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Phenological and physicochemical properties of *Pereskia aculeata* during cultivation in south Brazil

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ABSTRACT

Pereskia aculeata, known as ora-pro-nobis in Brazil, is native from tropical dry forests. This *Cactaceae* plant possesses succulent and edible leaves, which contain high amounts of protein, minerals, vitamins and fiber. Nutritional properties and ability to grow under limited water supply of ora-pro-nobis are known, but little information is available about the growth behavior and nutritional composition of this plant when cultivated under temperate humid climate. Therefore, we evaluated the phenology of the plant, including observation of new leaves, flowering, fruiting and relating it with the climate changes. Also, we analyzed some physicochemical characteristics (humidity, leaf area, height, protein, color, total phenolic content and antioxidant activity) of ora-pro-nobis cultivated in Pelotas, Rio Grande do Sul, Brazil. We observed that ora-pro-nobis developed normally, but with a quiescent state in the winter, without producing leaves. Flowering of the plant started in March and the fructification started one month later. All physicochemical characteristics varied through the period of cultivation. Our findings support that cultivation of ora-pro-nobis for production of leaves is feasible under temperate and humid climate.

Keywords: Ora-pro-nobis, *Cactaceae*, quiescent state, phenology.

RESUMO

***Pereskia aculeata* cultivada no sul do Brasil**

Pereskia aculeata, conhecida popularmente no Brasil como ora-pro-nobis, é nativa de regiões tropicais e de clima seco. Esta cactácea possui folhas suculentas e comestíveis, com altos teores de proteína, minerais, vitaminas e fibras. Propriedades nutricionais e caracterização de crescimento em regiões de clima seco já são conhecidas sobre essa planta, porém muito pouco se sabe sobre a possibilidade do seu cultivo e composição nutricional em áreas de clima temperado e úmido. Por isso, decidiu-se avaliar a fenologia da planta, incluindo a observação da formação de novas folhas, flores e frutos e a relação com as mudanças climáticas. Também analisou-se algumas das propriedades físico-químicas (umidade, área foliar, altura, proteína, cor, fenóis totais e atividade antioxidante) da planta ora-pro-nobis cultivada em Pelotas, Rio Grande do Sul, Brasil. Foi observado que ora-pro-nobis se desenvolveu normalmente, porém durante o inverno a planta permaneceu no estado de quiescência e não produziu folhas. O início da floração ocorreu no mês de março e a frutificação no mês seguinte. Todas as análises físico-químicas variaram durante os meses de cultivo avaliados. Os resultados sugerem que o cultivo da planta ora-pro-nobis para produção de folhas é possível em regiões de clima temperado e úmido.

Palavras-chave: Ora-pro-nobis, *Cactaceae*, quiescência, fenologia.

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Pereskia aculeata (ora-pro-nobis) is a plant member of the *Cactaceae* family and is found in tropical areas from south United States to south Brazil. It is known, popularly, as ora-pro-nobis and in some Latin American countries is known as Barbados Gooseberry (Takeiti *et al*., 2009; Sharif *et al*., 2013).

Ora-pro-nobis is a perennial shrub, very resistant to drought, and has scramble vine characteristics. Flowers are white and small, fruits are small yellow berries, and also, the plant has spines at the stems and large leaves (Brasil, 2010).

Ora-pro-nobis has succulent and edible leaves, which can be used in many preparations, such as salads, stews, flours, breads, pies and pastas (Rocha *et al*., 2008). Recent studies (Silva *et al*., 2017) show that ora-pro-nobis is safe for consumption in terms of acute toxicity and cytotoxicity. Other than food, the plant can be used ornamentally or cultivated for honey production once it is rich in pollen and nectar. Folk medicine practitioners have been known to use ora-pro-nobis as anti-inflammatory, emollient, expectorant and antisyphilitic (Sartor *et al*., 2010).

Ora-pro-nobis leaves are rich in protein (28.4 g 100 g^{-1} of dry weight) when compared with other vegetables source of protein, like black beans [8.8 g 100 g^{-1} of cooked weight (dw)], garbanzo beans (8.9 g 100 g^{-1} of dw) and lentils (9.0 g 100 g^{-1} of dw) (Takeiti *et al*., 2009; USDA, 2014).

