multiple range tests.

Results

At the end of each year, seed germination rate, seedling height at the end of the growing season, seedling diameter, and severity of heat stress damage were recorded and statistically analyzed (Table 1).

Table 1. Variance analysis of seed germination, seedling diameter and seedling height of pecan cultivars (2019-2021).

Source of variation	df	Mean square								
		2019			2020			2021		
		Seed germination	Seedling diameter	Seedling height	Seed germination	Seedling diameter	Seedling height	Seed germination	Seedling diameter	Seedling height
Replication	2	0.107^{ns}	0.097^{ns}	0.063^{ns}	0.342^{ns}	0.065^{ns}	0.983^{ns}	0.572^{ns}	0.965^{ns}	0.843^{ns}
Cultivars	14	27.32^{**}	6.324^{**}	20.236^{*}	23.41^{**}	9.364^{**}	29.236^{*}	33.51^{**}	12.643^{**}	25.431^{*}
Error	28	4.49	1.73	8.56	5.61	2.64	7.36	7.62	3.5	8.46
Total	44									
C.V%		4.57	11.76	5.1	5.17	12.92	3.15	6.05	13.55	3.03

ns, * and ** non-significant, significant at 5% and 1% respectively.

Seed germination rate of pecan cultivars (2019)

The seed germination rate of pecan cultivars was significantly different at the level of 5%. Seed germination rates in 'Stuart 4J', 'M6', 'Comanche 5M', 'Peruque', 'Wichita 7J', 'GraKing', and 'Apache' were more than 50%. A comparison of the average germination rate of pecan cultivars is shown in Fig. 5.

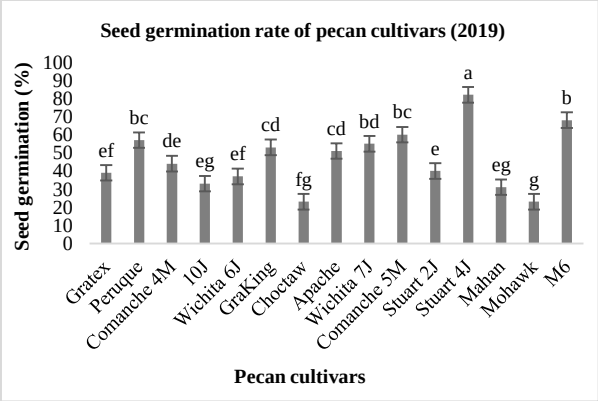

Fig. 5. Comparison of seed germination of pecan cultivars (2019). Means of columns followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.01).

Seedling diameter of pecan cultivars (2019)

Analysis of variance showed that the mean stem diameter of seedlings in different cultivars was statistically significant at the level of 5%. 'Choctaw' had the lowest stem diameter and 'Peruque', 'GraKing, and 'Mahan' had the highest diameter growth among other cultivars. The results of comparing the average stem diameter in 2019 using Duncan's multiple range test are shown in Fig. 6.

Height of seedlings of pecan cultivars (2019)

Pecan seedling height in 2019 was significantly different between cultivars at the level of 5%. 'Peruque', 'Apache', 'Wichita J7' and 'GraKing' had the highest stem growth among the cultivars. The results of comparing the average stem height in 2019 using Duncan's multiple range test are shown in Fig. 6. The vegetative growth status of seedlings of pecan cultivars in the autumn of the first year is shown in Fig. 7.

Evaluation of pecan cultivars seedling tolerance to warm weather stress (2019)

To evaluate the damage of seedlings due to summer warm weather stress, the temperature of the hours from May to the end of September 2019 was recorded from the Synoptic Station of Safiabad (Fig. 8).

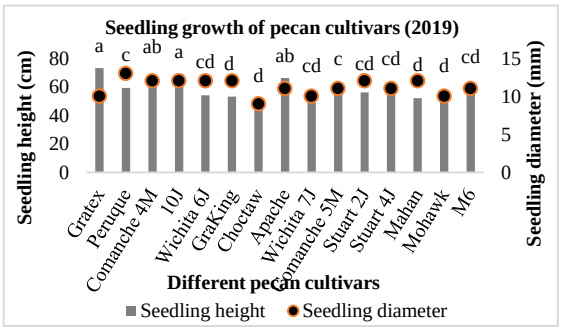

Fig. 6. Vegetative growth of seedlings pecan cultivars (2019). Means of columns followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.01).

