Factors affecting germination of *Diplotaxis erucoides* and their effect on selected quality properties of the germinated products

Carla GUIJARRO-REAL*, Ana María ADALID-MARTÍNEZ, Aroa GREGORI-MONTANER, Jaime PROHENS, Adrián RODRÍGUEZ-BURRUEZO, Ana FITA

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

*Corresponding author: Carla Guijarro-Real. *E-mail address:* carguire@etsia.upv.es, *telephone number* +34 96 3879418, *fax number* +34 96 3879422

E-mail addresses

C. Guijarro-Real: carguire@etsia.upv.es

A.M. Adalid-Martínez: anadmar@alumni.upv.es

A. Gregori-Montaner: aroa.gregori.18@um.edu.mt

J. Prohens: jprohens@btc.upv.es

A. Rodríguez-Burruezo: adrodbur@upvnet.upv.es

A. Fita: anfifer@btc.upv.es

Abstract

Wall rocket (*Diplotaxis erucoides*) is a wild vegetable with potential as a crop. Its seeds present secondary dormancy mechanisms that can become essential for the survival of wall rocket as a weed or in the wild. However, adaptation to crop conditions requires high and synchronised germination. The present work was aimed at studying whether different treatments improve the germination success of wall rocket seeds, and the effects on subsequent crop quality (morphology, yield, ascorbic acid and phenolics). By using of a L_8 orthogonal array design, the main effects of soaking the seeds, scarification with sodium hypochlorite (NaClO), gibberellic acid (GA_3), potassium nitrate (KNO_3), cold, and heat treatments on germination traits of wall rocket were evaluated. NaClO scarification was the most efficient treatment and significantly increased the early and final germination, the germination rate and the vigour index. The best germination results were obtained when the NaClO scarification was followed by application of GA_3. Thus, a protocol consisting on scarification with 2.5% NaClO for 5 min followed by treatment with 150 ppm GA_3 for 24 h was proposed to improve wall rocket germination success. In addition, the germination treatments did not greatly affect the agronomic characters of baby-leaf plants. Scarification with NaClO reduced the days to harvest but did not affect the yield, so its use could have commercial benefits. Moreover, the content in ascorbic acid increased in treatments using NaClO, which may increase the added value of the potential crop. Overall, this study contributes to the domestication of wall rocket by providing a simple germination method that in addition has potential beneficial effects for crop quality.

Keywords

Diplotaxis erucoides; germination; growth parameters; nutritional quality; secondary dormancy

Abbreviations. AA: ascorbic acid. CAE: chlorogenic acid equivalents. DW: dry weight. FW: fresh weight. GA_3: gibberellic acid. KNO_3: potassium nitrate. NaClO: sodium hypochlorite. TP: total phenolics. WEP: wild edible plant

1. Introduction

The term wild edible plants (WEPs) refers to species that are directly gathered from the wild for its consumption (Shin et al., 2018). These species may contribute to the diet with macro- and micronutrients like minerals and vitamins, and also represent an opportunity for adding new flavours and textures to the diet (e.g., Grivetti and Ogle, 2000; Molina et al., 2014; Morales et al., 2014; Guijarro-Real et al., 2019a, 2019b). For these reasons, the use, marketing and domestication of WEPs have been promoted during the last decades, as an alternative for improving human diet quality. This is the case, for example, of watercress (*Nasturtium officinale*) and wild and salad rocket (*Diplotaxis tenuifolia* and *Eruca sativa*), established as crops (Molina et al., 2016) and now found as usual ingredients on modern salads. However, there are still many other WEPs that can be considered as a source of new potential crops.

Wall rocket (*Diplotaxis erucoides* (L.) DC.) is an annual species belonging to the *Brassicaceae* family, traditionally gathered and consumed in different Mediterranean countries like Spain and Italy (Guarrera and Savo, 2016; Parada et al., 2011). The plant is consumed by the leaves and tender shoots, appreciated by its characteristic, mild pungent flavour resembling the aroma and taste of other *Brassicaceae* crops like mustard. The culinary use of wall rocket is mainly fresh as a complement to salads, or cooked in preparations like pasta, soups or mixtures of cooked vegetables (Guarrera and Savo, 2016). In addition, the small, white flowers can be also considered as a decorative component in high cuisine (Guijarro-Real et al., 2018). As food markets are increasing efforts in offering new and distinctive products, the particular taste and pungency of wall rocket make it a good candidate for its domestication and introduction into cultivation.

The introduction and commercial exploitation of WEPs possess, however, a series of critical points that must be evaluated, including germination traits and cultivation conditions (Ceccanti et al., 2018). One of the main problems for the domestication of wall rocket is the presence of secondary dormancy in the seeds (Martínez-Laborde et al., 2007) and the consequent discontinuous germination. Artificial selection in cultivated species has led to a reduction of seed dormancy, thus allowing a rapid and synchronised germination for adaptation to crop systems (Née et al., 2017). By contrast, secondary dormancy and irregular germination can become essential for the survival of weeds (Darmency et al., 2017). In the case of wall rocket, Martínez-Laborde et al. (2007) proposed that fresh seeds of wall rocket are presumably non dormant. However, mechanisms of secondary dormancy would be activated in those fresh seeds that did not germinate, thus remaining in the soil as part of the soil seed bank (Martínez-Laborde et al., 2007). This irregular germination over time has been previously proposed as an adaptive strategy to control the demographic populations of wall rocket (Sans and Masalles, 1994), thus decreasing the competition for water and nutrients for increasing the population's survival. The presence of secondary dormancy in the seeds hampers the breeding programs and commercial cultivation of wall rocket, since a fast and uniform germination is required in both cases. The release of secondary dormancy is controlled by various regulators including phytohormones, mainly abscisic acid and gibberellins, and other specific proteins, and it is highly influenced by environmental factors such as temperature, light, water potential or content in nitrates in the soil (Finch-Savage and Footitt, 2017). Treatments changing or modifying those factors have demonstrated to be useful for breaking dormancy in several wild and cultivated species, and can be used routinely (Hellier, 2018).

In addition, this potential new crop is aimed at being consumed mainly as baby-leaf, before the appearance of the flower bud. Due to its short life cycle, this stage can be reached in one to two months, depending on the cultivation conditions. Thus, the application of specific dormancy-breakdown treatments may affect the quality of the final product (Evans et al., 1996). In fact, changes in plants associated to the application of specific germination conditions has been reported elsewhere. For example, Handa et al. (2017) optimized the soaking and germination conditions of horsegram (*Macrotyloma uniflorum*) seeds to decrease the antinutritional factors but maintaining the nutritional properties. In the same way, Tavares et al. (2014) evaluated the treatment of rice seeds with salicylic acid, and the effect on produced seed quality and yield. Also the application of GA_3 can induce stem elongation (Taylor and Cosgrove, 1989). Therefore, the main objective of this work was to obtain a highly efficient germination protocol for wall rocket without impairing the baby-leaf quality. For that reason, the main effects of six factors were evaluated. The experimental design consisted in an orthogonal array and was adapted from Ranil et al. (2015), whom used it for evaluating up to seven factors for developing a germination protocol in *Solanum torvum*. In addition, the effect of the different treatments in selected agronomic and nutritional traits of the baby-leaves was evaluated. The results of this work will be useful for ensuring a quick, synchronised germination in breeding programs, thus facilitating the domestication of this WEP. Finally, studying the effect on different agronomic and nutritional traits can be relevant for selecting the most adequate germination protocol for ensuring the quality of wall rocket as a commercial baby-leaf vegetable.