Also, this plant is a remarkable source of nutritionally important minerals (Takeiti *et al*., 2009; Oliveira *et al*., 2013). Fresh ora-pro-nobis leaves contains more calcium (20.0 fold), iron (5.7 fold) and zinc (3.3 fold) than kale, spinach, and pumpkin seeds, respectively, considered rich vegetable sources for these minerals (Takeiti *et al*., 2009; USDA, 2014). Ora-pro-nobis leaves contain high levels of fiber (39.1 g 100 g^{-1} of dw), vitamin C (185.8 mg 100 g^{-1} of dw) and folic acid (19.3 mg 100 g^{-1} of dw) (Takeiti *et al*., 2009).

Therefore, ora-pro-nobis could be a good alternative to many common food sources, especially for vegetarians, because it has high levels of minerals and proteins (Takeiti *et al*., 2009).

Ora-pro-nobis is known to be native from tropical dry forests (Takeiti *et al*., 2009; Brasil, 2010). To our knowledge, growth behavior and composition of this plant when cultivated under a temperate humid climate is unknown. Therefore, the goal of this study was to evaluate phenological and physicochemical characteristics of ora-pro-nobis cultivated in Pelotas, Rio Grande do Sul, Brazil, under temperate and humid climate.

MATERIAL AND METHODS

Plant material

Ora-pro-nobis was cultivated at the Brazilian Agricultural Research Corporation (Embrapa Clima Temperado), located in Pelotas, Brazil. The 21 plants utilized in this experiment were divided into 3 lots. Plants were spaced 100 cm x 80 cm without irrigation and pest control. Harvest was done monthly, between December 2012 and June 2013. In the moment of harvest, the 21 plants were measured, to determine height. Also, phenological aspects were determined observing new leaves, flowering and fruiting. These processes were related with climate changes, as precipitation, radiation and temperature (Marques & Oliveira, 2004).

Harvested leaves were cleaned, milled to a fine powder in a ball mill in liquid nitrogen and stored at -80°C for future analyzes (antioxidant activity, phenol and protein). Fresh leaves were also analyzed for humidity, color and area.

Physicochemical evaluations

To determine humidity, 10 leaves per lot were weighted and dried during 24 hours in a forced air oven at 105°C, according AOAC (2012). Leaf area was determined in 10 leaves per lot, based on the use of an automated infrared imaging system, LI-COR-3100C (LI-COR Inc., Lincoln, Nebraska, USA). Plant height was assessed using a tape measure, according to Silva *et al*. (2012).

Color was determined using a colorimeter (Minolta®, Model CR 300). We measured lightness (L*), redness (a*) and yellowness (b*), in 3 parts of each leaf, and 10 leaves per lot, with a total of 30 leaves per month. The parameters of color were expressed in lightness, where L*= 0 is completely black, and L*= 100 is completely white; and Hue angle (H*), calculated from H*= [Arc tan (b/a)], where H*= 0 is red, H*= 90 is yellow, H*= 180 is green and H*= 270 is blue, as described by Cogo *et al.* (2011).

Antioxidant activity was estimated using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay method, adapted from Kedare & Singh (2011). Extraction was performed using methanol with 1:4 proportion and stored at 4°C for 24 h; after that, the extract was centrifuged (12000 rpm) for 15 minutes. The absorbance of samples was measured at 517 nm and antioxidant activity was expressed as g of trolox/kg of fresh leaves.

The total phenolic content of ora-pro-nobis was determined by Folin-Ciocalteu method, adapted from Medina (2011). Extraction lasted 2 h, using methanol in the proportion 1:10 and stirring every 15 minutes. Results were expressed as g of galic acid equivalent (GAE) per kg of fresh leaves.

Protein of fresh leaves was estimated using the microkjeldahl method, according to the AOAC (2012). The value 6.25 was used to convert nitrogen into protein.

Statistical analysis

All analyzes were performed in triplicate. Obtained data of all physicochemical evaluations were analyzed by descriptive statistics for every month and compiled into graphs using SigmaPlot 10.0 software. Person's correlation was used to compare antioxidant activity and total phenolic content, using the Statistical Analysis Software (SAS) for Windows V8, at 5% significance ($p < 0.05$).