Fig. 7. Vegetative status of annual seedlings of pecan cultivars (2019).

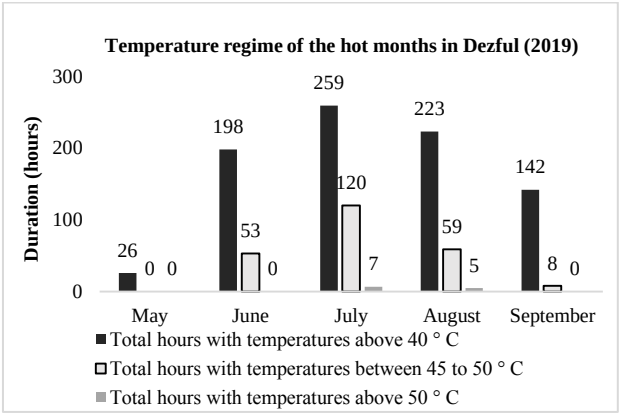

Fig. 8. Total hours of the day with temperatures above 40°C, 45°C, and 50°C (Safiabad, 2019).

Evaluation of the vegetative growth status of seedlings in the first year of the experiment showed that although vegetative growth was almost stopped in July and August, the symptoms of warm weather stress damage on leaves and terminal buds of most cultivars was not significant. The warm weather stress damage of summer 2019 in annual seedlings of pecan cultivars is shown in Fig. 9.

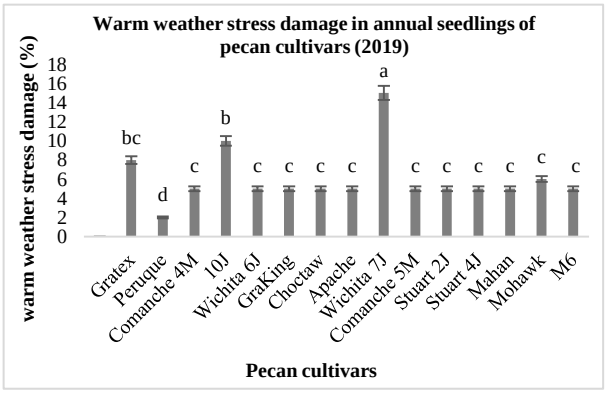

Fig. 9. Damage of warm weather stress in annual seedlings of pecan cultivars (2019). Means of columns followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.01).

Seed germination rate of pecan cultivars (2020)

The seed germination rate between cultivars was significantly different at the level of 5%. 'Stuart 4J', 'Peruque' and 'M6' had the highest seed germination rate. A comparison of the average germination rate of pecan cultivars is shown in Fig. 10.

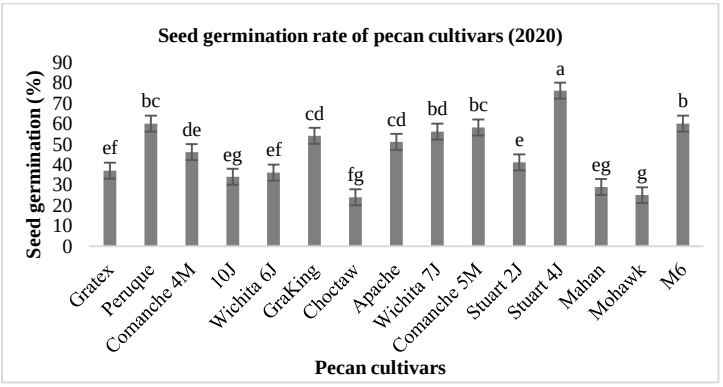

Fig. 10. Comparison of seed germination of pecan cultivars (2020). Means of columns followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.01)

Seedling diameter of pecan cultivars 2020

Analysis of variance showed that the stem diameter of seedlings in pecan cultivars was statistically significant at the level of 1%. 'Choctaw' had the lowest and 'perique' 'GraKing' and 'Mahan' had the highest increase in diameter among other cultivars. The results of comparing the average stem diameter at the end of the growing season in 2020 and also the increase in stem diameter compared to the previous year are shown in Figs. 11 and 12.

