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Article

Seed Germination Traits of Loquat (*Eriobotrya japonica* Lindl.) as Affected by Various Pre-Sowing Treatments (Cutting of Cotyledons, Removal of Perisperm, Moist Chilling and/or Exogenous Application of Gibberellin)

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Abstract: The purpose of this study was to investigate the effects of various pre-sowing treatments on the germinability (final germination percentage) and germination rate of loquat seeds in order to increase seedling production in nurseries (applied research) as well as provide answers for important physiological issues related to loquat seeds and their seed coat (basic research). Three experiments were carried out with various pre-sowing treatments. These treatments included full or partial removal of seed coat (perisperm), partial cutting of cotyledons as well as moist chilling at 5°C for 13 days and/or soaking the seeds in water or 250 ppm gibberellic acid (GA_3) solution for 24 h. According to the results, cotyledons excision resulted in delayed germination, regardless of the presence or absence of the seed coat in comparison with the decoated seeds that demonstrated the highest germination rate amongst them. In addition, even the partial excision of seed coats affected positively both the germinability and the germination rate, compared to the control-intact seeds. Furthermore, control-intact seeds had a higher germination percentage when exposed to moist chilling independently of the application or not of gibberellin; while the combination of gibberellin application and moist chilling improved both the percentage and the rate of germination of decoated seeds. In conclusion, the role of perisperm (seed coat) in the germination procedure of loquat seeds seems to be important, indicating the existence of seed coat-imposed dormancy on loquat seeds. Finally, the existence of a mild endogenous embryo-dormancy on loquat is also discussed.

Keywords: Cotyledon; gibberellin; seed coat; seed testa; *Eriobotrya japonica*; plant propagation; perisperm; sexual propagation; seedling; dormancy

1 Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a subtropical fruit tree (2n = 34) originating from the cool hill regions of Southwestern China [1]. Nowadays, it is widely cultivated in the subtropical regions of the Mediterranean area, Southern China, Japan and Northern India as a fruit tree and an ornamental plant. Ripe fruit flesh is soft and juicy [2,3] and the flesh contains nearly all the essential nutrients, particularly minerals, carotenoids and vitamin A, and it has a mild, sub-acid and sweet flavor [2].

Loquat is typically propagated asexually using grafting onto seedling rootstock. Seeds (between one and four in each fruit) have no embryo dormancy but they display an endogenous dormancy that can be broken by pre-sowing treatments [4,5]. Loquat seeds are sown immediately after their extraction from the fruit; however, in some cases their germination rates are low, and seed germination is usually slow and irregular. As a result, serious difficulties arise in the nurseries regarding the production planning of seedlings that will be used as rootstocks ideals for the grafting of well-known varieties.

Seed dormancy and germination in higher plants are influenced by a large number of genes as well as numerous environmental factors. Seed dormancy is defined as the failure of intact seeds to germinate under favourable conditions (e.g. water, temperature, light, exogenous hormones) [6–9]. The same mechanisms that are responsible for the dormancy, when repressed are able to positively affect seed germination [10]. There are two major types of dormancy mechanisms, the first one is “embryo dormancy” and is characterized by ingrained agents of the embryo that inhibit its germination. The second one is “coat-imposed dormancy” which protects the embryos from environmental conditions and is able to delay the germination due to the localization of the inhibition factor(s) in the seed envelopes [11].

According to Koornneef et al. [6], dormancy as well as germination are affected by the restraints imposed by the tissues surrounding the seed. In addition, there is evidence that water-soluble germination inhibitors are involved in the regulation of seed germination and dormancy. These inhibitors seem to be located in the coat, the embryos as well as other seed tissues [12].Taking this under consideration, it is important to examine the testa permeability when testing exogenous substances on embryos (e.g., GA_3) [11]. Seed dormancy can be broken through various treatments including seed coat disruption [13]. In particular, it has been reported that coat-imposed dormancy can be broken with the application of exogenous hormones or by scarifying the seed coat [9].

Even in species producing seeds without embryo dormancy and with a coat (testa) permeable to water, seed coat may still offer some resistance to germination. Both abscisic acid (ABA) and gibberellin (GA) are plant hormones that affect dormancy and germination. Genetic analysis has demonstrated the important role of ABA in seed dormancy and of GAs in germination [6,9,14]. In particular, GAs have been reported to control seed germination-usually by promoting seed germination—and to break the dormancy [15] of both the seeds and the buds of many plants [16].

