The Effect of Osmopriming on Seed Germination and Early Seedling Characteristics of *Carum carvi* L.

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Received: 29 January 2020; Accepted: 21 March 2020; Published: 30 March 2020

**Abstract:** Two experiments (in Petri dishes and in soil) were conducted to investigate the effects of osmopriming on seed germination and the early seedling characteristics of caraway (*Carum carvi* L. var. *annua*). The priming treatments in the Petri dish experiment were: polyethylene glycol (5%, 10% and 20%), KNO$_3$ (0.5%, 1% and 2%) and KCL (1%, 2% and 4%) with three different soaking times (12, 24 and 36 h) along with control (non-primed seeds). Only polyethylene glycol and H$_2$O were applied in the cell tray experiment, which were then compared with the non-primed seeds. In the Petri dish experiment, all three priming reagents significantly enhanced seedling length, with the most effective treatments being 5% PEG, 2% KNO$_3$ and 1% KCL for 12 h. The plumule dry weights were also increased significantly after PEG (20% for 36 h), KNO$_3$ (2% for 24 and 36 h) and KCL (1% for 12 h) treatments in comparison with the control. Only polyethylene glycol and H$_2$O were applied in the cell tray experiment, which were then compared with the non-primed seeds. In the Petri dish experiment, all three priming reagents significantly enhanced seedling length, with the most effective treatments being 5% PEG, 2% KNO$_3$ and 1% KCL for 12 h. The plumule dry weights were also increased significantly after PEG (20% for 36 h), KNO$_3$ (2% for 24 and 36 h) and KCL (1% for 12 h) treatments in comparison with the control. In the soil experiment, osmopriming with PEG significantly improved the germination rate (GR) and percentage, the plumule dry and fresh weights and the plumule length of caraway seedlings when compared with the control. A 23% higher germination percentage was recorded for the seeds treated with 5% PEG for 24 h as compared with the non-primed seeds. The PEG-primed seeds produced significantly longer seedlings when treated with 5% PEG for 24 h. All of the applied PEG treatments significantly enhanced the plumule fresh and dry weights, with the best outcomes being after 5% PEG (24 h) and 10% PEG (36 h) treatments, respectively. The 12-h hydro-priming also significantly enhanced all of the studied germination parameters when compared to the control. The results of the presented experiments show the significant positive effects of seed priming on caraway germination and how early seedling performance can easily be adopted by producers.

**Keywords:** Caraway; plumule; germination pretreatments; seed priming; PEG; KNO$_3$; KCL

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1. Introduction

Caraway is a common European name for the plant *Carum carvi* L., an Apiaceae plant family member. The plant is mainly known as a spice and the source of an essential oil for the cosmetic industry. Caraway contains a wide range of primary metabolites such as sugars (mono-, oligo- and polysaccharides), lipids, amino acids, proteins, free organic acids and secondary metabolites such as...
terpenes, flavonoids, steroids, coumarins, tannins and phenolic substances [1]. Caraway is often used as a component of herbal preparations with digestive, carminative and galactagogue effects. The orally ingested fruits of caraway enhance the function of the digestive tract, the bile ducts, the liver and the kidneys [2]. What is commonly referred to as caraway “seed” is botanically a small dry fruit with a 1000-seed weight of 2–7g [3]. The herb has been cultivated and grown for centuries as a cosmopolitan species in Europe, parts of Asia, northern Africa and northern US and Canada. Caraway essential oil has a unique fragrance and antioxidant properties that have been widely used as a flavoring agent for alcoholic drinks, in toothpaste and in several food products [4,5]. Caraway seed have been also used directly as a spice and as traditional herbal medicine in many countries around the world [6]. There are two botanical varieties of caraway (biennial and annual, or Carum carvi var. biennis and var. annua), which can be mainly distinguished by the length of vegetation period based on the time of flower initiation [7]. The annual variety is naturally spread in the Middle East and eastern Mediterranean [3], and is more commonly used in cultivation due to its faster growth index [8].

The only practical propagation method for caraway is the direct seed sowing of dried mericarps that may germinate unevenly in the field [9]. Uniformity, rapid germination and seedling emergence capacity of direct-seeded crops have a major impact on final yield, quality and ultimately profits in cropping [10]. Caraway usually has a germination capacity of around 60% [11], but the germination rate (GR) also plays an important role in maximizing seed performance by using all of the necessary elements adjacent to the seeds in time, especially in arid and semiarid regions.