RESULTS AND DISCUSSION

It is possible to cultivate ora-pro-nobis in temperate and humid areas. However, it is important to consider that, during winter, when exposed to low temperatures and frost, the plant loses leaves and stays in a quiescent state. Quiescence is a common state in seeds, but it can occur also in the entire or in parts of the plant. Normally, quiescence is a preparation for winter and is a strategy to conserve energy and carbohydrate by restraining growth (Luo *et al*., 2011). We started to analyze the plants in June 2012, but because of the quiescent state, we only had enough leaves to analyze between December 2012 and June 2013.

Phenology is the sequential developmental stages of the annual growth cycle and their timing. In our study we analyzed the occurrence of new leaves, flowering and fructification (Table 1).

Observations were made at the end of spring, in December, when temperature was higher, frost subsided, and new leaves started to grow back. After three months, in March, we noticed that flowering had started in the first plants, and one month later, in April, the fruiting started. Again, when temperature decreased, in June, leaves started to fall, ending the cycle of ora-pro-nobis in south Brazil. Temperature was found to be an essential factor for growth and development of this species. It is known that temperature affects photosynthesis and development of shoots (Ushio *et al*., 2008).

In temperate climates, warm temperatures often act as flowering triggers. Also, rain is an important timer for flowering in shrubs. There is a difference between fruiting in temperate and tropical areas. In temperate regions fruiting normally starts late in the summer or in the autumn, lasting for one and a half month in average. The fruit production is largely controlled by the accumulation of enough photosynthesis, which can only occur towards the end of the growing season (Fenner,

Table 1. Temperature, radiation, precipitation and phenological aspects during *Pereskia aculeata* cultivation from June 2012 to June 2013. Pelotas, UFPel, 2013.

Month	Temperature (°C)			Radiation (cal/cm²/d)	Precipitation (mm)	Phenological aspect
	Min	Max	Average			
July/2012	5.6	16.8	10.4	221.7	138.5	Quiescence
August/2012	12.6	22.9	16.7	263.3	103.1	Quiescence
September/2012	12.3	21.3	16.2	309.9	115.3	Quiescence
October/2012	15.9	23.6	19.2	338.5	106.5	Quiescence
November/2012	11.6	27.0	21.2	504.8	52.1	-
December/2012	18.7	29.3	23.6	502.0	175.1	New leaves
January/2013	17.5	27.5	22.3	548.5	69.2	New leaves
February/2013	19.1	28.0	22.8	454.9	177.3	-
March/2013	15.2	25.8	19.8	390.4	27.6	Flowering
April/2013	13.8	24.5	18.3	306.7	147.4	Flowering & Fruiting
May/2013	10.5	20.6	14.6	211.8	84.1	Flowering & Fruiting
June/2013	8.0	18.4	12.5	202.9	75.8	Quiescence Started

Source: Estação Agrometeorológica de Pelotas, RS (UFPEL/EMBRAPA/INMET: http://www.cpact.embrapa.br/agromet). The minimum (Min) and maximum (Max) temperatures correspond to the mean of minimum and maximum temperatures in each month, respectively.

1998). In our study we observed that temperature was the most important factor for flowering. Also, fruiting started in autumn, lasting two months in average. A single-year study was sufficient to demonstrate the ability of ora-pro-nobis to grow under temperate and humid climate, but cannot provide complete information on phenological changes among the years. According to Fenner (1998), long-term (3-5 year) investigation is required to determine phenological modifications accurately.

Color, expressed as lightness and Hue angle, is shown in Figure 1. Lightness presented the higher level in March (L= 51.32), meaning that this was the month when leaves had the lightest colors, coinciding with flowering start. The values of Hue angle showed that leaves presented colors between yellow and green during all months. In December and March, leaves were more yellowish and in January more greenish. These color changes of leaves are probably due to local climate conditions. In January, when leaves were more greenish, the solar radiation reached its maximum (548.5 cal/cm²/d) (Table 1). Therefore, it could be argued that solar radiation was a limiting factor for the plant development under temperate climate. Pereskia species have typically been described as drought deciduous, suggesting that Pereskia water relations are different from those of specialized core cacti and that Pereskia regulates water loss in the same way as a typical C3 woody plant (Edwards & Diaz, 2006). Therefore, in comparison to the stem color of specialized core cacti, the color of leaves from Pereskia species is expected to respond faster to changes in climate conditions (rain, temperature and solar radiation). Chlorophyll is the most common pigment found in plants and is responsible for their characteristic green color. The bright green color of vegetables is often associated to their freshness (Calvano *et al.*, 2015).