Graeber et al. [17] showed that DOG1 (Delay of Gemrination1) gene is involved in the dormancy mechanism by setting the optimal temperature conditions for germination. It is considered that the conserved coat dormancy of seeds, mediated by DOG1 gene, can be controlled by the regulation of GA metabolism in seeds [15]. According to Li et al. [14], DOG1 is responsible for controlling the primary seed dormancy with its function varying greatly from epigenetic modifications, transcriptional regulation, protein stability, posttranslational regulation as well as biochemical functions. Gibberellic acid as well as moist chilling seem to be very promising treatments for breaking seed dormancy in many woody species [18]. It has also been reported that cold stratification leads to the increase of GA_3 in seeds [16].

In some plant species that produce seeds of similar biology and morphology to loquat (embryo without dormancy and large cotyledons), removing a part of the cotyledons and/or seed coat helps germination [19]. Moreover, chilling loquat seeds and soaking them into gibberellin solutions can improve some quantitative

and qualitative germination traits [4]. Gibberellin treatments also promote seed germination in other species after endocarp removal [20] and significantly increase the overall germination percentage [21,22].

Only a few studies [18,23] have been carried out on loquat seed propagation. Practical applications aiming to enhance the percentage and/or rate of loquat seed germination would have positive economic effects for nurseries due to the increase of seedling production within a short period of time (applied research). Moreover, understanding loquat seed physiology is also of great significance (basic research). Therefore, the dual purpose of the present study concentrated on determining treatments that can enhance seed germination traits of loquat, thus improving the propagation efficiency of this plant species, as well as on investigating the presence or absence of an endogenous or exogenous dormancy on loquat seeds. The research focused on the role of various pre-sowing treatments including moist chilling, exogenous gibberellin application, cotyledon cutting and/or seed coat removal on the germinability and germination rate of loquat.

2 Materials and Methods

Three experiments were conducted for the investigation of the effects of various pre-sowing treatments on loquat seeds. The first experiment was conducted during the first year of the study while the other two (second and third) were carried out two years later. For all three experiments, mature fruits of different loquat genotypes were initially harvested (for each one experiment, fruits of a different genotype were used). After their removal from the fruits, the seeds were thoroughly washed with tap water and immediately afterwards they were either immersed for 24 h in water, with changes every 4–5 h (first experiment and some treatments of the third experiment), or stored overnight in a refrigerator (5°C), closed in polyethylene plastic bags between pre-moisturized perlite granules (second experiment and some treatments of the third experiment) and/or immersed in 250 ppm GA_3 solution for 24 h (some treatments of the third experiment). The procedures that were followed in each experiment are described separately below.

First experiment: 480 uniform seeds were randomly divided into four groups, each containing 120 seeds. The treatments involved the removal of the seed coat (seed testa; perisperm) and/or the partial excision of the cotyledons: (i) control-intact seeds, (ii) seeds with removed coat (decoated seeds), (iii) excision of 1/3 of the seed coat and cotyledons from the opposite of the radicle side, and (iv) excision of 1/3 of the cotyledons from the opposite of the radicle side and removal of the remaining seed coat (Fig. 1). Immediately afterwards, the seeds were sown individually at the cells of seed trays containing a peat: perlite (2:1, v/v) substrate. Each treatment comprised of 6 replicates, each containing 20 seeds.

The seed trays were kept under glasshouse conditions in order to observe the progress of seed germination. The measurements were conducted after the initiation of seed germination and up until the recording of the maximum germination percentage (germinability) per treatment. The sowing and after-sowing procedures were similar in all three experiments.

Second experiment: The treatments included the complete or partial (half) removal of the seed coat: (A) control-intact seeds, (B) seeds with removed coat (decoated seeds), (C) seeds with removed coat from 1/2 of the seed surface (1/4 right and 1/4 left of the longitudinal axis of the seed), (D) seeds with removed coat from 1/2 of the seed surface nearby the radicle, and (E) seeds with removed coat from 1/2 of the seed surface from the opposite of radicle side (Fig. 2). Totally, 480 uniform seeds were randomly divided into five groups corresponding to each treatment (4 replicates of 24 seeds per treatment). The sowing procedure was identical with the one reported above for the first experiment.