The term “germination” has a surprisingly large number of meanings but, in general, refers to dry seeds taking up water, which is completed when the embryonic axis elongates [12]. Priming in particular is the imbibition of seeds to a water content sufficient for pre-germinative metabolic activity to occur while preventing radicle emergence. Such seeds can be dried and will generally exhibit more rapid rates of radicle emergence and strong seedling establishment upon subsequent imbibition [13–15]. Different seed pretreatment techniques such as chemopriming, thermopriming, hydro-priming, osmopriming and biopriming (controlled hydration of seeds via the application of microorganisms or their biological compounds) have been applied for the germination enhancement of several crops [16,17]. In osmopriming—the most common priming method—the seeds are soaked in aerated osmotic solutions usually containing potassium nitrate, potassium phosphate or potassium chloride salts or polyethylene glycol (PEG) with different water potentials and time durations. The applied solutes are commonly dissolved in water at a range of concentrations in which the seeds can imbibe a limited quantity of water to initiate the pre-germination metabolism. The primed seeds are then removed from the osmoticum before the emergence of the primary root or the radicle [17]. The physiological pattern in which priming could be effective in important metabolic phases of seed germination is shown in Figure 1. Following the water uptake (imbibition) in phase I, a sufficient amount of water is used for metabolic processes to repair the cellular components damaged in the maturation drying period.
The cells of the mature dry seeds contain mitochondria that are poorly differentiated as a consequence of maturation drying, but still contain sufficient Kreb’s cycle enzymes and terminal oxidases to provide adequate amounts of ATP to support metabolism for several hours after imbibition [18,19]. Thornton et al. [20] suggested that damage to DNA that accumulates during seed aging is repaired by aerated hydration treatments during the early hours of germination.

Although several experiments have been conducted to investigate the effect of pretreatment on seed germination performance of commercially important crops, less effort has been dedicated to the application of seed priming for medicinal plant cultivation, especially in the case of caraway.

The present study was carried out to evaluate the effects of KNO₃, KCL and PEG solutions as osmopriming reagents on seed germination and the early seedling characteristics (germination percentage and rate, seedling and plumule length, and dry and fresh weight of plumules) of annual caraway in Petri dishes, as well as to compare the effects of PEG and hydro-priming on the same germination parameters in soil. The procedures were carried out at different concentrations with three different soaking times.

2. Materials and Methods

2.1. Plant Materials and Culture Condition

Seeds of annual *Carum carvi* L. var. *annua* were obtained from the department of medicinal and aromatic plants’ experimental farm (“SZKI” cultivar, the gene bank code: APICARU6, Soroksár), Szent István University, Budapest, Hungary. All experiments were carried out in a SGC-120 growth chamber (Weiss Technik UK, Loughborough, UK,) where the temperature and relative humidity were kept at 25/18°C and 60/80% day/night, respectively, with a 14-h photoperiod. The light was provided by fluorescent light tubes (12 × 36 W Philips 840 TL-D 1G; 4000K, Philips, Amsterdam, Netherlands) with a light intensity of 10 klx at the level of culture.
2.2. Priming Experiment in Petri Dishes

A completely randomized design was applied as a factorial block with four replications. KNO₃ (0.5%, 1% and 2%), KCl (1%, 2% and 4%) and PEG₆₀₀₀ (5%, 10% and 20%) were used as osmopriming reagents [21–23] with soaking times of 12, 24 and 36 h. Five grams of caraway seeds were primed in 100-mL flasks containing 80 mL of osmotic solutions. Priming solutions were prepared in distilled water. Caraway seeds were wrapped in a fabric net and marked by three different color threads indicating their individual soaking times. In order to ensure normal seed respiration, oxygen supply was prepared [24]. The flasks were sealed with a hole on the top through which a narrow tube (6 mm inner diameter) was connected to a small aquarium pump for insufflating air into each solution. The seeds were fully immersed in the priming media at a temperature of 15°C [25] in the dark. For the 24- and 12-h treatments, seeds batches were immersed 12 and 24 h after the first batch (36 h), respectively, so that all seeds were removed from the priming solutions at the same time after 36 h. The primed seeds were thoroughly rinsed with distilled water for 2 minutes. Then, the washed seeds were labeled and air-dried on blotting paper at room temperature (24°C) overnight [22]. Four replicates of 25 seeds from each treatment were placed on filter paper (Whatman No.1, sterilized at 105°C for 1 h) in 8-cm diameter acrylic plastic Petri dishes, then moistened with distilled water so that about half the volume of each seed was immersed. The dishes were placed in the growth chamber for germination. The germination was considered to have occurred when the primary root reached 5 mm [26]. Germination of individual seeds was measured at 24-h intervals and continued until no further germination occurred for 24 h after the last record. After the lapse of the experimental period (24 days), germination percentage (in %) and rate of germination were evaluated. Germination rate (GR) was determined using Maguire’s index [27] as follows:

$$GR = \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \ldots + \frac{\text{Number of germinated seeds}}{\text{Day of final count}}$$