2. Materials and methods

2.1. Plant material

Seeds from a wild population of wall rocket were collected in the spring of 2015 in Teulada, Alicante, Spain (coordinates 38º 43' 15" N; 0º 05' 06" E). Seeds were collected from dry siliques as an indicator of the proper ripeness of seeds. Once in the laboratory, the collected seeds were manually cleaned from the siliques and other vegetable organs, and dehydrated for two weeks at room temperature. The dehydrated, ripe seeds were then placed in a plastic bag and stored at 4 ºC in a hermetic jar until use, using silica gel for control of humidity. The moisture content of seeds at the moment of storage was 5.7%. The germination assay was performed during the next spring.

2.2. Germination assay

The germination assay was performed in Petri dishes (9.0 × 2.5 cm; Phoenix Biomedical, Mississauga, Ontario, Canada) filled with 1.5 cm of moistened commercial Neuhaus Humin-substrat N3 nursery growing substrate (Klasmann-Deilmann Gmbh, Geeste, Germany). In each Petri dish, twenty-five seeds, previously treated according to the specific treatment, were placed on top of the substrate. Seven replicates were used, with a total of 175 seeds evaluated in each treatment. Petri dishes were placed in a climatic chamber, organizing the experiment in order to ensure that the application of all treatments finished the same day, considered as day 0 or starting day for the germination evaluation (Table 1). The environmental conditions in the climatic chamber remained constant during the germination assay, with a photoperiod of 16 h light / 8 h dark at 25ºC (Martínez-Laborde et al., 2007), and maintaining the relative humidity at 50-60%. The substrate was watered as needed in order to keep adequate moisture.

The germination assay was designed according to the work of Ranil et al. (2015), with slight modifications. The effect of six factors on seed germination was evaluated: soaking, sodium hypochlorite (NaClO), gibberellic acid (GA_3), potassium nitrate (KNO_3), cold and heat. The

presence/absence of light was not evaluated as a factor, and seeds were placed on the top of the substrate in all treatments.

The effect of each factor was evaluated at two levels: a) level -, if the factor was not applied; or b) level +, if the factor was applied. Details of each factor were:

- Soaking: immersion of seeds in distilled water for 24 h at room temperature, prior to sown.
- NaClO: immersion of seeds in 2.5% commercial NaClO for 5 min, followed by three rinses with distilled water, 10 min each. The scarification was performed prior to sown at room temperature.
- GA_3: treatment of seeds with 150 ppm GA_3 (Duchefa Biochemie, Haarlem, The Netherlands) by immersion for 24 h at room temperature, with a final rinse with distilled water. Treatment with GA_3 was performed prior to sown.
- KNO_3: application of 1000 ppm KNO_3 (Panreac, Montcada i Reixac, Spain) in the plate for moistening the peat, at room temperature or climate chamber temperature.
- Cold: stratification of seeds for seven days at 4 ºC, after being sown.
- Heat: incubation of seeds for 24 h at 37 ºC, after being sown.

In order to analyse the effect of these factors, a L_8 orthogonal array matrix design (2^6) was followed. Eight different treatments were tested, using specific combinations of factors in order to ensure that all of them were applied in four of the treatments (Ranil et al., 2015). The resulting treatments are summarized in Table 1, in which factors were applied observing the following order: soaking, NaClO, GA_3, KNO_3, cold and heat application.

2.3. Evaluation of traits in the germination assay

At day 0 all treatments and replicates were placed in a climatic chamber in order to evaluate the germination. Evaluation started at day 3 and followed during seven consecutive days, with a final

evaluation at day 11. Seeds were considered as germinated when the radicle emerged. However, sprouts with erratic germination, it is, with failures in the subsequent radicle development, were not considered as viable and removed from the count.

The germination traits evaluated were: a) early germination, considered as percentage of seeds germinated at day 3; b) final germination, considered as percentage of germinated seed at day 11; c) germination rate, in percentage (%), calculated as $(S_1 \cdot t_1 + S_2 \cdot t_2 + ... + S_n \cdot t_n)/(t_1 + t_2 + ... + t_n)$, where S_n is the cumulative percentage of germinated seeds at day n and t_n is the number of days from day 0 at which the count was performed; and d) vigour index, calculated as $(S_1/t_1) + (S_2/t_2) + \ldots + (S_n/t_n)$ (Ranil et al., 2015). Germination rate determines the potential for a high final germination combined with a rapid germination, and vigour index determines the potential for a rapid germination. In addition, the hypocotyl length (cm) in the sprouts was measured. Measurement was performed when the first true leaf reached a size of one third the size of cotyledons.

2.4. Growing conditions and evaluation of baby-leaf plants

Germinated sprouts were individually transplanted into 7x7x8 cm plastic pots filled with the same commercial substrate used in the germination assay. Thirteen to thirty-six plants of each treatment, depending on germination success, were transplanted. Transplanted plants were adapted for one day to room temperature conditions and then moved to a glasshouse equipped with a cooler system. Plants were placed following a completely randomized design and grown until the appearance of the first flower bud, before stem elongation. During the growing period, water was supplied regularly to maintain the substrate moistened, with no addition of fertilizers. Once the baby-leaf plants reached the defined developmental stage, the aerial part was harvested, transported to the laboratory in sealed bags for avoiding excessive loss of moisture and stored at

4 ºC until analysis. The time elapsed between harvesting plants and placing under cooling conditions was less than one hour.

2.4.1. Agronomic traits

Characterization was performed within the next 24 h. Seven agronomic traits were evaluated: total height, in cm; stem length between the cotyledons and the first leaf, in mm; length of the first and second internodes, in mm; length of the largest leaf, in cm; total number of leaves per plant; and earliness, measured as the number of days after transplant needed for the appearance of the flower bud.

2.4.2. Determination of nutritional parameters

Weight of plants prior to the freeze-drying process was recorded as fresh weight (FW, g). Plants from each treatment were then used for analysing the content in ascorbic acid (AA) and total phenolics (TP). Three replicates were performed for each analysis.

Content in AA was determined as described in Cano and Bermejo (2011), with slight modifications. Briefly, 1 g of fresh leaf tissue was homogenized with 5 ml of 3.0% (w/v) cold *meta*-phosphoric acid (Sigma-Aldrich; Saint Louis, MO, USA) for 1 min in a mortar, filtered and centrifuged at 2,500 rpm for 10 min at 5 ºC. The supernatant was filtered through a 0.22 μm PVDF filter (Teknokroma, San Cugat del Vallès, Spain) and analysed by HPLC in a 1220 Infinity HPLC (Agilent Technologies; Santa Clara, CA, USA). A Brisa C_{18} column (150mm × 4.6 mm id, 3μm) (Teknokroma, San Cugat del Vallès, Spain) was used, with an isocratic phase of methanol: 1.0% acetic acid (5:95) for 15 min, an injection volume of 5 μL and a flow rate of 1 mL min^{-1}. Quantification was performed at 254 nm using an external standard calibration of ascorbic acid (Sigma-Aldrich), and results were expressed as mg AA 100 g^{-1} FW.

Content in TP was determined according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) as indicated in (Plazas et al., 2014). Briefly, 0.125 g of freeze-dried, finely ground material was extracted with 70% acetone (v/v) containing 0.5% glacial acetic acid (v/v) solution. An aliquot of 65 µl was incubated with 500 µl of diluted Folin-Ciocalteu reagent (1:10; Scharlab SL, Sentmenat, Barcelona, Spain) for 5 min, plus 500 µl of sodium carbonate solution (60 g/L) for other 90 minutes. Absorbance was measured at 750 nm in an iMark^{TM} Microplate Reader (Bio-Rad; Hercules, CA, USA). Chlorogenic acid (Sigma-Aldrich) was used as standard and results were expressed as mg of chlorogenic acid equivalents in each 100 g of freeze-dried material (mg CAE 100 g^{-1} DW).