Third experiment: Initially, 720 seeds were randomly separated in two groups, the first one consisted of intact seeds (treatments I-V) and the second one consisted of decoated seeds (treatments VI-X). Afterwards,

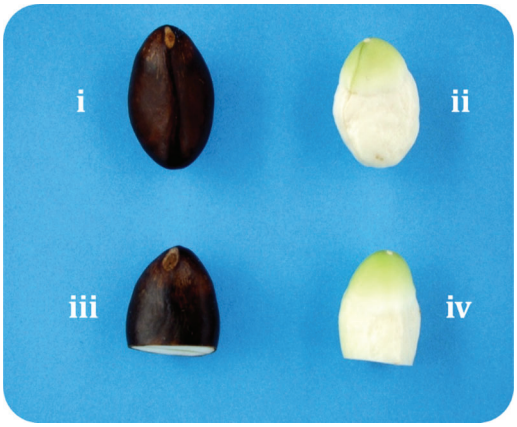

Figure 1: Treatments of the 1st experiment included the removal of the seed coats and the cutting of a part of the cotyledons: (i) control-intact seeds, (ii) seeds with removed coat (decoated seeds), (iii) excision of 1/3 of the seed coat and cotyledons from the opposite of the radicle side, (iv) excision of 1/3 of the cotyledons from the opposite of the radicle side and removal of the remaining seed coat

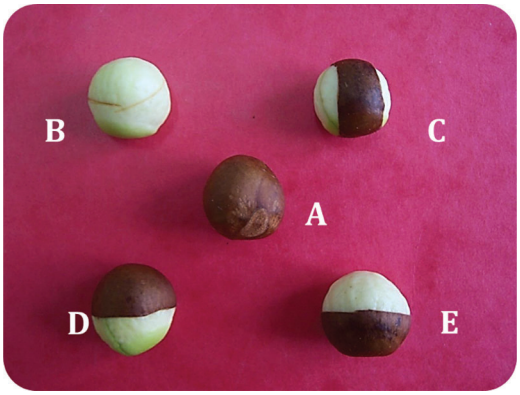

Figure 2: Treatments of the 2nd experiment included the complete or partial (half) removal of the seed coat: (A) control-intact seeds, (B) seeds with removed coat (decoated seeds), (C) seeds with removed coat from 1/2 of the seed surface (1/4 right and 1/4 left of the longitudinal axis of the seed), (D) seeds with removed coat from 1/2 of the seed surface nearby the radicle, and (E) seeds with removed coat from 1/2 of the seed surface from the opposite of the radicle side

the seeds of each group were further divided into five sub-groups of 72 seeds each. The seeds of each sub-group were treated as follows:

I and VI: 24 h in water + 13 days at 5°C + 24 h in water,

II and VII: 24 h in water + 13 days at 5°C + 24 h in 250 ppm GA_3,

III and VIII: 24 h in 250 ppm GA_3 + 13 days at 5°C + 24 h in water,

IV and IX: 24 h in water + 0 days at 5°C + 24 h in water,

V and X: 24 h in water + 0 days at 5°C + 24 h in 250 ppm GA_3.

Each one of the ten treatments comprised of 4 replicates of 18 seeds each. When the pre-sowing treatments were completed, the seeds were sown in seed trays and left to germinate under controlled glasshouse conditions.

Measurements: Irrespectively of the experiment and treatment, starting from the day the first seed germinated up until the stabilization of the germinating percentage, the number of germinated seeds per treatment and replicate were recorded every 2–3 days. In addition, the time (T) was computed in days needed for the germination of 10% (T10), 50% (T50) and 90% (T90) of the seeds that germinated per each treatment and experiment, based on the equation used previously by EI-Dengawy [4] for loquat: $Ty = [(t_2 - t_1) \times y\% + (p_2 \times t_1 - p_1 \times t_2)]/(p_2 - p_1)$, where y = 10, 50 or 90, t_1 = time at which the germination percentage is less than y%, t_2 = time at which the germination percentage is more than y%, and p_1 and p_2 are the measurements of germination percentage occurring at t_1 and t_2, respectively.