On the 25th day, the seedling lengths were measured. The plumules were separated from the roots; their fresh weights were recorded before putting the samples in an oven for 2 days at 60°C to determine the dry mass.

2.3. Priming Experiment in Soil

The priming effect on caraway germination in soil was carried out to study germination parameters in a condition closer to the field circumstances. The caraway seeds were primed with PEG (12, 24 and 36 h) plus a new treatment (hydro-priming) with distilled water (12, 24 and 36 h) and subsequently sown in the soil in cell trays. Of the non-primed and individual priming treatments, three replicates of 25 seeds were sown 0.5 cm deep and covered with soil in cell trays, then placed in the growth chamber with the same temperature, humidity and light intensity as the Petri dish experiment. The soil in trays consisted of a combined Rekyva Remix D soil mixture, black peat and perlite in a ratio of 7:2:1, respectively. The plants were irrigated with equal amounts of water (3 mL in each cell) every day. Germination rate and seedling emergence in percentage were determined using the same Maguire’s index. On the 25th day of the experiment, seedlings were cut from right before the plumule part, their length was measured and the fresh weight was recorded. Samples then were dried at 60°C for 2 days to obtain their dry mass.

2.4. Statistics

Priming experiment in Petri dishes was analyzed by a three-way multivariate analysis of variance (MANOVA) model with factor treatments (control, PEG, KCl and KNO₃), concentration (low, medium and high) and time (12, 24 or 36 h). The results of the priming experiment in soil were evaluated by a three-way MANOVA model with factor treatments (control and PEG), concentration (low, medium and high) and time (12, 24 and 36 h) while the hydro-priming experiment in soil was tested by a
two-way MANOVA model with factor treatments (control and PEG) and time (12, 24 and 36 h). In case the overall MANOVA result was significant, between-subjects follow-up analysis was performed with a Bonferroni’s adjustment. In order to normalize the data set, germination percentage was arsin (sqrt(x))-transformed in the case of the hydro-priming experiment, while plumule fresh weight was reciprocally transformed. The normality of the residuals was accepted by the absolute values of the skewness and kurtosis of the model error terms, as they were all below 1. The homogeneity of variances was tested by maximal and minimal variance ratios. Since it was in some cases slightly violated, a Games-Howell post hoc test was applied for all pairwise comparisons. For statistical analysis, statistical software IBM SPSS (v25) (IBM Corp, Armonk, NY, USA) [28] was used, while and for sketching the diagrams MS Excel was employed.

3. Results and Discussion

3.1. Priming Experiment in Petri Dishes

The three-way MANOVA model with germination rate, germination percentage, seedling fresh and dry weight and seedling length revealed significant treatment and time effects (Wilks’ λ = 0.14 and 0.75, resp. both with p < 0.001) and an insignificant concentration effect (Wilks’ λ = 0.93; p = 0.60). On the other hand, treatment*concentration and treatment*time interactions were both significant (Wilks’ λ = 0.69 and 0.67, resp. both with p < 0.05).

The follow-up analysis of caraway seed priming in Petri dishes did not result in significant differences for germination rate, germination percentage (%) and seedling fresh weight, as shown in Table 1 (treatment: F(3;112) < 3.50; p > 0.05; time: F(2;112) < 0.60; p > 0.20; treatment*concentration: F(6;112) < 0.60; p > 0.10; treatment*time: F(6;112) < 0.80; p > 0.20).

The follow-up analysis using a between-subjects effects test with Bonferroni’s correction indicated significant effects of treatment and time with significant treatment*concentration and treatment*time interactions for seedling length (treatment: F(3;112) = 106.36; p < 0.001; time: F(2;112) = 13.92; p < 0.001; treatment*concentration: F(6;112) = 8.63; p < 0.01; treatment*time: F(6;112) = 7.28; p < 0.05). Seedling length was significantly improved in comparison with the control after 5% PEG priming and 12 h soaking. All KNO₃ treatments (0.5%, 1% and 2% for 12, 24 and 36 h) significantly increased seedling length as compared with the control. The 2% KNO₃ treatment for 12 h resulted in the highest seedling length, not only among the three concentrations of KNO₃ treatments but also among the three priming reagents. Among the KCL-primed seeds, the highest seedling length was obtained from the seeds treated with 1% concentration for 12 h, which was significantly higher than the non-primed seeds (Table 1).