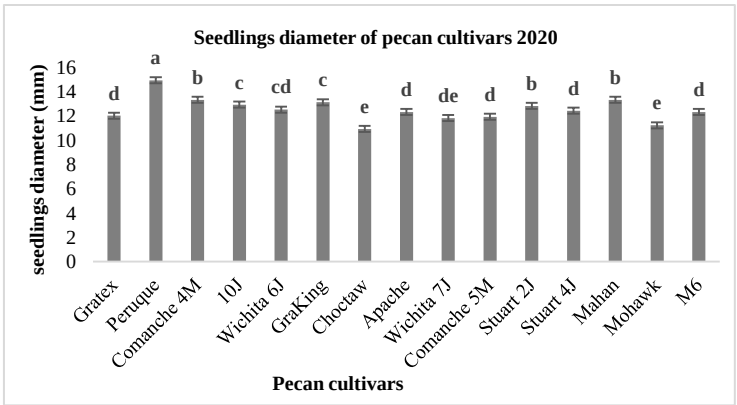

Fig. 11. Comparison of average stem diameter of seedlings of pecan cultivars 2020. Means of columns followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.01).

Seedlings height of pecan cultivars at the end of the second year

Seedling height at the end of the 2020 growing season was significantly different between cultivars at the level of 1%. 'Perique', 'Apache', 'Wichita 7J', and 'GraKing' had the highest stem growth among the other cultivars (Fig. 13). The results of comparing the average height in the second year and the average increase in seedling height compared to the previous year are shown in Fig. 14.

Fig. 12. Seedling height of 'Apache' 21 months after planting.

Fig. 13. Pecan stem diameter of 'Apache' 21 months after planting.

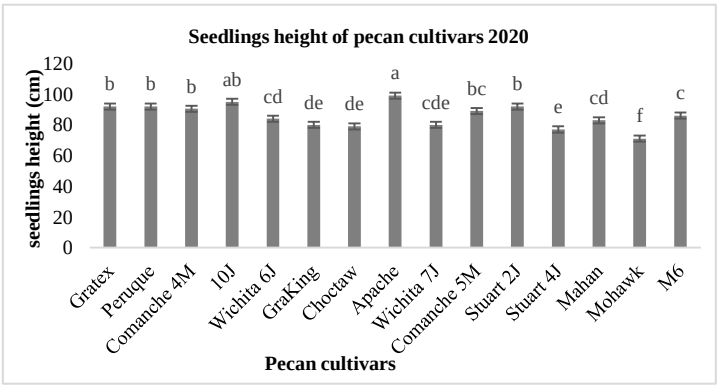

Fig. 14. Comparison of average Seedlings height of pecan cultivars (2020). Means of columns followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.01).

Evaluation of pecan cultivars seedling tolerance to warm weather stress (2020)

To evaluate the damage of seedlings due to summer warm weather stress, the temperature of the hours from May to the end of September 2020 was recorded from the Safiabad Synoptic Station (Fig. 15).

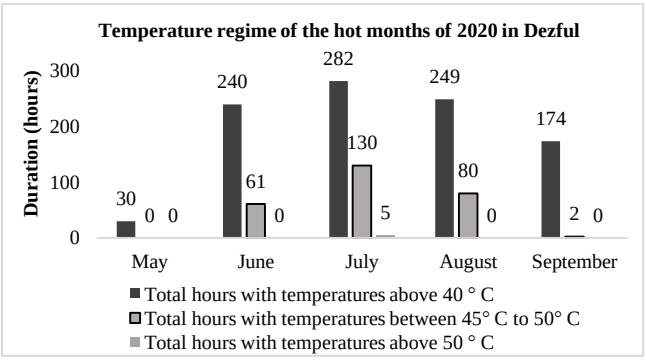

Fig. 15. Total hours of the day with temperatures above 40° C, 45° C and 50° C in the Safiabad (2020).