Statistics: The experiments were designed according to the model of a completely randomized design. The Duncan's multiple range test was used to compare the means ($p \leq 0.05$). Prior to statistical analysis, seed germination percentages were subjected to square root transformation.

3 Results

In the first experiment, the final seed germination percentage was up to 90–95%, with no statistically significant differences among the five treatments (i–iv) (Tab. 1). Referring to the number of days after sowing (DAS) needed for the germination of 10% (T10), 50% (T50) and 90% (T90) of the seeds; the fastest germination (less days to germinate) was recorded in fully decoated seeds (treatment ii, Tab. 1). In addition, the time required for seed germination to reach 100% (T100), was significantly reduced in seeds with removed coats (treatment ii) compared to the combined treatment of excision of 1/3 of the cotyledons from the opposite of the radicle side and removal of the remaining seed coat (treatment iv) (data not shown). The highest germination rate (time course of germination percentages of germinated seeds in DAS) was recorded for treatment ii (decoated seeds), while the lowest percentages of germinated seeds were recorded for treatment iii (seeds with excised 1/3 of their seed coat and cotyledons from the opposite from the radicle side) (Fig. 3).

Table 1: Final seed germination percentage (%) and time in days after sowing (DAS) for the germination of 10% (T10), 50% (T50) and 90% (T90) of the seeds as observed during the treatments of the 1st experiment.

	Treatments			
	i	ii	iii	iv
Seed germination (%)	91.7 ± 5.3 (a)	90.0 ± 4.3 (a)	94.2 ± 4.9 (a)	95.0 ± 3.2 (a)
T10 (DAS)	36.6 ± 0.7 (c)	26.3 ± 0.9 (a)	42.1 ± 0.9 (d)	32.5 ± 0.8 (b)
T50 (DAS)	46.7 ± 1.8 (b)	36.3 ± 2.0 (a)	58.5 ± 2.3 (c)	47.0 ± 0.7 (b)
T90 (DAS)	70.9 ± 3.9 (b)	49.0 ± 1.4 (a)	74.2 ± 0.5 (b)	74.5 ± 4.3 (b)

(i) control-intact seeds, (ii) seeds with removed coat (decoated seeds), (iii) excision of 1/3 of the seed coat and cotyledons from the opposite of the radicle side, and (iv) excision of 1/3 of the cotyledons from the opposite of the radicle side and removal of the remaining seed coat. Average ± Standard Error (S.E.). Each treatment had six replications (n = 6) with 20 seeds per replication (480 seeds in total). The means of each parameter marked with the same letter(s) don't differ statistically from each other (Duncan's multiple range test, $p \leq 0.05$).

In the second experiment, the germination percentage of decoated seeds (treatment B) was significantly increased from day 36 to day 61 compared to intact seeds (treatment A). During the same period, intermediate percentages were recorded in treatments C, D and E, where the seed coat had been partially removed (Fig. 4). When grouping the treatments of the second experiment, depending on the complete, partial or non-removal of the seed coat, it appeared that intact seeds had a significantly lower final germination percentage (78.12%) than seeds with partial removal (92.72%) or complete removal (89.58%) of their seed coat (Tab. 2). In addition, the time in days needed for the germination of 10% or 50% of the seeds was significantly higher in intact seeds (47.6 and 61.4, respectively), compared to seeds