The follow-up statistical analysis showed a significant treatment effect with an insignificant time effect, and treatment*concentration and treatment*time interactions for seedling dry weight (treatment: F(3;112) = 43.14; p < 0.001; time: F(2;112) = 1.60; p = 0.21; treatment*concentration: F(6;112) = 2.48; p = 0.13; treatment*time: F(6;112) = 1.26; p = 0.28).

The plumule dry weights showed significant differences as a consequence of seed priming. Among the PEG-primed seeds, the highest and most significantly different plumule dry weight was measured from the seeds treated with 20% PEG for 36 h. The KNO₃-primed seeds showed their highest plumule dry weights, which were significantly higher than the control, when treated with 2% KNO₃ for 24 and 36 h. The 1% KCL-primed seeds for 12 h were the only KCL treatment that significantly increased the plumule dry weights when compared to the control.
Table 1. Comparative analysis of caraway seed priming with PEG, KNO$_3$ and KCL on germination and seedlings characteristics in the Petri dish experiment.

| Treatment | Time | Germination percent (GR) | Germination rate (GR) | Seedling length (cm) | Plumule fresh weight (mg) | Plumule dry weight (mg) |
|-----------|------|--------------------------|----------------------|---------------------|---------------------------|------------------------|
| PEG 5%    | 12 h | 54.00 ± 19.73            | 1.64 ± 0.27          | 8.95 ± 0.89         | 29.14 ± 5.45              | 1.62 ± 0.06            |
|           | 24 h | 46.00 ± 14.79            | 1.41 ± 0.43          | 7.48 ± 0.96A        | 23.98 ± 1.64              | 1.92 ± 0.06            |
|           | 36 h | 51.00 ± 2.00             | 1.44 ± 0.09          | 6.58 ± 0.87         | 24.67 ± 3.40              | 1.93 ± 0.07            |
| PEG* 10%  | 12 h | 53.00 ± 15.10            | 1.62 ± 0.40          | 7.90 ± 0.35B        | 25.70 ± 1.36              | 1.79 ± 0.07            |
|           | 24 h | 51.00 ± 11.94            | 1.50 ± 0.24          | 7.96 ± 0.48AB       | 24.49 ± 4.39              | 1.86 ± 0.15B           |
|           | 36 h | 61.00 ± 11.94            | 1.71 ± 0.31          | 7.40 ± 0.28A        | 23.43 ± 2.72              | 2.00 ± 0.10B           |
| PEG 20%   | 12 h | 52.00 ± 6.64             | 1.46 ± 0.28          | 7.86 ± 0.65A        | 21.37 ± 5.26              | 1.92 ± 0.20            |
|           | 24 h | 54.00 ± 6.93             | 1.62 ± 0.11          | 7.61 ± 0.51         | 25.80 ± 4.04              | 1.70 ± 0.12B           |
|           | 36 h | 48.00 ± 13.47            | 1.42 ± 0.34          | 6.66 ± 0.59A        | 26.43 ± 3.46              | 2.01 ± 0.12B           |
| KNO$_3$ 0.5% | 12 h | 52.00 ± 9.80             | 1.61 ± 0.27          | 9.11 ± 0.26         | 20.70 ± 2.83              | 1.78 ± 0.27            |
|           | 24 h | 55.00 ± 10.52            | 1.61 ± 0.36          | 8.93 ± 0.28B        | 26.20 ± 5.80              | 2.02 ± 0.31            |
|           | 36 h | 48.00 ± 16.97            | 1.43 ± 0.42          | 7.77 ± 0.47a        | 27.31 ± 6.24              | 2.08 ± 0.22            |
| KNO$_3$ 1% | 12 h | 48.00 ± 9.80             | 1.47 ± 0.22          | 9.47 ± 0.40B        | 25.40 ± 1.87              | 1.92 ± 0.18            |
|           | 24 h | 39.00 ± 8.25             | 1.38 ± 0.19          | 9.03 ± 0.66B        | 24.18 ± 3.44              | 2.02 ± 0.21B           |
|           | 36 h | 51.00 ± 8.25             | 1.61 ± 0.22          | 9.48 ± 0.53B        | 24.00 ± 5.52              | 2.01 ± 0.20AB          |
| KNO$_3$ 2% | 12 h | 47.00 ± 13.22            | 1.42 ± 0.36          | 9.96 ± 0.76B        | 24.55 ± 2.59              | 2.04 ± 0.19            |
|           | 24 h | 57.00 ± 16.77            | 1.71 ± 0.35          | 9.08 ± 0.96         | 27.13 ± 0.94              | 2.13 ± 0.06B           |
|           | 36 h | 49.00 ± 10.52            | 1.52 ± 0.31          | 9.12 ± 0.28B        | 30.13 ± 2.53              | 2.11 ± 0.14A           |
| KCL 1%    | 12 h | 54.00 ± 17.74            | 1.71 ± 0.52          | 8.32 ± 0.66B        | 25.93 ± 3.47              | 2.10 ± 0.12            |
|           | 24 h | 48.00 ± 7.12             | 1.43 ± 0.37          | 8.15 ± 0.32AB       | 31.24 ± 2.48              | 1.86 ± 0.18            |
|           | 36 h | 51.00 ± 8.25             | 1.53 ± 0.28          | 7.98 ± 0.68         | 26.94 ± 2.68              | 2.05 ± 0.10            |
| KCL* 2%   | 12 h | 40.00 ± 11.78            | 1.31 ± 0.18          | 8.31 ± 0.28B        | 27.11 ± 1.08              | 1.68 ± 0.07            |
|           | 24 h | 52.00 ± 10.83            | 1.56 ± 0.36          | 6.73 ± 0.83A        | 23.11 ± 2.36              | 1.80 ± 0.13A           |
|           | 36 h | 46.00 ± 7.66             | 1.46 ± 0.24          | 7.23 ± 0.15A        | 24.88 ± 1.22              | 1.46 ± 0.08A           |
| KCL 4%    | 12 h | 55.00 ± 3.83             | 1.72 ± 0.07          | 7.77 ± 0.16A        | 27.71 ± 4.59              | 1.66 ± 0.21            |
|           | 24 h | 48.00 ± 6.53             | 1.46 ± 0.20          | 7.66 ± 0.66B        | 26.90 ± 2.45              | 1.82 ± 0.07A           |
|           | 36 h | 46.00 ± 13.27            | 1.46 ± 0.38          | 8.01 ± 1.00 AB      | 29.84 ± 3.66              | 1.72 ± 0.04B           |
| Control   |      | 46.00 ± 10.58            | 1.42 ± 0.34          | 6.66 ± 0.32         | 24.48 ± 3.73              | 1.62 ± 0.19            |