2.5. Statistical analysis

Data from the four germination traits, agronomic characters and nutritional traits were submitted to a one-way analysis of variance (ANOVA). Data from the germination parameters, expressed as percentage (for early germination, final germination and germination rate) or as the proportion of the maximum possible value (for vigour index), were arcsine-transformed prior to the analysis (McDonald, 2014). On the other hand, normality was tested as well for agronomic and nutritional data, and log-transformed if needed. Differences between treatments were studied with the Duncan multiple range test at $P = 0.05$. Significance of the six factors effects was tested by partitioning the degrees of freedom and sums of squares of the ANOVA, and the magnitude was measured as the difference between treatments in which the factor was applied (level +) and those ones in which it was not applied (level -) (Ranil et al., 2015). Finally, Pearson pairwise comparisons were performed in order to evaluate the correlation between AA and TP.

3. Results

3.1. Germination assay

The treatment effect was highly significant for the four germination parameters evaluated (Table S1), with significant differences among means (Fig. 1). Early germination was high for treatments T2, T3, T6 and T7, with values ranging between 49.7% (T7) to 79.4% (T3). By contrast, control, T1, T4 and T5 displayed low germination percentages, ranging from 2.3% (T1) to 22.3% (T4). This large difference between treatments displaying high and low response was also obtained for final germination, germination rate, and vigour index (Fig. 1). Treatments T2 and T3 displayed the greatest percentage for final germination (80.0% and 91.4%, respectively), whereas the percentage of germinated seeds in control and low-response treatments was below 35%. In the same way, T2 and T3 displayed the highest germination rates (86.0% and 76.5%, respectively), while this rate was < 30.0% for low-response treatments with T1 giving the lowest value (4.8%). Finally, T2, T3 and T6 showed the best vigour index values, close to 200 (Fig. 1). The effect of each factor was analysed as the difference between average values when the factor was applied (level +) or not (level -) (Table 2, S1). Scarification with NaClO was the only factor having a highly significant effect on the four germination traits, with a positive effect when it was applied (Table 2). Moreover, this factor had the highest positive effect, and its application increased the early germination, final germination and germination rate values in more than 50.0%, and the vigour index in 147.6 units. In addition, the effect of treating with GA_3 was significant ($P < 0.05$) for final germination and highly significant ($P < 0.01$) for the other germination traits (Table 2, S1). GA_3 application induced an increase between 7.5% (final germination) and 14.3% (early germination), and an increase of 39.0 units in the vigour index (Table 2). For the rest of factors evaluated, the effect was non-significant, or when significant, negative for the germination traits (Table 2, S1).

3.2. Agronomic characterization and nutritional value

For the agronomic characterization, hypocotyl length of the sprouts, and earliness, total height, number of leaves per plant, stem length from cotyledons to 1st leaf, length of 1st and 2nd internodes and length of the largest leaf in the pre-flowering stage were measured.

The hypocotyl length of sprouts had no differences among treatments, with an average value of 1.3 cm (Table S2). In the pre-flowering stage, no significant differences were determined among treatments for the number of leaves per plant, first internode and leaf length. The mean values for these traits were, respectively, 8.8 leaves, 3.7 mm and 8.7 cm (Table S2). On the other hand, differences were determined among treatments for total height, length from cotyledons to 1st leaf and 2nd internode (Fig. 2). Plant height was on average 10.8 cm. Treatments T4 and T7 developed plants between 1.0 and 2.0 cm higher than the control. For the cotyledon-to-1st leaf trait, the mean value was 2.0 mm. Plants from T1 displayed the lowest value (1.0 mm), similar to the control, while T2 developed plants with the greatest values (3.7 mm). On the contrary, plants from T2 developed the shortest 2nd internode (2.8 mm) while treatment T1 developed plants with the longest values (8.6 mm). Thus, addition of cotyledon-to-1st leaf, 1st internode and 2nd internode measurements resulted in a loss of significance among treatments ($P = 0.368$), so factor effects were not analysed. Finally, earliness was analysed (Fig. 2). The days needed to develop the flower bud was, on average, 21.4 days, with values ranging between 18.9 (T3) and 23.8 (T5) days. Treatments T2, T3, T6 and T7 gave the lowest values, with around 20 days needed for appearance of the flower bud. These treatments had in common the scarification of seeds with NaClO. By contrast, T1, T4 and T5 needed around three days more for reaching that developmental stage.

Effect of the individual factors used in the germination test were analysed for plant height and earliness (Table S3). The only germination factor with a significant effect on plant height was

soaking the seeds prior to the sown. Treatments that included this factor developed plants on average 0.87 cm higher than those ones that did not include the soaking step (Table 3). For earliness, factors with significant effect included soaking the seeds, the treatment with NaClO and the application of heat (Table S3). While soaking the seeds and applying heat resulted in an increase of 1.1 and 0.3 days, respectively, treatment with NaClO was the only factor that reduced the time needed for the appearance of the flower bud, in almost 3 days (Table 3).

On the other hand, the fresh weight, content in AA and content in TP were analysed. No significant differences were determined for the fresh weight, with an average value of 1.5 ± 0.0 g (Table S2). By contrast, significant differences between germination treatments were detected for the content in AA and TP (Table S3). Application of the different germination treatments did not negatively affected the content in AA with respect to the control (Fig. 3). The greatest difference was detected for plants germinated with treatments T2 (96.2 mg AA $100g^{-1}$ FW) and T6 (101.1 mg AA $100g^{-1}$ FW) compared to the control (64.16 mg AA $100g^{-1}$ FW). On the other hand, the only germination factor with a significant effect in the AA content was the scarification of the seeds with NaClO (Table S3). Thus, treatments including scarification with NaClO had on average an increase of 21.0 mg AA $100g^{-1}$ FW with respect to the no application (Table 3).

In addition, plants germinated with different treatments also displayed significant differences for the content in TP (Fig. 3). The average content was 1,309.8 mg CAE $100g^{-1}$ DW, with values ranging between 1,224.2 (T1) and 1,371.1 (T6) mg CAE $100g^{-1}$ DW. Treatments T1 and T3 (1,224.2 and 1,263.7 mg CAE $100g^{-1}$ DW, respectively) displayed values significantly lower than the content determined for the control (1,341.4 mg CAE $100g^{-1}$ DW). All treatment factors, except for heat, had a significant effect on TP content (Table S3). The application of GA_3, KNO_3 and cold had negative effects, decreasing TP content between 3.9 (cold) and 42.8 (KNO_3) mg

CAE 100g^{-1} DW (Table 3). By contrast, scarification with NaClO resulted in the highest increase (47.4 mg CAE 100g^{-1} DW). Finally, the linear correlation between the content in AA and TP in each treatment was evaluated (Fig. S1), with no significant correlations determined among these nutritional traits ($P > 0.05$).

4. Discussion

4.1. Effect of individual factors on germination

Wall rocket has great potential to be marketed and introduced into the diet as a baby-leaf vegetable for salads (Di Gioia et al., 2018). However, its domestication and exploitation as a crop and the necessary adaptation to an agricultural large scale production requires early, vigorous, high and synchronised germination (Née et al., 2017). Thus, the present study analysed the response of a wild population of wall rocket to eight germination treatments, through the evaluation of four germination traits. In addition, the effect of treatments on baby-leaf plants was also evaluated.

