



Southern highland papaya (*Vasconcellea quercifolia* A. St.-Hil.): Do fruit ripening and harvesting time affect seed germination?

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ABSTRACT. In this work, we report the effects of the harvesting time, the stages in fruit ripening and the influence of potassium nitrate in *V. quercifolia* seed germination. In addition, information about the storage period and light requirements is provided. Fruits were harvested at the beginning and at the end of the fruiting season, and they were classified into five ripening categories. Seed germination was evaluated with two factorial experiments: 1) harvesting time, fruit ripening, and pre-germination treatment; 2) storage and light requirements. The response variables were germination percentage, mean germination time, and seedling vigor. Seeds harvested at the beginning of the season appeared to be less dormant and they were not influenced by fruit ripening or pre-germination treatments. By contrast, seeds harvested at the end of the season were influenced by fruit ripening and pre-germination treatments. Light and seed storage had a positive effect on germination. Mean germination time varied from 12 to 40 days, and vigor index was positively influenced by potassium nitrate. *V. quercifolia* seeds are photoblastic positive at constant temperatures and their dormancy can be influenced by harvest time, fruit ripening and a storage period.

Keywords: caricaceae; dormancy; photoblastism; potassium nitrate.

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Introduction

Vasconcellea species, often known as ‘highland papayas’ or ‘mountain papayas’, are widely distributed in tropical America, from Mexico to Argentina. Most of them are grown in Colombia, Ecuador and Peru, countries that present cool climates with limited seasonality (Scheldeman et al., 2007). The genus is the largest within Caricaceae family and comprises 21 species, five of them included in a risk category (International Union for Conservation of Nature [IUCN], 2018). Highland papayas are wild relatives of common papaya (*Carica papaya*), and have a great potential as genetic resources for papaya improvement and as a source of chemical compounds, like papain (Scheldeman et al., 2007). Moreover, their edible fruits have appealing organoleptic properties (e.g. aroma, taste, color) of importance for the tropical fruit industry (d’Eeckenbrugge, Drew, Kyndt, & Scheldeman, 2014). *V. quercifolia* is the southernmost species of the genus and its distribution in Argentina includes the provinces of the North-west, North-east and Santa Fe (Zuloaga, Morrone, & Belgrano, 2008). It is a pioneer species and it plays an important ecological role such as colonization of degraded soils and as food supply for wild animals. Furthermore, their orange and sweet fruits produce latex, a milky solution with high levels of proteases (Torres et al., 2012). This species is resistant to ringspot papaya virus and to cold temperatures; thus, it has been reported to be the best option for intergeneric hybridizations for common papaya improvement (Siar, Beligan, Sajise, Villegas, & Drew, 2011).

Seed dormancy is a well-known strategy to optimize germination, seedling emergence and establishment. It is defined as the incapacity of a viable seed to germinate under favorable conditions. Therefore, seedling emergence can occur when environmental conditions are favorable for plant survival and growth. Hence, seed dormancy is more frequent in species growing in seasonal and/or unpredictable environments (Baskin & Baskin, 2014; Née, Xiang, & Soppe, 2017).

The dormancy level is affected by the mother plant’s environmental conditions like temperature, light, and nitrate levels, experienced during fruit development. In fact, seeds in the soil bank are able to

sense changes in the local soil environment and are able to adjust their dormancy levels. In that sense, the study of dormancy and the mechanisms involved in its release are of biological and economical importance (Tokuhisa, Dias, Alvarenga, Dias, & Marin, 2008; Baskin & Baskin, 2014; Née et al., 2017).

Vasconcellea species, like most of the family, have dormant seeds. Their low and erratic germination has been attributed to inhibitory compounds present in the sarcotesta (jellied envelop of seeds) or sclerotesta (middle hard layer of the testa) (Aroucha, Silva, Oliveira, Viana, & Gonzaga, 2005; Tokuhisa, Dias, Alvarenga, Hilst, & Demuner, 2007a; Freitas et al., 2011; Benítez, Lobo, Delgado, & Medina, 2013; Melo & Seleguini, 2013; Dias, Dias, Lima, & Dias, 2015). The concentration of inhibitory compounds is associated to fruit ripening and harvesting season, and these are related to weather conditions and physiological aspects of fruit ripening. In general, when fruits change from green to orange, seeds are in their maximum physiological quality and should be collected (Melo & Seleguini, 2013). However, fruit and seed development are heterogeneous (Aroucha et al., 2005).