*underlined: seedling length is significantly longer after 12 h after 12 h than after 36 h of priming time (Games-Howell: $p < 0.05$). Different letters are for significantly different groups if significant results were detected (Games-Howell: $p < 0.05$). Lower case letter: comparison of concentration for the same treatment and time effect. Upper case letter: comparison of treatment for the same concentration and time effect. Bold numbers: significantly different from control; (Games-Howell: $p < 0.05$); n = 4.

The relatively higher lengths of KNO$_3$–primed seedlings compared to other applied priming reagents could be due to the effect of oxidized forms of nitrogen, which can cause a shift in respiratory metabolism to the pentose phosphate pathway [29] and therefore may have enhanced germination. When studying the effect of KNO$_3$ priming on Apiaceae species, Pérez-Fernández et al. [30] reported an enhanced germination % after *Foeniculum vulgare* Mill. and *Thapsia villosa* L., but not in the case of *Daucus carota*. Similar to our findings, they could not find any positive effect on the germination rate of the KNO$_3$-primed seeds in Petri dishes [30]. No significant enhancement in embryo growth rate was reported when *Conopodium majus* (Apiaceae) seeds were treated with different concentrations of KNO$_3$ [31]. In another study on fennel, Tahaei et al. did not find any significant enhancement on seed germination when 0.4% KNO$_3$ was applied in Petri dishes [32]. Seedling dry weight is a reliable parameter by which to evaluate seedling performance and quality, especially in laboratory conditions where water availability is not a limiting factor. There have been several reports on the significant positive effects of KNO$_3$ priming on the seedling dry weights of several crops [33–37]. Based on an overall view of our result, the studied parameters in the Petri dish experiment did not show a linear tendency in response to different osmotic potentials or soaking times. Therefore, another experiment was conducted to study the priming effects on caraway germination in soil.
3.2. PEG Priming Experiment in Soil