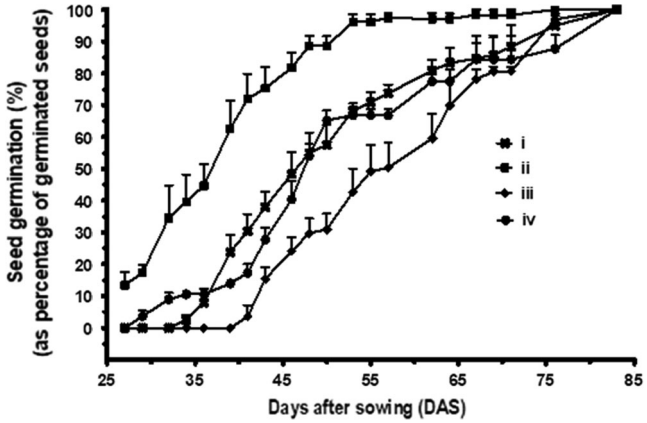

Figure 3: Percent of seed germination (expressed as the percentage of the total number of germinated seeds of each treatment separately) in the 1st experiment over time, days after sowing (DAS). Treatments: (i) control-intact seeds, (ii) seeds with removed coat (decoated seeds), (iii) excision of 1/3 of the seed coat and cotyledons from the opposite of the radicle side, and (iv) excision of 1/3 of the cotyledons from the opposite of the radicle side and removal of the remaining seed coat. Average + Standard Error (S.E.). Each treatment had six replications (n = 6) with 20 seeds per replication (480 seeds in total)

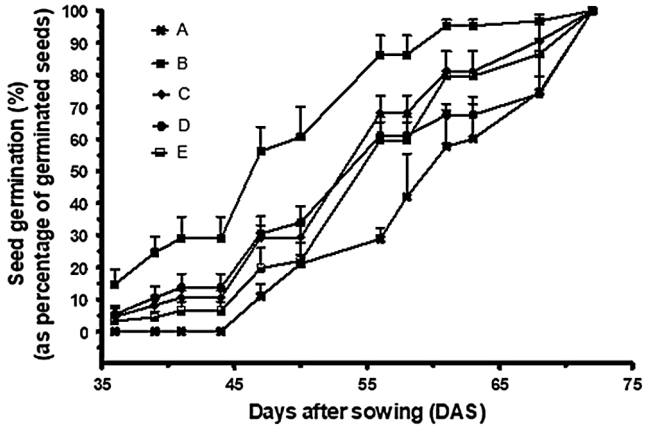

Figure 4: Percent of seed germination (expressed as the percentage of the total number of germinated seeds of each treatment separately) in the 2nd experiment over time, days after sowing (DAS). Treatments: (A) control-intact seeds, (B) seeds with removed coat (decoated seeds), (C) seeds with removed coat from 1/2 of the seed surface (1/4 right and 1/4 left of the longitudinal axis of the seed), (D) seeds with removed coat from 1/2 of the seed surface nearby the radicle and (E) seeds with removed coat from 1/2 of the seed surface from the opposite of the radicle side. Average + Standard Error (S.E.). Each treatment had four replications (n = 4) with 24 seeds per replication (480 seeds in total)

with partial (41.6 and 53.7, respectively) or complete (34.6 and 47.2, respectively) removal of their seed coat. Regarding T90 timing point, no differences were observed between the groups of intact seeds and those whose coat was partially removed. Finally, at timing points T10, T50 and T90 the fastest seed germination was observed in fully decoated seeds, compared to intact seeds or seeds with partially removed seed coat (Tab. 2).

According to the results of the third experiment, the final seed germination percentage ranged from 37.5% up to 90.3% (Fig. 5A). Decreased germination percentages were observed in treatments IV and V

Table 2: Seed germination (final germination percentage) and time in days after sowing (DAS) for the germination of 10% (T10), 50% (T50) and 90% (T90) of the seeds in the 2nd experiment

Treatments	Intact seeds (Treatment A)[1]	Complete removal of seed coat (Treatment B)[2]	Partial removal of seed coat (Treatments C, D, E)[3]
Seed germination (%)	78.12 ± 5.48 (a)	89.58 ± 6.48 (b)	92.72 ± 5.06 (b)
T10 (DAS)	47.57 ± 1.52 (c)	34.55 ± 1.01 (a)	41.55 ± 1.14 (b)
T50 (DAS)	61.35 ± 2.50 (c)	47.21 ± 1.57 (a)	53.74 ± 0.68 (b)
T90 (DAS)	69.60 ± 0.89 (b)	58.06 ± 2.69 (a)	67.63 ± 1.65 (b)

Treatments: (A) control-intact seeds, (B) seeds with removed coat (decoated seeds), (C) seeds with removed coat from 1/2 of the seed surface (1/4 right and 1/4 left of the longitudinal axis of the seed), (D) seeds with removed coat from 1/2 of the seed surface nearby the radicle, and (E) seeds with removed coat from 1/2 of the seed surface from the opposite of the radicle side. Average ± Standard Error (S.E.). Each replication (n) had 24 seeds; (Treatment A)[1] n = 4, (Treatment B)[2] n = 4, (Treatments C, D and E)[3] n = 12. The means of each parameter marked with the same letter (s) don't differ statistically from each other (Duncan's multiple range test, $p \leq 0.05$).