The best treatments for increasing the germination parameters (T2, T3, T6 and T7) had in common the scarification of seeds with NaClO. These results differ considerably from the work of Ranil et al. (2015), whom found a negative effect of NaClO scarification on the germination of *Solanum torvum*. On the contrary, our results are consistent with previous works, in which treatment with bleach increased the germination rates of different species (e.g., Marty and Kettenring, 2017; Wagner and Oplinger, 2017; Jones et al., 2016), although the exposure times were commonly greater. Moreover, the use of NaClO is a common treatment for the scarification in tomato and tomato wild relatives' seeds (Gordillo et al., 2008), and it is also used for disinfection of seeds in this crop (Figàs et al., 2018a, 2018b; Mehalaine et al., 2017). Our results suggest that the seed coat structure may be implied in the secondary dormancy of wall rocket.

Thus, using a chemical scarification as the treatment with NaClO would help to break the barrier between embryo and environment (Wagner and Oplinger, 2017), presumably by weakening the seed coat tissues and/or increasing the permeability (Katzman et al., 2001). Moreover, these results suggest that scarification with NaClO might be also useful to break dormancy in other related species from the *Brassicaceae* family, although specific studies should be conducted in order to ensure the positive effect in those species.

On the other hand, the treatment with GA_3 had also a positive effect on the seed germination of wall rocket, in line with the results of Martínez-Laborde et al. (2007). The application of GA_3 for increasing seed germination has provided successful results along the family *Brassicaceae*, especially for wild species including weeds. Thus, it has been used in germination studies for species such as *Isatis violascens* (Zhou et al., 2015), *Thlaspi arvense* and *Sinapis arvensis* L. (Hsiao, 1980) or *Brassica torunedortii* (Chauhan et al., 2006). Moreover, GA_3 has been suggested as a dormancy-breaking treatment to be applied in seeds of the *Brassicaceae* family by genebanks (González-Benito et al., 2011). GA_3 is part of the gibberellins group (GA), a group of phytohormones known for its enhancing germination effects (Graeber et al., 2012; Née et al., 2017). In fact, the dormancy-germination mechanisms are regulated by the balance between GA_3 and abscisic acid (Finkelstein et al., 2008). While abscisic acid is required to induce and maintain dormancy during seed maturation, a positive balance for GA_3 overcomes dormancy and stimulates germination (Finkelstein et al., 2008; Née et al., 2017).

However, significant differences on germination were found according to the factor applied before the treatment with GA_3. Thus, when the treatment with GA_3 was preceded by soaking the seeds for 24 h (T4 and T5), values of the germination parameters were very low and close to the control. Here we hypothesize that long soaking treatment may cause a saturation of water inside

the seeds, reducing the subsequent absorption of GA_3. On the contrary, preceding the treatment with GA_3 by a scarification with NaClO (T2 and T3) significantly increased the germination traits. This increase may correspond to the synergistic effect of both factors. Scarification with NaClO can affect the coat, facilitating the later penetration of GA_3 (Hsiao et al., 1979a, b) and therefore increasing the dormancy-breaking effect of this hormone. However, it is also possible that NaClO may act against other germination inhibitors, what would explain the great germination rates also when it is applied with no GA_3 (T6 and T7). More physiological studies should be conducted in this sense to clarify the mechanisms activated in wall rocket dormancy, and how the NaClO scarification can break them.

The rest of the factors had no effect, or negatively affected the germination traits. According to these results, we suggest the use of a simplified protocol for the germination of wall rocket seeds, consisting of scarification of seeds with 2.5% NaClO for 5 min, then rinse the seeds in three changes of distilled water, 10 min each, remove the excess of water, and treat with 150 ppm GA_3 for 24 h. At this point, one short rinse with water prior to sowing would be appropriate in order to remove the excess of GA_3 from the seed surface (Small et al., 2019). This protocol would ensure a proper germination of wall rocket even during the greatest dormancy period, considered after one year storage of seeds (Pérez-García et al., 1995; Laborde et al., 2007). The germination protocol described in this study has been used and validated in subsequent experiments developed by the group, providing germination rate success above 80% (unpublished results). Thus, it provides an efficient alternative that guarantees an effective, fast and uniform germination, with the need of only one day of pre-sown treatment.

4.2. Treatment and germination factor effects on plant quality

Rocket crops are highly appreciated as baby-leaf products and marketed as whole leaves, for what the mechanical harvest is commonly used (Caruso et al., 2018). Adaptation of wall rocket to a similar production system and marketing can promote its acceptance by consumers and also by producers. For that reason, producing plants short in height may be desirable for automatic harvesting, as the presence of long stems would require manual harvesting or subsequent manipulations for removing them.

Application of different germination factors can affect the plant growth. The best studied factor in literature is GA_3 as it is a plant regulator. Gibberellic acid can affect the epigenetics of plants and modify different enzyme activities, so it produces changes in phenotype for different growth and developmental stages including stem elongation, leaf expansion and even induction of flowering (Kaur et al., 1999; Liang et al., 2014; Yamamuro et al., 2016). In fact, the application of GA_3 has shown to induce stem elongation in different species (e.g., Silk and Jones, 1975; Taylor and Cosgrove, 1989). In our case, no significant elongation of the hypocotyls was detected in the sprouts when seeds were treated with GA_3. On the contrary, in the case of the baby-leaf plant, only one of the treatments using GA_3 (T4) produced an increase in plant height with respect to the control. Thus, our results were ambiguous to declare that seed treatment with this hormone increases the plant height of wall rocket at the pre-flowering stage.

Earliness was also affected by the treatment. Rocket species can reach the flowering stage in a short period, which varies depending on the season, region and growing conditions (e.g., greenhouse instead of field), and plants should be commercially harvested prior to reach this stage (Bell et al., 2015; Caruso et al., 2018). In this respect, increasing the vegetative cycle would be of interest for producers if it derives in an increase of yield. The lowest values for earliness were obtained for plants treated with NaClO, while no differences were determined for

fresh weight. Chun et al. (1997) found that the application of NaClO increased seedlings growth in rice. Moreover, Hall (1985) observed a reduction in the time to harvest for sweet potato due to the use of calcium hypochlorite. In the same way, our results suggested that the scarification with NaClO would reduce the vegetative period of wall rocket without affecting yield. Moreover, this difference in days needed for crop development may have considerable impacts in reducing production costs.

Regarding the nutritional quality, the demand of healthy foods, and foods rich in bioactive compounds, have increased in the last decades as consequence of an increasing number of consumers are aware of the linkage between diet and health (Olayanju, 2018). Application of different germination treatments may affect compositional quality, especially in products of short age such as sprouts although it could also affect plants of higher developmental stage as well (Mostafa and Alhamd, 2011; Dueñas et l., 2015). Thus, studying the levels of bioactive molecules and the effect of germination treatments can be useful for future cultivation and marketing strategies. According to our results, applying different germination treatments did not negatively affect the accumulation of AA in wall rocket leaves. Moreover, the use of adequate treatments may increase the content in AA, in particular for treatments that include NaClO as scarification product. On the contrary, results were not clear for the content in TP. In fact, all factors except for heat had a significant effect on TP accumulation in wall rocket leaves. Thus, new studies focused on comparing the improved germination protocol suggested in this work, with control plants, may be addressed in order to help clarifying whether using this protocol would significantly affect the quality of the final product. Finally, a lack of correlation between AA and TP was determined. These results suggest that it would be difficult to improve both parameters by the application of germination treatments. In this sense, we suggest that future

studies should especially focus on the content in AA, since this compound is of particular relevance in rocket crops (Cavaiuolo and Ferrante, 2014).

5. Conclusions

This study provides a germination protocol for wall rocket in order to break the secondary dormancy of this potential new vegetable crop. The proposed protocol for improving germination success consists combining the scarification of seeds using 2.5% NaClO for 5 min followed by a treatment with 150 ppm GA_3 for 24 h. This simple, short method has been validated in subsequent studies in our laboratory increasing the germination rate above 80% (unpublished results).