Evaluation of the vegetative growth status of seedlings in the second year of the experiment showed that their vegetative growth had stopped in July and August and the symptoms of warm weather stress damage on leaves and terminal buds of most cultivars were significant. Hourly meteorological data in 2020 showed that the total hours with temperature more than 45°C in summer reached 271 hours, which compared to 2019 caused more damage to seedlings. Hot weather stress damage in seedlings of Pecan cultivars in 2020 is shown in Fig. 16.

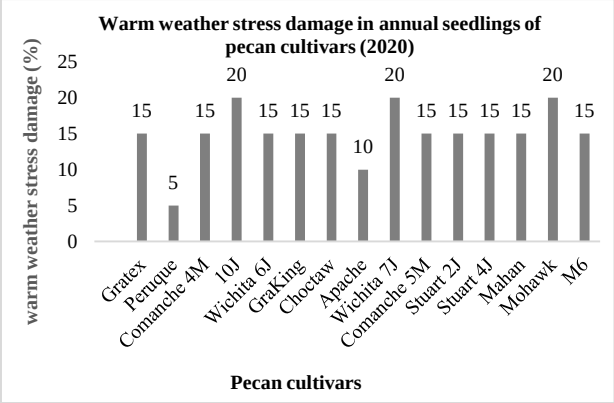

Fig. 16. Damage of warm weather stress in two-year-old seedlings of pecan cultivars (2020).

Seed germination rate of pecan cultivars (2021)

The seed germination rate between cultivars was significantly different at the level of 5%. Seed germination rate as in 2020 in 'Stuart 4J', 'M6', 'Comanche 5M', 'Peruque', 'Wichita 7J', 'GraKing' and 'Apache' was more than 50%. A comparison of the average germination rate of seeds of pecan cultivars is shown in Fig. 17.

Seedlings that can be produced from one kilogram of seeds

In pecan nurseries, cultivars with smaller seeds and high germination rates are preferred. Because more seedlings are produced from one kilogram of their seeds.

This formula was used to determine the index of the number of seedlings that can be produced from one kilogram of seeds Pecan cultivars.

$$\text{Index} = 1000 / \text{Average seed weight (gr)} \times \text{seed germination rate}$$

Index of the number of seedlings that can be produced from one kilogram of seeds pecan cultivars is shown in Fig. 18.

Seedling diameter of pecan cultivars in the third year (2021)

Analysis of variance showed that the stem diameter of seedlings in pecan cultivars was statistically significant at the level of 1%. 'Choctaw' had the lowest stem diameter and 'peruque', 'GraKing' and 'Mahan' had the highest diameter growth among other cultivars. The results of comparing the mean stem diameter at the end of the 2021 growing season using Duncan's multiple range test are shown in Fig. 19.

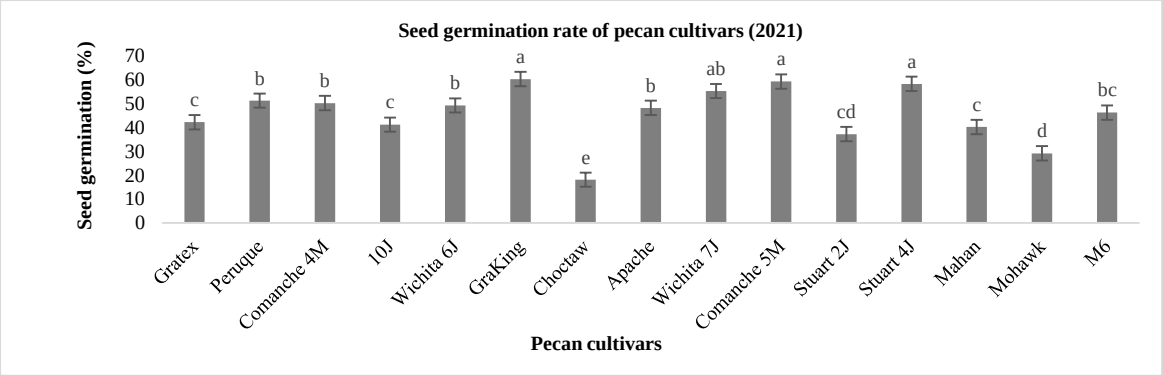

Fig. 17. Comparison of seed germination of pecan cultivars (2021). Means of columns followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.01).