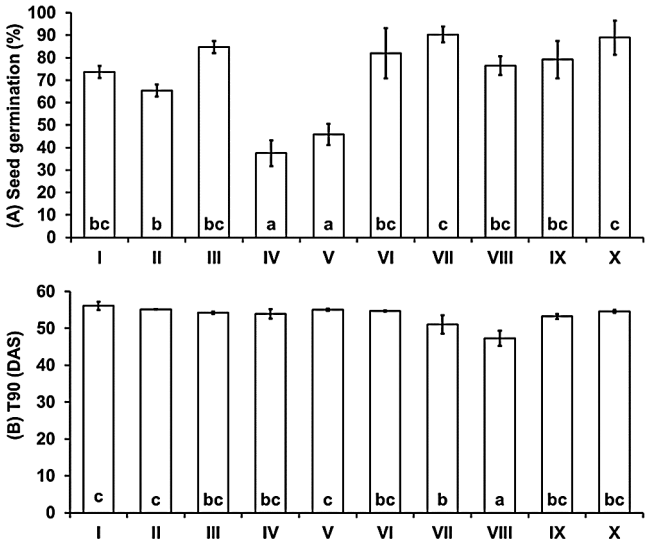

Figure 5: (A) Seed germination (final germination percentage) and (B) the time in days after sowing (DAS) for the germination of 90% (T90) of the seeds that germinated in the 3rd experiment. Treatments: I-V (intact seeds) and VI-X (decoated seeds); I and VI: 24 h in water + 13 days at 5°C + 24 h in water, II and VII: 24 h in water + 13 days at 5°C + 24 h in 250 ppm GA_3, III and VIII: 24 h in 250 ppm GA_3 + 13 days at 5°C + 24 h in water, IV and IX: 24 h in water + 0 days at 5°C + 24 h in water, and V and X: 24 h in water + 0 days at 5°C + 24 h in 250 ppm GA_3. Average ± Standard Error (S.E.). Each treatment had four replications (n = 4) with 18 seeds per replication (720 seeds in total). The means of each parameter marked with the same letter(s) don't differ statistically from each other (Duncan's multiple range test, $p \leq 0.05$)

(where intact seeds were not exposed to low temperatures), compared to treatments I, II and III (where intact seeds were exposed to 13 days of low temperatures). No significant differences were observed among the decoated seeds in treatments VI-X (Fig. 5A). The number of days from sowing to (T90), was 47.3–56.1 days (Fig. 5B). No significant differences were observed among the treatments of seeds with intact seed coat (Fig. 5B). On the contrary, the decoated seeds of treatment VIII germinated in significantly less time (DAS) compared to the decoated seeds of treatments VI, IX and X (Fig. 5B). Fig. 6 shows the seed germination rate (percentage over time) for all treatments. The seeds used in treatments VI-X, which had their seed coat removed, germinated faster than seeds used in treatments I-V that had intact seed coat.