The germination treatments generally did not present significant differences for agronomic characters at commercial level. Earliness increased with the application of NaClO, probably due to a greater vigour of plants germinated in these conditions. However, this shortage of growing period did not reduce the yield of wall rocket plants. In addition, results of AA and TP contents suggest that scarification with NaClO in wall rocket seeds could be used to increase the content in AA, increasing the marketing value of the plants. Overall, our results make an effective contribution of the domestication of wall rocket, by providing an efficient and simple method for seed germination, which in addition has beneficial effects by increasing earliness and improving AA content.

Acknowledgments

C. Guijarro-Real thanks the Ministerio de Educación, Cultura y Deporte (MECD) of Spain for financial support by means of a predoctoral grant (FPU14-06798). The research did not receive

any other specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

Authors declare that there is not conflict of interest.

References

Bell, L., Oruna-Concha, M.J., Wagstaff, C., 2015. Identification and quantification of glucosinolate and flavonol compounds in rocket salad (*Eruca sativa*, *Eruca vesicaria* and *Diplotaxis tenuifolia*) by LC–MS: Highlighting the potential for improving nutritional value of rocket crops. Food Chem. 172, 852–861. DOI:10.1016/j.foodchem.2014.09.116

Cano, A., Bermejo, A., 2011. Influence of rootstock and cultivar on bioactive compounds in citrus peels. J. Sci. Food Agric. 91, 1702–1711. DOI:10.1002/jsfa.4375

Caruso, G., Parrella, G., Giorgini, M., Nicoletti, R., 2018. Crop systems, quality and protection of *Diplotaxis tenuifolia*. Agriculture 8, 55. DOI:10.3390/agriculture8040055

Cavaiuolo, M., Ferrante, A., 2014. Nitrates and glucosinolates as strong determinants of the nutritional quality in rocket leafy salads. Nutrients 6, 1519–1538. DOI:10.3390/nu6041519

Ceccanti, C., Landi, M., Benvenuti, S., Pardossi, A., Guidi, L., 2018. Mediterranean wild edible plants: Weeds or "new functional crops"?. Molecules 23, 2299. DOI:10.3390/molecules23092299

Chauhan, B.S., Gill, G., Preston, C., 2006. African mustard (*Brassica tournefortii*) germination in southern Australia. Weed Sci. 54, 891–897. DOI: 10.1614/WS-06-053R.1

Chun, S.C., Schneider, R.W., Cohn, M.A., 1997. Sodium hypochlorite: Effect of solution pH on rice seed disinfestation and its direct effect on seedling growth. Plant Dis. 81, 821–824. DOI:10.1094/PDIS.1997.81.7.821

Darmency, H., Colbach, N., Le Corre, V., 2017. Relationship between weed dormancy and herbicide

rotations: implications in resistance evolution. Pest Manag. Sci. 73, 1994–1999. DOI:10.1002/ps.4611

Di Gioia, F., Avato, P., Serio, F., Argentieri, M.P. 2018. Glucosinolate profile of *Eruca sativa*, *Diplotaxis tenuifolia* and *Diplotaxis erucoides* grown in soil and soilles systems. J. Food Compos. Anal. 69, 197–204. DOI: 10.1016/j.jfca.2018.01.022

Dueñas, M., Martínez-Villaluenga, C., Limón, R.I., Peñas, E., Frias, J. 2015. Effect of germination and elicitation on phenolic composition and bioactivity of kidney beans. Food Res. Int. 70, 55–63. DOI: 10.1016/j.foodres.2015.01.018

Evans, A.S., Mitchell, R.J., Cabin, R.J. 1996. Morphological side effects of using gibberellic acid to induce germination: Consequences for the study of seed dormancy. Am. J. Bot. 83, 543–549

Figàs, M.R., Prohens, J., Casanova, C., Fernández-de-Córdova, P., Soler, S., 2018a. Variation of morphological descriptors for the evaluation of tomato germplasm and their stability across different growing conditions. Sci. Hortic. 238, 107–115. DOI:10.1016/j.scienta.2018.04.039

Figàs, M.R., Prohens, J., Raigón, M.D., Pereira-Dias, L., Casanova, C., García-Martínez, M.D., Rosa, E., Soler, E., Plazas, M., Soler, S., 2018b. Insights into the adaptation to greenhouse cultivation of the traditional Mediterranean long shelf-life tomato carrying the *alc* mutation: A multi-trait comparison of landraces, selections, and hybrids in open field and greenhouse. Front. Plant Sci. 9, 1774. DOI:10.3389/fpls.2018.01774

Finch-Savage, W.E., Footitt, S., 2017. Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. J. Exp. Bot. 68, 843–856. DOI:10.1093/jxb/erw477

Finkelstein, R., Reeves, W., Ariizumi, T.I., Steber, C., 2008. Molecular aspects of seed dormancy. Annu. Rev. Plant Biol., 59: 387-415. DOI:10.1146/annurev.arplant.59.032607.092740

González-Benito, M.E., Pérez-García, F., Tejeda, G., Gómez-Campo, C., 2011. Effect of the gaseous environment and water content on seed viability of four *Brassicaceae* species after 36 years storage. Seed Sci. Technol. 39, 443–451. DOI:10.15258/sst.2011.39.2.16

476 Gordillo, L.F., Stevens, M.R., Millard, M.A., Geary, B., 2008. Screening two *Lycopersicon peruvianum*
477 collections for resistance to Tomato spotted wilt virus. Plant Dis. 92, 694–704. DOI:10.1094/pdis-
478 92-5-0694
479 Graeber, K., Nakabayashi, K., Miatton, E., Leubner-Metzger, G., Soppe, W.J.J., 2012. Molecular
480 mechanisms of seed dormancy. Plant, Cell Environ 35, 1769–1786. DOI:10.1111/j.1365-
481 3040.2012.02542.x.
482 Grivetti, L., Ogle, B., 2000. Value of traditional foods in meeting macro- and micronutrient needs: The
483 wild plant connection. Nutr. Res. Rev. 13, 31–46. DOI:10.1079/095442200108728990
484 Guarrera, P.M., Savo, V., 2016. Wild food plants used in traditional vegetable mixtures in Italy. J.
485 Ethnopharmacol. 185, 202–234. DOI:10.1016/j.jep.2016.02.050
486 Guijarro-Real, C., Prohens, J., Rodriguez-Burruezo, A., Adalid-Martínez, A.M., López-Gresa, M.P., Fita,
487 A., 2019a. Wild edible fool's watercress, a potential crop with high nutraceutical properties. PeerJ
488 7, e6296. DOI:10.7717/peerj.6296
489 Guijarro-Real, C., Rodríguez-Burruezo, A., Prohens, J., Fita, A., 2018. Importance of the growing system
490 in the leaf morphology of *Diplotaxis erucoides*. Acta Hortic. 25–32.
491 DOI:10.17660/ActaHortic.2018.1202.4
492 Guijarro-Real, C., Rodríguez-Burruezo, A., Prohens, J., Raigón, M.D., Fita, A., 2019b. HS-SPME
493 analysis of the volatiles profile of water celery (*Apium nodiflorum*), a wild vegetable with
494 increasing culinary interest. Food Res. Int. 121, 765–775. DOI:10.1016/j.foodres.2018.12.054
495 Hall, M.R., 1985. Influence of calcium hypochlorite and genotype on plant production by bedded sweet
496 potato roots. HortScience 20, 692–693
497 Handa, V., Kumar, V., Panghal, A., Suri, S., Kaur, J., 2017. Effect of soaking and germination on
498 physicochemical and functional attributes of horsegram flour. J. Food Sci. Technol. 54, 4229–4239.
499 DOI:10.1007/s13197-017-2892-1
500 Hellier, B.C., 2018. Practical considerations for increasing seed samples of wild species. In: Greene, S.,
501 Williams, K., Khoury, C., Kantar, M., Marek, L. (Eds.), North American Crop Wild Relatives,