Average height of plants increased almost 4 folds during the seven months period of analyzes. The least average leaf area (14.82 cm²) was observed in December, due to the quiescence state period months before. After that, leaves started to grow, reaching the largest

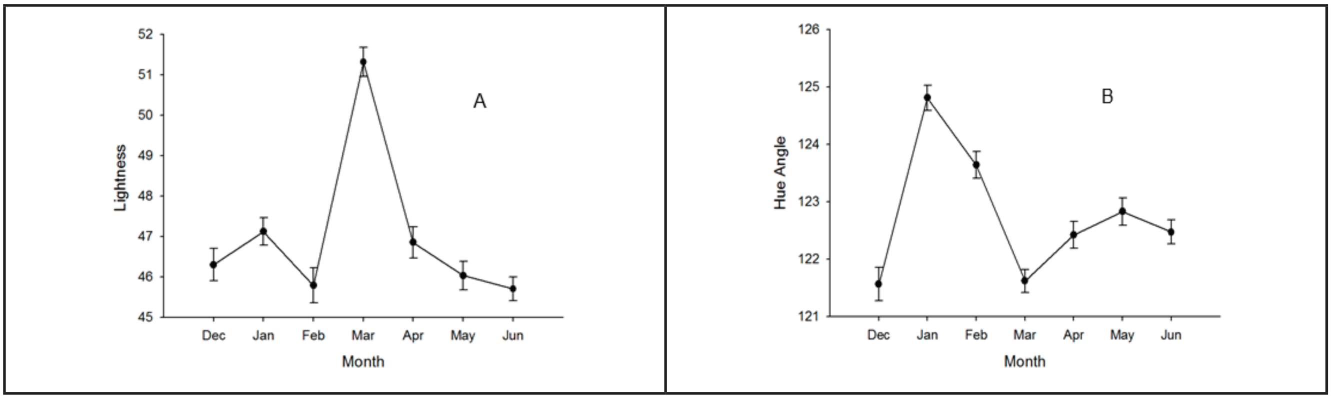

Figure 1. Color, expressed in lightness and Hue angle, of *Pereskia aculeata* leaves from December 2012 to June 2013. Pelotas, UFPel, 2013.

average size (33.11 cm^2) in February (Figure 2). Monitoring changes of leaf area is important for assessing growth and vigor of plants. Frost, storm, defoliation, drought, and management practice commonly cause reduction of leaf area, therefore decreasing the productivity of the plant (Breâda, 2003).

Humidity of ora-pro-nobis leaves remained around 880 g kg^{-1} for 7 months, reaching the lowest average value (861.11 g kg^{-1}) in February. Protein content was higher in December 2012 (27.23 g kg^{-1}) and June 2013 (27.22 g kg^{-1}) and lower in February (21.35 g kg^{-1}) (Figure 2). Maintenance respiration is known to be increased by higher temperatures (Modi, 2007). In this study, higher leaf protein turnover under higher temperature conditions may have been responsible for tendency of lower protein contents under higher temperature growth conditions. Antioxidant activity, measured with the DPPH scavenging assay, as well as total phenolic content, reached the highest level, 44.99 g of Trolox/kg of fresh plant and 2.66 g of GAE/kg of fresh plant , respectively, on April (Figures 2E and F). Antioxidant activity and total phenolic content had some correlation (r= 0.71; p<0.0001).

Pinto *et al*. (2012), researching ora-pro-nobis leaves, found by thin layer chromatography, that phenol was the main antioxidant compound. There are many studies showing that total phenol compound has stronger positive correlation with antioxidant activity in vegetables (Aires *et al*., 2011; Bhandari & Kwak, 2015). Phenolic compounds are among the most important components on the quality of vegetables and fruits. They contribute to organoleptic characteristics like color and taste and promote beneficial effect to human health (Sancho *et al*., 2011; Zielinski *et al*., 2014).