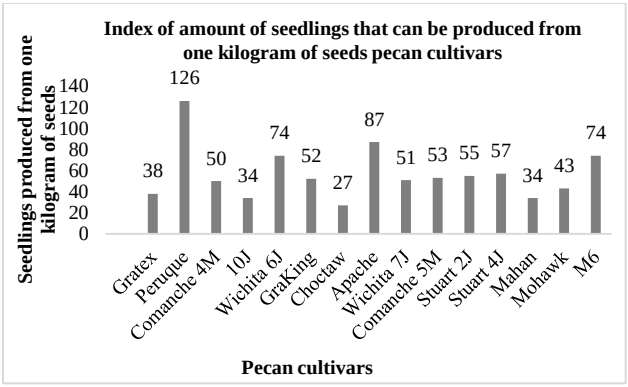

Fig. 18. Index of the amount of seedlings that can be produced from one kilogram of seeds pecan cultivars.

Seedling height pecan cultivars in the third year (2021)

The height of seedlings at the end of the growing season of 2021 was significantly different between cultivars at the level of 5%. 'Peruque', 'Apache', 'Wichita J7' and 'GracKing' had the highest stem among the cultivars. The results of comparing the average stem height in 2021 using Duncan's multiple range test are shown in Fig. 20.

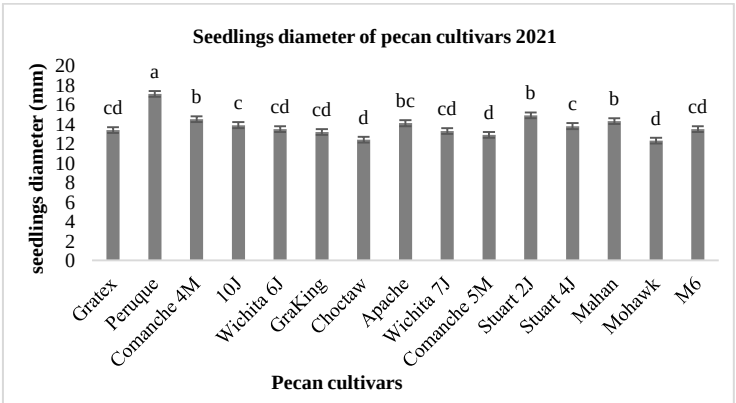

Fig. 19. Comparison of stem diameter of seedlings of pecan cultivars (2021). Means of columns followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.01).

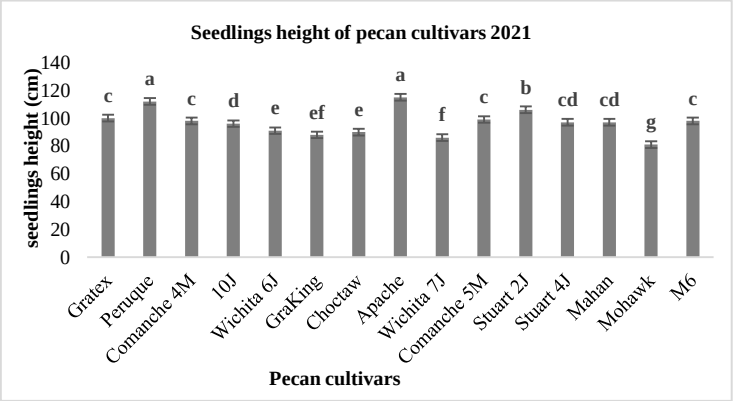

Fig. 20. Comparison of average Seedlings height of pecan cultivars (2021). Means of columns followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.01).