Volume 1. Springer, pp. 281–309. DOI:10.1007/978-3-319-95101-0_1

Hsiao, A.I., 1979a. The effect of sodium hypochlorite and gibberellic acid on seed dormancy and germination of wild oats (*Avena fatua* L.). Can. J. Bot. 57, 1729–1734. DOI: 10.1139/b79-212

Hsiao, A.I., 1979b. The effect of sodium hypochlorite, gibberellic acid, and light on seed dormancy and germination of wild buckwheat (*Polygonum convolvulus*) and cow cockle (*Saponaria vaccaria*). Can. J. Bot. 57, 1735–1739. DOI:10.1139/b79-213

Hsiao, A.I., 1980. The effect of sodium hypochlorite, gibberellic acid and light on seed dormancy and germination of stinkweed and wild mustard. Can. J. Plant Sci. 60, 643–649. DOI: 10.4141/cjps80-091

Jones, C.D., Stevens, M.R., Jolley, V.D., Hopkins, B.G., Jensen, S.L., Turner, D., Stettler, J.M., 2016. Evaluation of thermal, chemical, and mechanical seed scarification methods for 4 Great Basin lupine species. Nativ. Plants J. 17, 5–18. DOI:10.3368/npj.17.1.5

Katzman, L.S., Taylor, A.G., Langhans, R.W., 2001. Seed enhancements to improve spinach germination. HortScience 36, 979–981. DOI:10.21273/HORTSCI.36.5.979

Kaur, S., Guplta, A.K., Kaur, N. 2000. Effect of GA_3, kinetin and indole acetic acid on carbohydrate metabolism in chickpea seedlings germinating under water stress. Plant Growth Regul. 30, 61–70. DOI: 10.1023/A:1006371219048

Liang, Y-C., Reid, M.S., Jiang, C-Z. 2014. Controlling plant architecture by manipulation of gibberellic acid signaling in petunia. Horticulture Research 1, 14061. DOI: 10.1038/hortres.2014.61

Martínez-Laborde, J.B., Pita-Villamil, J.M., Pérez-García, F., 2007. Secondary dormancy in *Diplotaxis erucoides*: A possible adaptative strategy as an annual weed. Spanish J. Agric. Res. 5, 402–406. DOI:10.5424/sjar/2007053-265

Marty, J. E., Kettenring, K. M., 2017. Seed dormancy break and germination for restoration of three globally important wetland bulrushes. Ecological Rest. 35, 138-147. DOI:10.3368/er.35.2.138

McDonald, J.H., 2014. Handbook of biological statistics (3rd ed.). Sparky House Publishing, Baltimore, Maryland.

528 Mehalaine, S., Menasria, T., Bouguessa, S., Yahia, A., 2017. *In vitro* seed germination of some Algerian
529 medicinal plants and the effect of gibberellic acid (GA_3) on breaking dormancy. J. Mater. Environ.
530 Sci. 8, 2034–2039.
531 Molina, M., Pardo-de-Santayana, M., Tardío, Javier, 2016. Natural production and cultivation of
532 Mediterranean wild edibles. In: Sánchez-Mata, M., Tardío, J (Eds.), Mediterranean Wild Edible
533 Plants: Ethnobotany and food composition tables. Springer New York, New York, pp. 81–107.
534 Molina, M., Tardío, J., Aceituno-Mata, L., Morales, R., Reyes-García, V., Pardo-de-Santayana, M., 2014.
535 Weeds and food diversity natural yield assessment and future alternatives for traditionally
536 consumed wild vegetables. J. Ethnobiol. 34, 44–67. DOI:10.2993/0278-0771-34.1.44
537 Morales, P., Ferreira, I., Carvalho, A.M., Sánchez-Mata, M.C., Cámara, M., Fernández-Ruiz, V., Pardo-
538 de-Santayana, M., Tardío, J., 2014. Mediterranean non-cultivated vegetables as dietary sources of
539 compounds with antioxidant and biological activity. LWT- Food Sci. Technol. 55, 389–396.
540 DOI:10.1016/j.lwt.2013.08.017
541 Mostafa, G.G., Alhamd, M.F.A. 2011. Effect of gibberellic acid and indole 3-acetic acid on improving
542 growth and accummulation of phytochemical composition in *Balanites aegyptiaca* plants. Am. J.
543 Plant Physiol. 6, 36–43. DOI: 10.3923/ajpp.2011.36.43
544 Née, G., Xiang, Y., Soppe, W.J.J., 2017. The release of dormancy, a wake-up call for seeds to germinate.
545 Curr. Opin. Plant Biol. 35, 8–14. DOI:10.1016/j.pbi.2016.09.002
546 Olayanju, J.B., 2018. Perspectives on factors driving new trends in the food & drink.
547 https://www.forbes.com/sites/juliabolayanju/2018/10/01/perspectives-on-factors-driving-new-
548 trends-in-the-food-drink/. Accessed 6th February 2019.
549 Parada, M., Carrió, E., Vallès, J., 2011. Ethnobotany of food plants in the Alt Empordà region (Catalonia,
550 Iberian Peninsula). J. Appl. Bot. Food Qual. 84, 11–25.
551 Pérez-García, F., Iriondo, J.M., Martínez-Laborde, J.B., 1995. Germination behaviour in seeds of
552 *Diplotaxis erucoides* and *D. virgata*. Weed Res. 35, 495–502. DOI:10.1111/j.1365-
553 3180.1995.tb01647.x

554 Plazas, M., Prohens, J., Cuñat, A.N., Vilanova, S., Gramazio, P., Herraiz, F.J., Andújar, I., 2014.
555 Reducing capacity, chlorogenic acid content and biological activity in a collection of scarlet
556 (*Solanum aethiopicum*) and gboma (*S. macrocarpon*) eggplants. Int. J. Mol. Sci. 15, 17221–17241.
557 DOI:10.3390/ijms151017221
558 Ranil, R.H.G., Niran, H.M.L., Plazas, M., Fonseka, R.M., Fonseka, H.H., Vilanova, S., Andújar, I.,
559 Gramazio, P., Fita, A., Prohens, J., 2015. Improving seed germination of the eggplant rootstock
560 *Solanum torvum* by testing multiple factors using an orthogonal array design. Sci. Hortic. 193,
561 174–181. DOI:10.1016/j.scienta.2015.07.030
562 Sans, F.X., Masalles, R.M., 1994. Life-history variation in the annual arable weed *Diplotaxis erucoides*
563 (*Cruciferae*). Can. J. Bot. 72, 10–19. DOI:10.1139/b94-003
564 Shin, T., Fujikawa, K., Moe, A.Z., Uchiyama, H., 2018. Traditional knowledge of wild edible plants with
565 special emphasis on medicinal uses in Southern Shan State, Myanmar. J. Ethnobiol. Ethnomed. 14,
566 48. DOI:10.1186/s13002-018-0248-1
567 Silk, W.K., Jones, R.L., 1975. Gibberellin response in lettuce hypocotyl sections. Plant Physiol. 56, 267–
568 272.
569 Singleton, V., Rossi, J., 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid
570 reagents. Am. J. Enol. Vitic. 16, 144–158. DOI:10.1098/rspa.1963.0204
571 Small, C.C., Degenhardt, D., McDonald, T., 2019. Plant growth regulators for enhancing Alberta native
572 grass and forb seed germination. Ecological Eng.:X 1, 100003. DOI:10.1016/j.ecoena.2019.100003
573 Tavares, L.C., Rufino, C.A., De Oliveira, S., Pich, A., Villela, F.A., 2014. Treatment of rice seeds with
574 salicylic acid: seed physiological quality and yield. J. Seed Sci. 36, 352–356. DOI:10.1590/2317-
575 1545v36n3636
576 Taylor, A., Cosgrove, D.J., 1989. Gibberellic acid stimulation of cucumber hypcotyl elongation. Effects
577 on growth, turgor, osmotic pressure, and cell wall properties. Plant Physiol. 90, 1335–1340.
578 Wagner, E.J., Oplinger, R.W., 2017. Effect of overwinter hydration, seed storage time, temperature,
579 photoperiod, water depth, and scarification on seed germination of some *Schoenoplectus*,