It was shown that growth promoters eliminate or counteract the effects of lipoproteins that inhibit germination and cause dormancy in papaya seeds. Soaking the seeds in potassium nitrate improve the percentages and the uniformity of seedling emergence. Moreover, potassium nitrate also promotes greener and vigorous plants and its use was currently recommended by the International Seed Testing Association to overcome dormancy of light requirement seeds (Furutani & Nagao, 1987; Tokuhisa, Dias, Alvarenga, Dias, & Marin, 2007b; Andrade-Rodríguez et al., 2008; Zanotti, Dias, Barros, Damatta, & Oliveira, 2014).

Furthermore, light is proposed as an extremely important factor for releasing seeds from dormancy but its requirement may change as seeds come out of dormancy (with variations in temperature and seed moisture content). For that reason, it is important to test the influence of light in fresh seeds and during the dormancy-breaking period (Bewley, Bradford, Hilhorst, & Nonogaki, 2013; Baskin & Baskin, 2014).

In this work we address the following questions: do fruit ripening and harvesting time affect seed dormancy? Has the potassium nitrate a positive effect in germination response? Do *V. quercifolia* seeds need light and a storage period to germinate?

Material and methods

V. quercifolia is a tree that may reach 12 m height and 1 m in diameter. It is a dioecious and a xenogamous species that requires pollinators (moths) (Cerino, Torretta, Gutiérrez, Richard, & Pensiero, 2014). It grows best in full sunlight and in moist ground. Therefore, it can be found near rivers or in slopes. It is a deciduous species and has simple but polymorphic leaves (Figure 1A and B), greenish flowers (Figure 1C and D) and orange fruits (Figure 1E). The flowering season occurs from October to February and the fruiting season from January to March (Scheldeman et al., 2007). In the Northwestern region of Argentina, it grows in Yungas and transition forests (Grau, Malizia, & Brown, 2016). The fruits for this study were collected from ten female trees randomly chosen from a wild population located at Las Costas natural reserve (24° 45' S, 65° 30' W), near Salta city (Northwestern Argentina).

Harvesting time and fruit ripening

To test the effect of fruit ripening and harvesting time on seed germination, fruits were collected at the beginning and at the end of the 2017 fruiting season, February and March respectively. In each period, fruits were grouped into 5 developing stages of ripening: 1. unripe (green) fruits with white seeds; 2. unripe fruits with brown seeds; 3. unripe fruits stored until full ripening (from 2 to 8 days at room temperature); 4. almost ripe fruits (up to 50 % yellow tinge peel), and 5. ripe fruits (more than 75% yellow tinge peel) (Figure 1E, from left to right respectively).

After fruit collection, seeds were extracted immediately and the sarcotesta was removed by mixing and shaking the seeds with dry sand for 24 hours. Then, the seeds were washed and were left to dry on absorbent paper before germination. To assess germination response, a factorial assay was performed. The factors were: 1. harvesting time (February and March), 2. fruit ripening (1, 2, 3, 4, 5), and 3. pre-germination treatments (control and potassium nitrate 1 M for 30 minutes) (Tokuhisa et al., 2007b; Andrade-Rodríguez et al., 2008).