Evaluation of Pecan cultivars seedling tolerance against warm weather stress (2021)

To evaluate the damage of seedlings due to summer warm weather stress, the temperature of the hours from May to the end of September 2021 was recorded from the Safiabad Synoptic Station (Fig. 21).

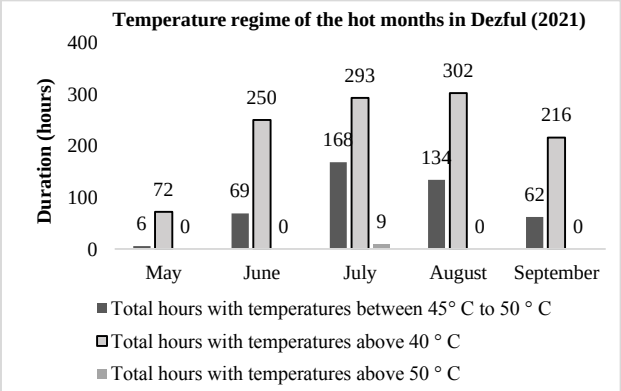

Fig. 21. Total hours of the day with temperatures above 40°C, 45°C and 50°C in Safiabad (2021).

Evaluation of seedling growth status in the third year of the experiment showed that their vegetative growth was completely stopped in July and August and the symptoms of warm weather stress damage on leaves and terminal buds of most pecan cultivars were very severe. Hourly meteorological statistics in 2021 showed that the total hours with temperature more than 45°C in summer was more than 430 hours, which compared to 2020 caused Three-year-old seedlings of all cultivars to show severe symptoms of warm weather stress. Warm weather stress damage in seedlings of pecan cultivars in 2021 is shown in Fig. 22.

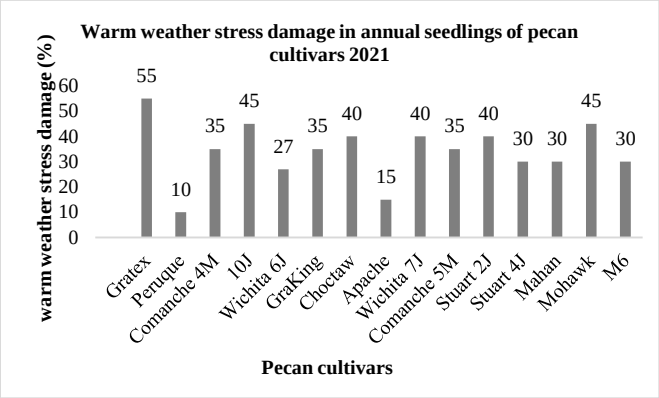

Fig. 22. Damage of warm weather stress in annual seedlings of pecan cultivars (2021).

Comparison of warm weather stress intensity in Safiabad (2019 – 2021)

Meteorological statistics from 2019 to 2021 showed that the intensity of warm weather stress in the summer of 2021 was very severe and most plants, even large and old pecan trees were damaged. It is obvious that young seedlings were more affected by this stress and caused severe symptoms of drying of leaves and terminal buds (Fig. 23). The total hours with temperatures above 40, 45, and 50°C are shown in Fig. 24.

Fig. 23. Symptoms of leaf drying in three-year-old pecan seedlings due to warm weather stress (2021).

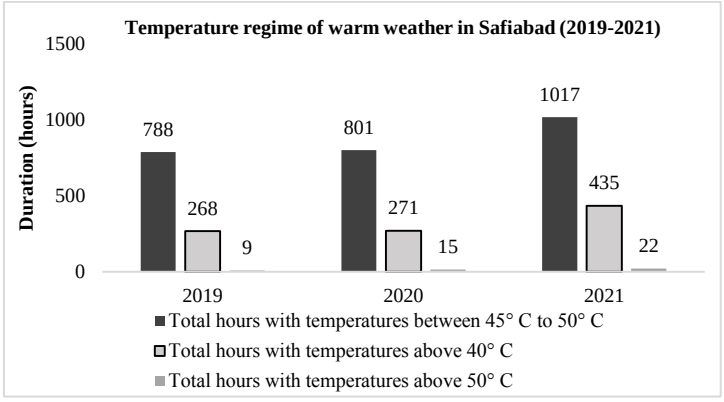

Fig. 24. Total hours of the day with temperatures above 40°C, 45°C and 50°C in Safiabad (2019-2021).