580 *Polygonum*, *Eleocharis* and *Alisma* species. Aquat. Bot. 136, 164–174.
581 DOI:10.1016/j.aquabot.2016.10.004
582 Yamamuro, C., Zhu, J-K., Yang, Z. 2016. Epigenetic modifications and plant hormone action. Mol. Plant.
583 9, 57–70
584 Zhou, Y.M., Lu, J.J., Tan, D.Y., Baskin, C.C., Baskin, J.M. 2015. Seed germination ecology of the cold
585 desert annual *Isatis violascens* (Brassicaceae): Two levels of physiological dormancy and role of
586 the pericarp. PLoS One 10, e0140983

 DOI: 10.1016/j.scienta.2019.109013

587 **Table 1.** Orthogonal matrix L_8 (2^6) indicating the eight treatments applied for testing the
588 germination of wall rocket seeds. The six factors evaluated (soaking, NaClO, GA_3, KNO_3, cold
589 and heat application) were applied at two possible levels (-, no application; +, application) in
590 each of the eight treatments.

		Factors					
Treatment	**Starting day[a]**	**Soaking**	**NaClO**	**GA_3**	**KNO_3**	**Cold**	**Heat**
Control	0	-	-	-	-	-	-
T1	-8	-	-	-	+	+	+
T2	-2	-	+	+	-	-	+
T3	-8	-	+	+	+	+	-
T4	-9	+	-	+	-	+	-
T5	-3	+	-	+	+	-	+
T6	-9	+	+	-	-	+	+
T7	-1	+	+	-	+	-	-

591 [a]The starting day of application of treatment was adjusted in order to synchronize the day 0 (starting day
592 of the germination evaluation) for the eight treatments.

593

DOI: 10.1016/j.scienta.2019.109013

Table 2. Average values for the germination traits analysed in wall rocket seeds, when each germination factor (soaking, NaClO, GA3, KNO3, cold and heat) was applied at each level (- no application; + application). Difference between the two levels is also indicated (Δ +/-).

	Early germination (day 3, %)			Final germination (day 11, %)			Germination rate (%)			Vigour index		
Factor	**-**	**+**	**Δ**	**-**	**+**	**Δ**	**-**	**+**	**Δ**	**-**	**+**	**Δ**
Soaking	39.6	37.6	-2.0^{ns}	54.0	43.7	-10.3**	46.6	40.0	-6.6^{ns}	104.6	95.2	-9.4^{ns}
NaClO	10.4	66.7	56.3***	20.9	76.9	56.0***	14.7	71.9	57.1***	26.1	173.7	147.6***
GA_3	31.4	45.7	14.3***	45.1	52.6	7.5*	37.2	49.4	12.3***	80.5	119.4	39.0***
KNO_3	41.6	35.6	-6.0^{ns}	52.6	45.1	-7.5*	46.8	39.8	-7.1**	113.0	86.9	-26.1**
Cold	34.3	42.9	8.6^{ns}	48.3	49.4	1.1^{ns}	40.9	45.7	4.8^{ns}	93.9	106.0	12.1^{ns}
Heat	39.4	37.7	-1.7^{ns}	54.6	43.1	-11.4**	46.4	40.2	-6.2*	93.7	106.2	12.5^{ns}

ns, *, ** and *** indicate non significant, or significant at P = 0.05, 0.01 and 0.001, respectively.

599

DOI: 10.1016/j.scienta.2019.109013

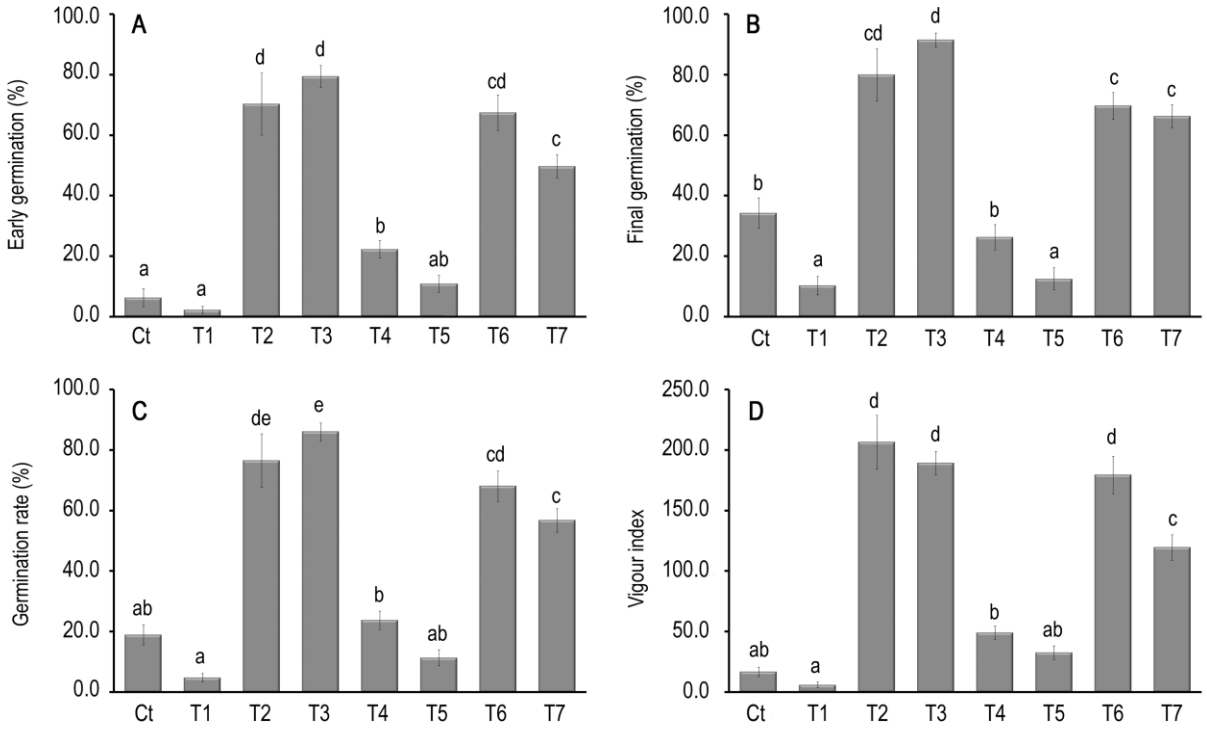
Table 3. Average values for the agronomic and nutritional traits in baby-leaf plants of wall rocket, when each germination factor (soaking, NaClO, GA3, KNO3, cold and heat) was applied at each level (- no application; + application). Difference between the two levels is also indicated (Δ +/-) during the germination test. Only traits with significant differences among germination treatments are considered: plant height, earliness, content in ascorbic acid (AA) and content in total phenolics (TP).