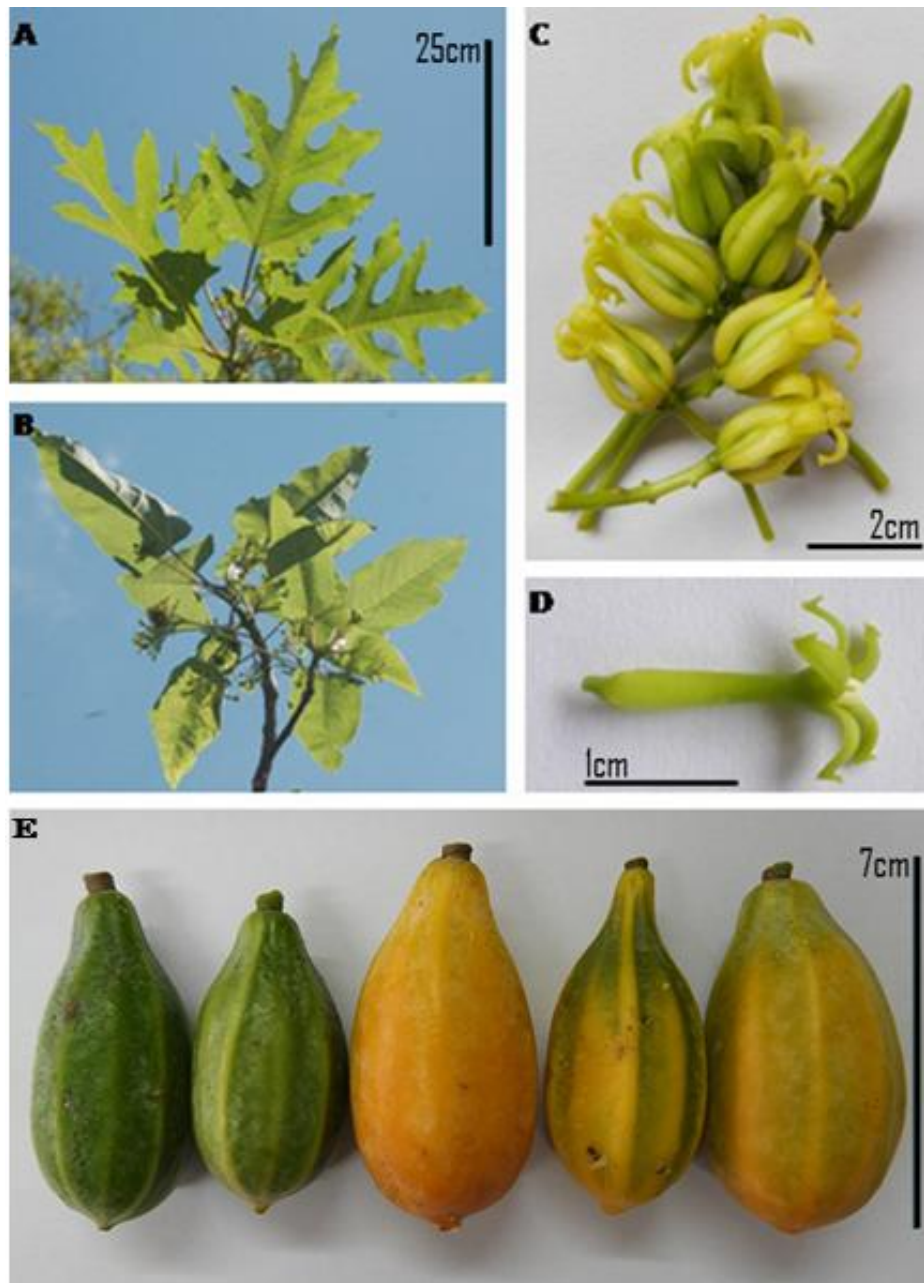


Figure 1. *V. quercifolia* (A) segmented leaves (B) whole leaves (C) female flowers (D) male flower (E) unripe and ripe fruits (see below, stages of ripening).

Seed storage and photoblastism

In order to test the effect of storage time and light on seed germination, we collected ripe fruits in February 2016 and 2017. The seeds from 2016 harvest were stored in paper bags at ambient temperature for one year. In March 2017, we run a germination assay with fresh seeds (2017) and stored seeds (2016), which were exposed to 16 hours photoperiod or complete darkness until the end of the experiment.