The results of priming were more pronounced in the soil experiment. The three-way MANOVA with germination percentage, germination rate, plumule length and plumule fresh and dry weight resulted in significant treatment, time and concentration effects with a significant concentration*time interaction (treatment: Wilk’s λ = 0.60; p < 0.001; time: Wilk’s λ = 0.60; p < 0.05; concentration: Wilk’s λ = 0.62; p < 0.001; concentration*time: Wilk’s λ = 0.30; p < 0.001). A significant treatment effect was manifested in all of the studied parameters in this experiment (F(1;20) = 3.26; p < 0.05). The concentration effect was significant for plumule fresh weight only (F(2;20) = 5.80; p < 0.05), while the time effect was significant in cases of germination percentage and germination rate (F(2;20) = 8.13; p < 0.05; F(2;20) = 7.88; p < 0.01, respectively). The concentration*time interaction, however, was significant for the plumule fresh and dry weight, as well as for the germination rate (F(2;20) > 4.2; p < 0.05). The effect of PEG treatment on the germination percentage for primed caraway seeds sown in cell trays is shown in Table 2. The highest germination percentage (63%) was recorded after 24 h of treatment with a 5% PEG concentration, which corresponded to a −0.35 MPa water potential. The highest germination rate also was measured after 5% PEG treatment for 24 h, which was significantly effective in decreasing the emergence time of the caraway seedlings when compared with non-primed seeds (p < 0.001).

Table 2. Comparative analysis of caraway seed priming with PEG on germination and seedling characteristics in the soil experiment.

| Treatment | Time | Germination percent | Germination rate | Plumule length (cm) | Plumule fresh weight (mg) | Plumule dry weight (mg) |
|-----------|------|---------------------|-----------------|--------------------|--------------------------|------------------------|
|           | 12 h | 56.63 ± 3.35B       | 1.61 ± 0.03B    | 5.89 ± 0.27        | 94 ± 0.006b              | 12.2 ± 0.1             |
| PEG 5%    | 24 h | 63.30 ± 3.30B       | 1.91 ± 0.03C    | 6.17 ± 0.09        | 105 ± 0.007b             | 11.1 ± 0.4             |
|           | 36 h | 33.30 ± 3.30A       | 0.84 ± 0.05A    | 6.01 ± 0.31        | 074 ± 0.006a             | 9.8 ± 0.12             |
|           | 12 h | 36.63 ± 3.35A       | 1.44 ± 0.04A    | 6.08 ± 0.52        | 92 ± 0.001b              | 11.2 ± 0.2             |
| PEG 10%   | 24 h | 56.63 ± 3.35B       | 1.59 ± 0.02B    | 5.78 ± 0.54        | 79 ± 0.006a              | 10.8 ± 0.3             |
|           | 36 h | 43.30 ± 3.30A       | 1.39 ± 0.04A    | 5.94 ± 0.72        | 101 ± 0.004b             | 13.8 ± 0.12            |
|           | 12 h | 49.97 ± 3.35A       | 1.36 ± 0.02A    | 6.19 ± 0.29        | 64 ± 0.001a              | 9.5 ± 0.7              |
| PEG 20%   | 24 h | 53.30 ± 3.30A       | 1.49 ± 0.02B    | 5.31 ± 0.23        | 81 ± 0.008a              | 11.0 ± 0.12            |
|           | 36 h | 49.97 ± 3.35A       | 1.55 ± 0.05B    | 5.62 ± 0.23        | 80 ± 0.011ab             | 10.8 ± 0.7             |
| Control   |      | 39.97 ± 3.35       | 1.01 ± 0.02     | 4.49 ± 0.05        | 53 ± 0.1                 | 7.0 ± 0.04             |

Different letters are for significantly different groups if a significant result was detected (Games-Howell: p < 0.05). Lower case letters: comparison of low, medium and high concentrations with the same time; Upper case letters: comparison of time effects with the same concentration effect. Bold numbers: significantly different from control; n = 3.