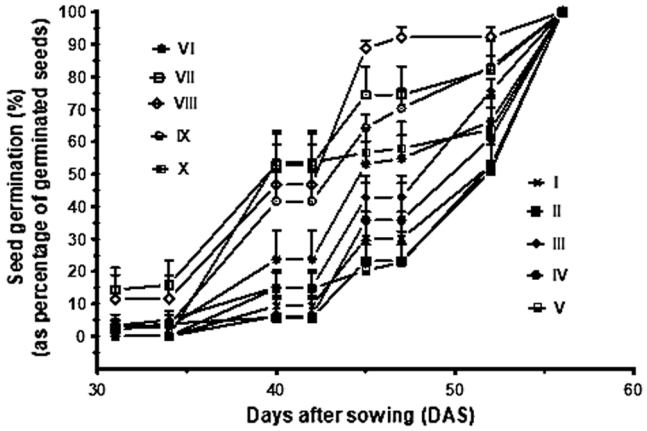

Figure 6: Percent of seed germination (expressed as the percentage of the total number of germinated seeds of each treatment separately) in the 3rd experiment as observed in time, days after sowing (DAS). Treatments: I-V (intact seeds) and VI-X (decoated seeds); I and VI: 24 h in water + 13 days at 5°C + 24 h in water, II and VII: 24 h in water + 13 days at 5°C + 24 h in 250 ppm GA_3, III and VIII: 24 h in 250 ppm GA_3 + 13 days at 5° C + 24 h in water, IV and IX: 24 h in water + 0 days at 5°C + 24 h in water, and V and X: 24 h in water + 0 days at 5°C + 24 h in 250 ppm GA_3. Average + Standard Error (S.E.). Each treatment had four replications (n = 4) with 18 seeds per replication (720 seeds in total)

4 Discussion

According to our results, complete seed coat removal led to increased seed germination rate for both whole and cut seeds. Moreover, excising a part of the cotyledons delayed seed germination, regardless of the removal or not of the seed coat (Tabs. 1 and 2, Figs. 3–6). To our knowledge, these results, which derived from three different experiments and different presowing treatments of the seeds, are the first to be reported for loquat.

These results are in accordance with the results of Whiley et al. [19] who cut a part of the cotyledons and/or removed the seed coat from avocado seeds. In addition, our results showed that the removal of the entire or a part of the seed coat accelerates seed germination and increases the germination rate (Fig. 3, Tab. 2). Similarly, Kim's results [24] showed that seed coat removal has the ability of overcoming *Prunus yedoensis'* seed dormancy. Seed dormancy has also been reported to be broken through seed coat removal in pear [9]. Seed coat removal may be so efficient in enhancing seed germination due to the fact that seed coat produces on its own chemical substrates—such as ABA—which seem to directly inhibit seed germination [9,25]. Another type of plant hormone, GAs, are metabolized in seeds and affect seed coat-imposed dormancy [14,15]. Particularly, GAs are responsible for negatively regulating seed dormancy through the release of seed coat-imposed dormancy [26]. In general, some germination inhibitors are considered to be located in the seed coat because their extract is capable of inhibiting the germination of decoated seeds (seeds with removed seed coat). It has been speculated that the role of seed coat in the inhibition of germination may include the limitation of gas exchange between the embryo and the external environment, the suppression of embryo swelling, the embryo growth as well as the excretion of toxins from it [9,27–29]. The possibility of seed coat preventing the entry of water and oxygen into loquat seeds and thus delaying the germination is little, since even partial seed coat removal seemed to delay the germination compared to decoated seeds (Fig. 2, Tab. 2). This delay may be due to chemical limitations caused by substances of the seed coat and/or mechanical inhibition due to the presence of seed coat (2nd experiment, treatments C, D, E; Fig. 2, Tab. 2). In fact, the chemical cause seems to be more likely than the mechanical one since no significant differences were observed among the three treatments

of partial seed coat removal (treatments C, D, E). In addition, T50 (the number of days after sowing (DAS) needed for the germination of 50% of the seeds) was reached about 1.5 months later in all three experiments and for all the different treatments applied—even for the decoated seeds-, thus suggesting that loquat demonstrates a mild endogenous embryo dormancy, independent from any chemical and/or mechanical causes encountered in the seed coat. However, this issue needs to be further investigated in the future.