	Plant height (cm)			**Earliness (days)**			**AA content (mg AA 100g^{-1} FW)**			**TP content (mg CAE 100g^{-1} DW)** [a]		
Factor	**-**	**+**	**Δ**	**-**	**+**	**Δ**	**-**	**+**	**Δ**	**-**	**+**	**Δ**
Soaking	10.4	11.3	0.9*	20.9	22.0	1.1*	79.0	79.7	0.7^{ns}	1291.1	1328.6	37.6*
NaClO	10.7	11.0	0.4^{ns}	22.9	20.0	-2.9***	68.8	89.8	21.0***	1286.2	1333.5	47.4**
GA_3	10.9	10.8	-0.1^{ns}	21.2	21.7	0.5^{ns}	78.5	80.2	1.7^{ns}	1325.3	1294.4	-30.9*
KNO_3	11.0	10.7	-0.2^{ns}	21.7	21.2	-0.5^{ns}	83.4	75.3	-8.0^{ns}	1331.3	1288.4	-42.8**
Cold	11.1	10.6	-0.5^{ns}	21.0	21.9	0.9^{ns}	74.5	84.2	9.6^{ns}	1311.8	1307.9	-3.9**
Heat	10.4	11.3	0.8^{ns}	21.3	21.6	0.3*	79.5	79.2	-0.3^{ns}	1319.4	1300.3	-19.1^{ns}

ns, *, ** and *** indicate non significant, or significant at P = 0.05, 0.01 and 0.001, respectively.

[a]CAE: equivalents of chlorogenic acid.

DOI: 10.1016/j.scienta.2019.109013



606

607 **Fig. 1.** Mean values ± SE in the eight treatments (control, Ct, or treatments T1 to T7) tested
608 using a L_8 orthogonal array design, for the four germination traits evaluated in seeds of wall
609 rocket. A) Percentage of early germination (at day 3 after sown). B) Percentage of final
610 germination (at day 11 after sown). C) Percentage of the germination rate. D) Value of the vigour
611 index.

612 Means separated by different letters are significantly different according to the Duncan multiple range test
613 (P = 0.05), with significance obtained using the arcsine-transformation of data.

DOI: 10.1016/j.scienta.2019.109013

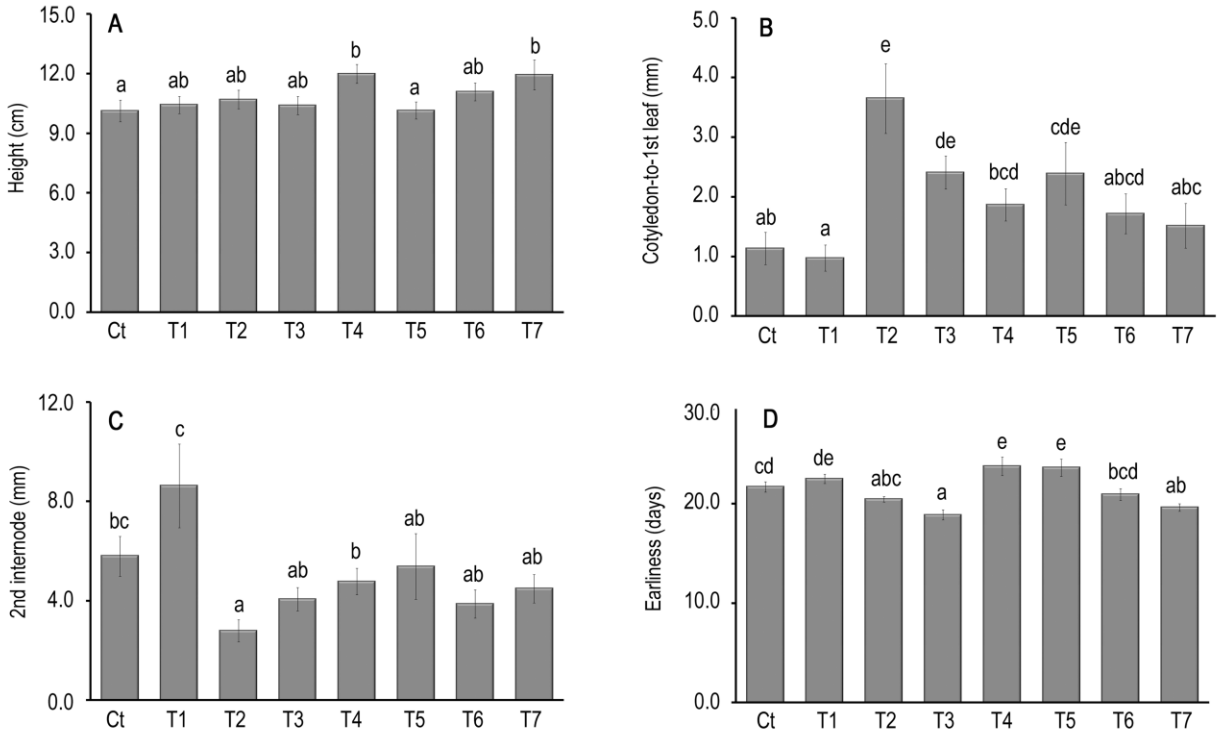
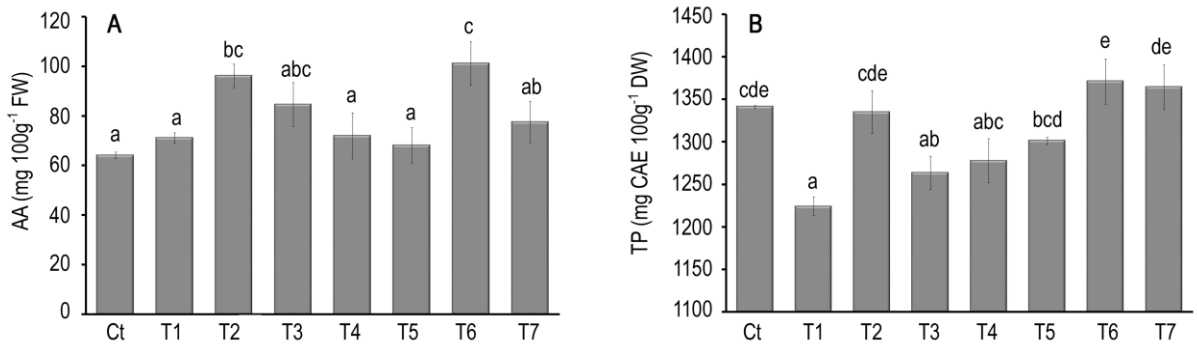


Fig. 2. Mean values ± SE of agronomic traits for baby-leaf plants of wall rocket germinated with the different germination treatments (control, Ct, or treatments T1 to T7). Only the agronomic traits displaying differences among treatments are included. A) Plant height (cm). B) Cotyledon-to-1st leaf length (mm). C) 2nd internode length (mm). D) Earliness or days needed for the appearance of the flower bud.

Means separated by different letters are significantly different according to the Duncan test ($P = 0.05$). Data were tested for normality and log-transformed when needed for the calculation of significance.

DOI: 10.1016/j.scienta.2019.109013



622

623 **Fig. 3.** Mean values ± SE of nutritional traits for baby-leaf plants of wall rocket germinated with
624 the different germination treatments (control, Ct, or treatments T1 to T7). A). Content in ascorbic
625 acid (AA, mg $100g^{-1}$ FW). B) Content in total phenolics (TP, mg of chlorogenic acid equivalents
626 $100g^{-1}$ DW).

627 Means separated by different letters are significantly different according to the Duncan test ($P = 0.05$).
628 Data were tested for normality and log-transformed when needed for the calculation of significance.