In both experiments four replicates with 25 seeds per treatment were kept for 60 days in a germination chamber (25°C, 50% RH, 16 hours light/ 8 hours darkness). The seeds were sown on sterilized sand (240°C for 24 hours) in plastic trays. The germination criterion was root emergence. The response variables were germination percentage ($GP = (n/N) \cdot 100$), mean germination time ($MGT = [(\sum ni \cdot ti) / Ni]$), seedling vigor ($VI = \text{root length} + \text{stem length (in cm)} \cdot GP$) and photoblastism ($GRL = GL / (GO + GL)$). Where n: total number of germinated seeds, N: number of seeds per replicate, ni: number of germinated seeds in ti days, ti: days since the start of the experiment, Ni: total number of germinated seeds, GL = GP of seeds exposed to light, GO = GP of seeds exposed to darkness (Milberg, Andersson, & Thompson, 2000; Kumar, Kak, & Singh, 2011; Soltani, Ghaderi-Far, Baskin, & Baskin, 2015).

Differences in germination percentages, average of germination time and vigor index between harvesting times, fruit ripening stages, pre-germination treatments, photoblastic effect, and storage period were tested with ANOVA. Treatments were compared with Tukey test. When the data did not adjust to ANOVA assumptions, Kruskal Wallis test was used. All the analyses were performed with Infostat software (Di Rienzo et al., 2009).

Results

Harvesting time and fruit ripening

Germination percentages were low in all treatments, less than 39% (Table 1). At the beginning of the fruiting season (February), seed germination was higher than at the end ($F = 32.34$, $p < 0.0001$), and there were no differences on fruit ripening stages ($F = 2.18$, $p = 0.09$) nor in pre-germination treatments ($F = 0.71$, $p = 0.40$). Conversely, at the end of the fruiting season, we found significant differences between fruit ripening stages ($F = 34.57$, $p < 0.0001$) and pre-germination treatments ($F = 31.78$, $p < 0.0001$). Ripe and unripe fruits with brown seeds soaked in potassium nitrate had the highest GP (39 and 36% respectively) (Table 1).

Mean germination time varied from 12 to 40 days. It was not affected by harvesting time ($F = 2.46$, $p = 0.12$) nor pre-germination treatment ($F = 0.09$, $p = 0.76$). At the beginning of the fruiting season, MGT was higher in seeds from unripe fruits with white seeds and unripe fruits stored until ripening than in the others fruit ripening stages ($F = 9.32$, $p < 0.0001$). By contrast, at the end of the fruiting season, there were no differences in MGT between ripening stages ($F = 1.39$, $p = 0.26$) (Table 2).

Vigor index was similar in seedlings of fruits harvested at the beginning or the end of the fruiting season (KW, $p = 0.26$). However, at the end of the fruiting season, the VI was higher in seedlings of ripe and unripe fruits with brown seeds soaked in potassium nitrate ($F = 31.43$, $p < 0.0001$) (Table 3) than in the others fruit ripening stages.

Table 1. Germination percentage of *V. quercifolia* seeds according to harvest time (beginning: February; end: March), fruit ripening and potassium nitrate soaking (mean \pm standard error). Lower-cases are used to show differences between fruit ripening stages and asterisk is used to show differences between pre-germination treatments (Tukey $p < 0.05$).

Fruit ripening	Harvesting time			
	Beginning		End	
	Control	KNO ₃	Control	KNO ₃
Unripe with white seeds	11 \pm 6.2	20 \pm 6.1	0	1 \pm 1 a
Unripe with brown seeds	25 \pm 5.7	23 \pm 5.4	10 \pm 3.8*	36 \pm 2.8* b
Unripe and stored	24 \pm 2.8	26 \pm 4.8	10 \pm 0.3	5 \pm 3.1 a
Almost ripe	30 \pm 3.5	32 \pm 11.1	1 \pm 1*	11 \pm 1.9* a
Ripe	28 \pm 6.7	33 \pm 5.5	19 \pm 4.4*	39 \pm 5.3* b

Table 2. Mean germination time of *V. quercifolia* seeds according to harvest time (beginning: February; end: March), fruit ripening and potassium nitrate soaking (mean \pm standard error). Lower-cases are used to show differences between fruit ripening stages (Tukey $p < 0.05$).