Except for the 10% PEG treatment at 24 and 36 h, all treatments significantly enhanced plumule length, with the highest value (6.19 cm) being recorded after 20% PEG treatment for 12 h as compared to the control (4.49 cm). In the cases of plumule fresh and dry weights, the best treatments were 5% PEG with 24 h soaking and 10% PEG with 36 h soaking, respectively. Considering the statistical analysis of the results, the 5% PEG treatment with 24 h soaking can be recommended as the best treatment for improving the germination percentage, germination rate, seedling length and seedling fresh weight of annual caraway. Caraway seedlings grown after 5% PEG treatment for 24 h are compared with the controls in Figure 2.
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3.3. Hydro-priming Experiment in Soil

The two-way MANOVA model with germination percentage, germination rate, plumule length, plumule fresh weight and plumule dry weight revealed a significant treatment effect with an insignificant time effect (treatment: Wilk’s $\lambda = 0.004; p < 0.001$; time: Wilk’s $\lambda = 0.30; p = 0.15$). The follow-up between-subjects effects test with Bonferroni’s correction resulted in a highly significant treatment effect for all five parameters ($F(1;8) > 30.14; p < 0.001$). The results of hydro-priming on the early seedling characteristics of caraway are presented in Table 3. The highest germination percentage was obtained after 12 and 24 h of soaking, which was significantly higher than the control. When compared to the non-primed caraway seeds, the 12-h hydro-primed seeds had significantly enhanced germination rates, plumule lengths and plumule dry and fresh weights. A decreasing tendency was observed in the case of all studied parameters when the hydro-priming time was increased. A similar trend has previously been reported in the case of broad bean (Vicia faba L.) hydro-priming where, aside from significant enhancements in comparison with non-primed seeds, the increase in hydro-priming time from 8 to 24 and 36 h gradually decreased the seedling fresh weight and germination %, respectively [38]. According to the results, the 12 h of hydro-priming was the best soaking time for caraway germination performance, although still not as effective as PEG pre-treatment.

Table 3. The effect of caraway seed hydro-priming with distilled water (DW) germination percentage, germination rate, plumule length, plumule fresh weight and plumule dry weight.

| Treatment | Time | Germination percent | Germination rate | Plumule length (cm) | Plumule fresh weight (mg) | Plumule dry weight (mg) |
|-----------|------|---------------------|------------------|---------------------|---------------------------|------------------------|
| H$_2$O    | 12 h | 53.3 ± 3.35*        | 1.62 ± 0.01***   | 5.75 ± 0.12***      | 85.66 ± 0.9**             | 12.1 ± 0.05***         |
|           | 24 h | 53.3 ± 3.30*        | 1.52 ± 0.02***   | 5.61 ± 0.42         | 81.33 ± 0.05***           | 11.0 ± 0.06**          |
|           | 36 h | 46.63 ± 3.30        | 1.50 ± 0.03***   | 4.73 ± 0.37         | 75.33 ± 0.57**            | 9.8 ± 0.08*            |
| Control   |      | 39.97 ± 3.35        | 1.01±0.02        | 4.49 ± 0.05         | 53.0 ± 0.1                | 7.0 ± 0.04             |

Comparisons to control: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n = 3.

Figure 2. Caraway seedlings (25 days old) (A) from non-primed seeds and (B) from primed seeds with 5% PEG for 24 h.

Typical slow germination in Apiaceae species is perhaps due to the embryo’s growth pattern, along with the variable presence of endogenous inhibitory materials. In mature celery and carrot seeds,
for instance, the embryo is underdeveloped and entirely embedded in the endosperm, and must grow about two to three times at the expense of both its cell expansion and cell division before visible radicle emergence occurs [39,40]. This phenomenon is referred to as morphophysiological dormancy and recognized in 96% of Apiaceae species [41,42].

Although immature seeds are in general more responsive to priming than mature seeds, the overall degree of response would be strongly influenced by the diversification of seed ages within a seed lot. At biochemical and cytological levels, germination and priming mechanisms may differ substantially amongst seed types due to the structural nature of their embryos and the embryos’ enclosing tissues. Seed populations naturally conceal considerable differences in structure and physiological state (e.g., due to indeterminate flowering and seed development patterns) as well as genetic variation, and these factors determine the time required by a seed to complete germination. From point of view of the seed industry, understanding such species-specific physiological patterns can provide practical insights to optimize hydration and drying procedures. An important factor that may contradict the experimental results is the unavoidable differences between seed lots, which can result in different outcomes in the field. The reported effects of seed soaking in water on germination and subsequent seedling growth vary from improving to diminishing or to having no effect at all-factors that depend on the kind of seed used, the conditions of priming, the duration of soaking and the seed-water content. In spite of this, the applied methods in our work are easily applicable for farmers and provide results that can be obtained in a short period of time, allowing one to test the best-suggested priming reagents and soaking time before the commencement of any large scale cultivation.