Ora-pro-nobis developed adequately, but with a quiescent state in the winter (without producing leaves). Flowering of the plant started in March and

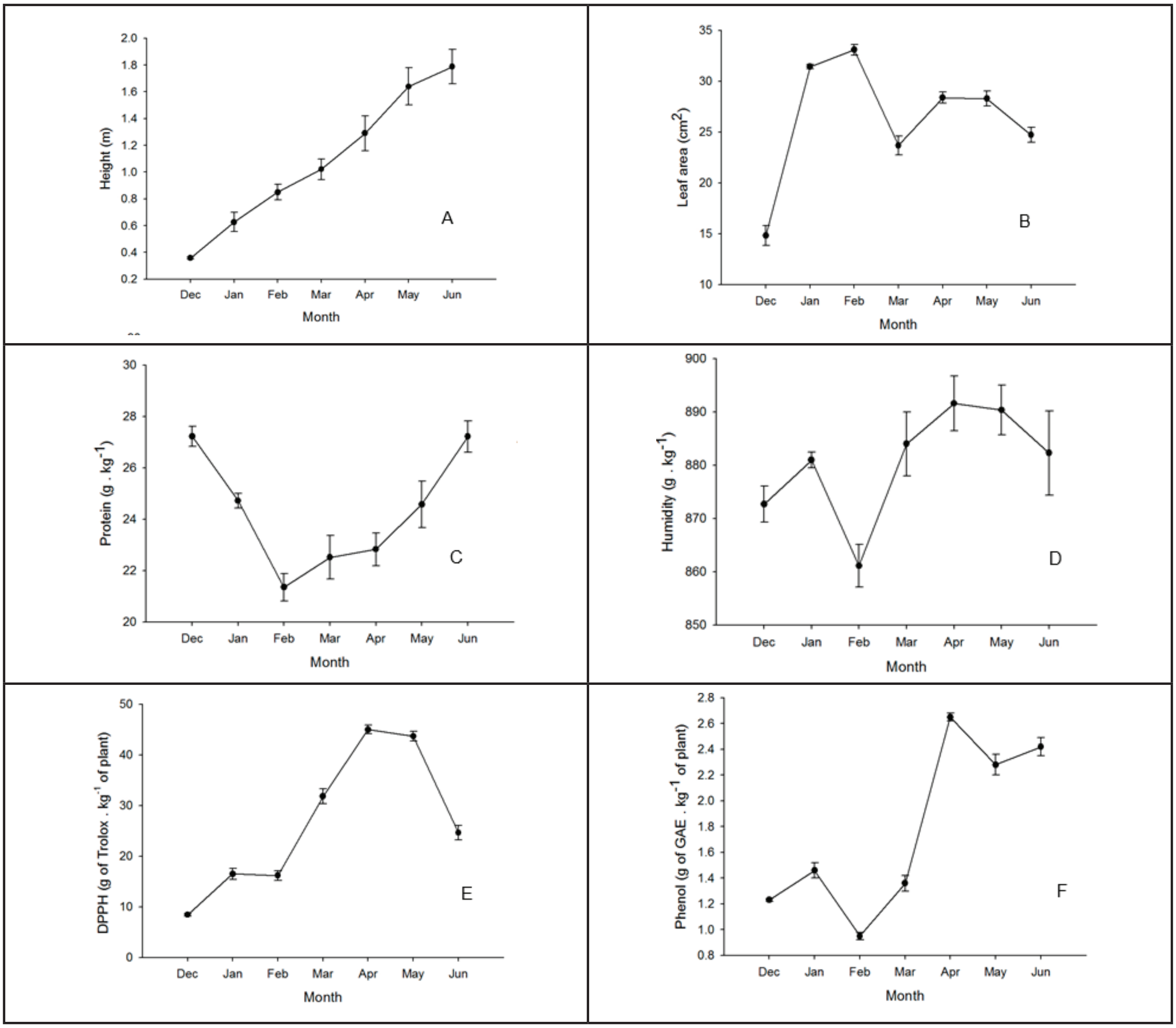

Figure 2. *Pereskia aculeata* during cultivation in southern Brazil (December 2012 to June 2013). A= height; B= leaf area; C= protein; D= humidity; E= antioxidant activity (DPPH); F= total phenolic content (phenol). Pelotas, UFPel, 2013.

fructification one month later. The physicochemical characteristics (humidity, leaf area, protein, color, total phenolic content and antioxidant activity) varied throughout cultivation period. All our findings support that cultivation of ora-pro-nobis for production of leaves is feasible under temperate and humid climate in south Brazil.

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