Discussion

With the development of pecan orchards in different provinces of Iran in recent years, it is very important to choose vigor rootstocks for grafting that are adapted to different regions. Especially in subtropical provinces such as Khuzestan and the southern province of Fars, the selection of hot weather tolerant rootstock is very important. Therefore, knowing the adaptation of different rootstocks to different environments becomes important for pecan breeders and growers (Poletto *et al.,* 2016). Selecting the right cultivar to produce pecan rootstocks not only provides scion resistance to environmental stresses but also increases growth vigor to reduce both the vegetative and reproductive times of the scion (Grauke *et al.,* 2003). In addition, a proper rootstock can reduce orchard management costs and increase profits for pecan growers. (Cao *et al.*, 2019).

From the point of most commercial nurseries, several important parameters are considered to select seed to propagate pecan rootstocks such as seed germination rate, seed size and uniformity (Andersen, 2004), and seedling vegetative growth rate (Reid, 2010). In nurseries, smaller seeds with a higher germination rate are considered. The result of this study showed that 'Peruque' and 'Apache' were preferred with the possibility of producing 126 and 86 seedlings per kilogram of their seeds respectively which is consistent with Andersen, 2004 reports.

The results of seedling growth vigor of pecan cultivars showed that 'Peruque', 'Stuart 2J', 'Comanche 4M', 'Mahan' and 'Apache' grew more than other cultivars and were suitable for grafting in a shorter time. According to Reid, 2010 reports, the vigor of seedlings are very important in the success of pecan grafting.

Tolerance pecan rootstock to biotic stresses such as diseases and pests and abiotic stresses such as heat and cold weather, salinity, and drought soil (Casales *et al.,* 2018) and compatibility in grafting (McEachern, 2010) are other characteristics that should be considered.

To select the pecan rootstock, all these parameters must be considered, but in this study, due to the special conditions of subtropical regions of Iran, especially Khuzestan province, special attention to seedlings of pecan cultivars that have tolerated warm weather stress. According to Cao *et al., 2019* reports, the most critical limitation for a pecan rootstock in any region is its adaptation to the climate of that region.

The warm weather in the summer of 2021 was so severe that even several decades old and large pecan trees were damaged. Prolonged daytime warmer than 45°C to 50°C caused the leaves and terminal buds of the seedlings to damage. For example, a thermometer on July 11, 2021, in Safiabad, showed a temperature above 45°C to 50°C for 12 hours, indicating the intensity of heat stress from morning to after sunset on this day.

The most important achievement of this study was to find out the high tolerance of 'Peruque' seedlings to warm weather stress. The damage of branches and leaves of this cultivar against severe summer warm weather stress was less than 10%. Seedlings of 'Apache' also showed less damage than other cultivars.

Evaluation of characteristics of different pecan cultivars for rootstock including seed germination rate, seed size, seedling vigor and resistance to hot weather stress in the years 2019 to 2020 showed that 'Peruque' and 'Apache' are two suitable cultivars for pecan rootstock in Khuzestan province.

It is suggested that more research be done to determine the best rootstock, especially disease-resistant rootstock for the northern regions of Iran, including Gilan, Mazandaran and Golestan provinces.

Conclusions

According to the results of seedling reaction of different pecan cultivars in the years 2019 to 2021 to increase the commercial orchards of pecan in subtropical regions of the country, including the

provinces of Khuzestan, south of Fars, south of Kermanshah, and other areas with hot summers, use ‘Peruque’ and ‘Apache’ rootstock. For other provinces, more research should be done to determine cold-tolerant as well as disease-resistant rootstock.

Acknowledgements

The author is grateful to Mr. Yavari Nejad, Dr. Hassani, Dr. Soleimani, Dr. Shafiei Zargar, Dr. Shooshi and Dr. Keshavarznia.

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