Fruit ripening	Harvesting time			
	Beginning		End	
	Control	KNO ₃	Control	KNO ₃
Unripe with white seeds	35.3 \pm 2.4 a	39.8 \pm 2.1 a	-	34.0 \pm 0
Unripe with brown seeds	25.8 \pm 1.0 b	30.2 \pm 4.5 b	39.2 \pm 3.7	32.4 \pm 1.1
Unripe and stored	34.3 \pm 1.8 a	36.2 \pm 2.5 a	34.8 \pm 2.7	29.0 \pm 0
Almost ripe	27.1 \pm 2.6 b	26.3 \pm 1.8 b	12.0 \pm 0	38.9 \pm 2.1
Ripe	28.5 \pm 0.1 b	23.6 \pm 2.5 b	29.8 \pm 2.3	26.3 \pm 2.9

Table 3. Vigor index of *V. quercifolia* seeds according to harvest time (beginning: February; end: March), fruit ripening and potassium nitrate soaking (mean \pm standard error). Lower-cases are used to show differences between fruit ripening stages and asterisk is used to show differences between pre-germination treatments (Tukey $p < 0.05$).

Fruit ripening	Harvesting time			
	Beginning		End	
	Control	KNO ₃	Control	KNO ₃
Unripe with white seeds	82.5 \pm 31.3	85.8 \pm 22.8	-	18.8 \pm 0 a
Unripe with brown seeds	308.1 \pm 114.4	124.8 \pm 32.3	49.1 \pm 23.5*	210.4 \pm 17.4* b
Unripe and stored	102.6 \pm 12.2	100.9 \pm 24.3	65.4 \pm 7.5	85.9 \pm 18.3 ab
Almost ripe	156.2 \pm 27.2	177.4 \pm 46.5	21.6 \pm 0	68.6 \pm 7.2 a
Ripe	150.5 \pm 28.2	215.1 \pm 29.5	159.3 \pm 45*	343.2 \pm 30.6* c

Storage and photoblastism

Germination percentages were low, less than 28%, in all treatments. Stored seeds had a higher germination percentage than the fresh ones ($F = 4.62, p = 0.05$). Furthermore, the seeds are photoblastic positive. Mean germination time varied from 13 to 32 days and there were no differences between stored and fresh seeds ($F = 2.85, p = 0.15$). Also, vigor index was similar in stored and in fresh seeds ($F = 1.55, 0.28$) (Table 4).

Discussion

Dormancy can be adaptive in seasonal environments and can vary temporally in individual seeds, especially in the case of physiological dormancy. The major environmental factors involved in dormancy are temperature, darkness, light, gases, water, and chemicals. Nevertheless, dormancy can be caused by a combination of exogenous and endogenous conditions (Baskin & Baskin, 2014; Née et al., 2017). *V. quercifolia* seeds probably present physiological dormancy. In previous studies we could confirm that this species does not present physical dormancy. However, we are uncertain about all the conditions needed for seed germination because of the low and fluctuating germination rates that never reach the percentage of viable seeds obtained with tetrazolium test (60-70%). Moreover, our results show that *V. quercifolia* dormancy can also be influenced by harvest time and fruit ripening.

Studies on the optimum harvesting season allow the identification of the maximum physiological quality of the seeds, i.e. when germination and vigor are at their maximum, key aspects to a successful seedling establishment. A few studies have been done to assess the optimum harvesting time of *C. papaya* fruits and overall, high seed germination and vigor were obtained from fruits collected in the warmest period (Aroucha et al., 2005; Tokuhisa et al., 2008; Dias et al., 2015). Likewise, we found similar results in the Northwestern region of Argentina, although the fruiting season is shorter. The low germination response at the end of the fruiting season could be related to the decrease in temperature and precipitation at the end of summer and their influence on dormancy depth. These environmental factors promote the synthesis of phenolic compounds in the sarcotesta and sclerotesta of *C.papaya* seeds which are important in dormancy regulation (Tokuhisa et al., 2007a; Dias et al., 2015). A similar behavior can occur in *V.quercifolia* seeds, a feature that needs further study.