The application of GA_3 has also demonstrated the ability of breaking seed dormancy of intact *Prunus yedoensis* seeds (seeds whose seed coat has not been removed) [24]. Furthermore, GAs and especially GA_3, have been shown to be able to break seed dormancy in many other species [30]. According to Kirmizi et al. [31], the dormancy of *Linum olympicum* may be suppressed by GA_3 application and/or cold stratification treatment. It is believed that gibberellins have the ability to substitute the chilling requirements of some plant seeds and enhance their germination rates [4]. GA_3 has been used in pretreatments of various highly dormant seeds—which are widely used for the production of seedling rootstocks during the propagation of fruit tree species—and has led to the increase of their germination [7]. According to the results of Al-Hawezy [32], the highest germination in loquat was reported during the treatment of the seeds with 250 ppm of GA_3 solution during the 1st, 2nd and 3rd week of the experiment. According to Kim [24], the most efficient method of overcoming *Prunus yedoensis'* seed dormancy is to apply exogenous GA_3 on intact seeds under vigorous shaking. This treatment led to the reduction of the mean germination time and to the improvement of the seed germination percentage. For the species *Abies pindrow* and *Picea smithiana* the most effective treatment for seed germination that was reported by Rawat et al. [16], included soaking the seeds in 10 ppm solution of GA_3 for 24 h, followed by moist chilling for a fortnight and finally the germination taking place at 10°C. Moreover, Shahi-Gharahlar et al. [18] showed that one way of breaking loquat seed dormancy is to soak the seeds in water, followed by soaking them in a KNO_3 solution, then apply moist-chilling conditions for a week or another day of soaking them in water, followed—in both cases—by soaking them in GA_3 solution for a week and finally applying another fortnight of moist chilling. This combination of pre-sowing treatments gave high germination percentages in comparison to the rest of the treatments of Shahi-Gharahlar et al. [18], but it lasted approximately 3 weeks. These 2–3 weeks though are very important for nurseries and it is a time that should be exploited more efficiently when trying to enhance the propagation of the species.

Moist cold stratification has been widely used for breaking dormancy and promoting the maximum percentage and rate of germination [20]. According to El-Dengawy [4], seeds of loquat stratified for 30 days at 4°C germinated at higher percentages and faster than the control. In the present study, seeds with intact seed coat had a higher rate of germination when moist-chilled at 5°C for 13 days, regardless of exogenous GA_3 application compared to un-chilled ones. On the contrary, the germination percentage of decoated seeds was not significantly different when exposed to GA_3 and/or moist-chilling. Therefore, chilling seems to affect germination percentage only when seeds maintain their seed coat intact. These results may indicate a form of dormancy that is not related to the embryo but to the seed coat—seed coat-imposed dormancy [12]. If dormancy was related to the embryo, germination percentages and rates would increase by moist-chilling for both seeds with intact seed coat and the decoated ones. However, the possibility of moist-chilling being capable of enhancing the germination of loquat seeds is not strong as the seed coat does not seem to soften in order to enable the required germination procedures to occur. This fact is confirmed by the results of the 1st experiment, according to which, partial seed coat removal delayed the germination in comparison to decoated seeds. Therefore, moist-chilling may contribute to the decomposition of inhibitory substances which delay loquat seed germination and are mainly located in the seed coat.

The simultaneous effect of GA_3 and moist-chilling (treatment VIII) significantly reduced the T90 germination time of seeds without seed coat, compared to the treatments of GA_3 without moist-chilling (treatment X) or moist-chilling without GA_3 (treatment VI). In other words, the combination of

moist-chilling and GA_3 accelerated the germination of loquat decoated seeds, rather than the intact seeds. According to Baskin et al. [33], gibberellin suspends effectively the mild physiological dormancy, but is not effective against normally intense dormancy. Therefore, in the case of intact loquat seeds, GA_3 is not as effective as on decoated seeds. This indicates that either GA_3 must overcome an additional dormancy due to the seed coat, or that seed coat acts as a barrier to the GA_3 entrance into the embryo. Gibberellin is directly related to interruption of embryo dormancy, as it activates the nutrients of the seed stored in the cotyledons [34]. It can be concluded that seed coat may have prevented the entry of GA_3 into the seed embryo and therefore could not contribute to the overcoming of even a mild, endogenous embryo dormancy, which may exist on loquat seeds.

In conclusion, the role of seed coat is important for loquat seed germination, since both the germination percentage and its rate increased by the removal of the seed coat. Moreover, moist-chilling improves the germination percentage of intact seeds without affecting the germination rate. Finally, the combination of GA_3 and moist-chilling improves both the percentage and the rate of germination of decoated seeds as seen by their pre-sowing treatment: 24 h in 250 ppm GA_3 + 13 days at 5°C + 24 h in water. Our results clearly demonstrate the existence of seed coat-imposed dormancy on loquat seeds without eliminating the possibility of the existence of a mild endogenous embryo-dormancy.

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