Besides harvesting season, germination response can also be affected by fruit ripening because of the relationship to seed maturity. Nonetheless, in some species, mature seeds can be dormant (Tokuhisa et al., 2008; Lopes, Seleguini, Boliani, & Correa, 2009; Melo & Seleguini, 2013; Baskin & Baskin, 2014; Dias, Estanislau, Finger, Alvarenga & Dias, 2010). In our work, germination was affected by fruit ripening in seeds collected at the end of the fruiting season. Ripe and unripe fruits with brown seeds had the highest germination (36-39%) when soaked in potassium nitrate. Similar results were reported by Lopes et al. (2009), Zanotti et al. (2014), and Dias et al. (2015) for *C. papaya* seeds treated with potassium nitrate and/or gibberellic acid. However, with an increase in gibberellins concentration, seeds from almost ripe fruits showed a higher germination than seeds from ripe ones. These results suggest that, for both species, fruit ripening influences seed germination, but the results do not show a marked trend. This can be a strategy to ensure seedling establishment in fluctuating environments. This study is relevant because it is the first report on the effect of fruit ripening in *V. quercifolia* germination.

Table 4. Response variables of fresh and stored seeds exposed to light and darkness. Germination percentage (GP), mean germination time (MGT), vigor index (VI) and germination relative to light index (GRL) (mean ± standard error). Lower-cases are used to show differences between light and darkness treatments and asterisk is used to show differences between fresh and stored seeds (Tukey $p < 0.05$).

Response variable	Storage	Light	Darkness
GP	Fresh*	13±5.5 a	0 b
	Stored*	28±5.9 a	9±7.7 b
MGT	Fresh	23±5	-
	Stored	16±2	-
VI	Fresh	151±14	-
	Stored	121±14	-
GRL	Fresh		1
	Stored		0.75

Seed storage may increase germination percentages in some species (Baskin & Baskin, 2014), as it was reported for *V. quercifolia* (this work) and *C. papaya*. Seeds stored for 12 months had better germination response than the fresh ones (Dias et al., 2010). This result can be attributed to dormancy breakdown. In general, freshly harvested seeds have a high dormancy level that is gradually released during dry storage (Baskin & Baskin, 2014). However, seed moisture content and storage temperature are key factors to dormancy release and seed viability.

Light quantity and quality are important ecological factors in regulating germination response. Many seeds can germinate in both, light and darkness, but many require a specific condition for dormancy break and/or germination (Baskin & Baskin, 2014). *V. quercifolia* seeds are photoblastic positive and need light to germinate at constant temperatures. However, seed photoblastic response can vary with dormancy release, maturation, storage, concentration of nitrates and temperature exposure (Cardoso, 2004; Baskin & Baskin, 2014).

Average of germination time is a measure of the rate and time-spread of germination (Soltani et al., 2015). In this study, germination began after a week, MGT varied from 12 to 40 days in all treatments, and it was higher in seeds from unripe fruits harvested at the beginning of the fruiting season. This might be related to the time required to break dormancy. Moreover, seed vigor varied with fruit maturity and was higher in seeds from ripe fruits. Similar results were reported by Dias et al. (2015) in mature *C. papaya* fruits. They found an inverse relationship between seed vigor and phenolic concentration in seed structures (Tokuhisa et al., 2007a; Dias et al., 2015).

Conclusion

Dormancy release studies are essential to the conservation and management of seeds, especially of wild species related to crops that provide opportunities to increase crop production and ensure food security in the face of global and climate change. *V. quercifolia* seed dormancy is influenced by fruit harvesting season, fruit ripening and seed storage. Furthermore, light is a requirement for germination at constant temperatures. This is the first study of *V. quercifolia* seed dormancy during fruit physiological changes. However, further researches are needed to understand the mechanisms involved in seed dormancy and improve germination response.

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