4. Conclusions

Different combinations of soaking times and potential water changes caused by solutes such as PEG have been reported in the scientific literature as effective for enhancing germination, although different specimens seem to show different behaviors when exposed to different priming reagents and treatment times. From our study case, it can be concluded that the germination rate and germination percentage were more responsive to the soaking time than the water potential caused by the priming reagent with different concentrations. Our results show a range of achievable enhancements in the case of caraway germination and its early growth performance. The osmopriming of caraway seeds with 5% PEG for 24 h soaking and hydro-priming for 12 h can be recommended as the most effective treatments. Such an easy to use and practical pretreatment technique can be utilized by farmers and plant growers to help increase crop quality and ultimately achieve a higher yield.

Author Contributions: Conceptualization, I.M. and P.R.; methodology, É.Z.N.; statistical analysis, M.L. and É.E.; investigation, I.M.; resources, É.Z.N.; writing—original draft preparation, I.M.; writing—review and editing, I.M. and A.K.; visualization, I.M. and P.R.; supervision, P.R.; funding acquisition, É.Z.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry for Innovation and Technology within the framework of the Higher Education Institutional Excellence Program (NKFIH-1159-6/2019) in the scope of plant breeding and plant protection research of Szent István University and by the Human Resources Development Operational Program of the European Social Fund and Ministry of Human Capacities under grant number EFOP-3.4.3-16-2016-00012.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ruszkowska, J. Main chemical constituents of Carum. In Caraway -The Genus Carum; Németh, É., Ed.; Harwood Academic Publisher: Amsterdam, The Netherlands, 1998; pp. 38–60.
2. Sadowska, A.; Obidoska, G. Pharmacological uses and toxicology of caraway. In Caraway -The Genus Carum; Németh, É., Ed.; Harwood Academic Publisher: Amsterdam, The Netherlands, 1998; pp. 186–196.
3. Toxopeus, H.; Lubberts, H. A century of breeding caraway in the Netherlands. In Caraway -The Genus Carum; Németh, É., Ed.; Harwood Academic Publisher: Amsterdam, The Netherlands, 1998; pp. 117–143.
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4. Samojlik, I.; Lakic, N.; Mimica-Dukic, N.; Dakovic-S#x160;vajcer, K.; Bozin, B. Antioxidant and hepatoprotective potential of essential oils of cariander (Coriandrum sativum L.) and caraway (Carum carvi L.) (Apiaceae). J. Agr. Food Chem. 2010, 58, 8848–8853. [CrossRef] [PubMed]

5. Javed, R.; Hanif, M.A.; Rehman, R.; Hanif, M.; Tung, B.T. Caraway. In Medicinal Plants of South Asia; Asif Hanif, M., Mumtaz Khan, M., Nawaz, H.J., Byrne, H., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 87–100.

6. Bouwmeester, H.H. Regulation of essential oil formation of caraway. In Caraway -The Genus Carum; Németh, É., Ed.; Harwood Academic Publisher: Amsterdam, The Netherlands, 1998; pp. 92–116.

7. Németh, E. Questions of the generative development in caraway. In Caraway -The Genus Carum; Németh, É., Ed.; Harwood Academic Publisher: Amsterdam, The Netherlands, 1998; pp. 79–91.

8. Omidbaigi, R.

9. Weglarz, Z. Production of biennial caraway for seed and essential oil. In Caraway -The Genus Carum; Németh, É., Ed.; Harwood Academic Publisher: Amsterdam, The Netherlands, 1998; pp. 144–157.

10. Tsotrzakis, N.G. Effect of pre-sowing treatment on seed germination and seedling vigour in endive and chicory. Hort. Sci. (Prague) 2009, 36, 117–125. [CrossRef]

11. Galambosi, B.; Peura, P. Agrobotanical features and oil content of wild and cultivated forms of caraway (Carum carvi L.). J. Essent. Oil Res. 1996, 8, 389–397. [CrossRef]

12. Bewleyl, J.D. Seed germination and dormancy. Plant Cell 1997, 9, 1055–1066. [CrossRef] [PubMed]

13. Heydecker, W.; Coolbear, P. Seed treatment for improved performance survey and attempted prognosis. Seed Sci. Technol. 1977, 13, 299–335.

14. Bradford, K.J. Manipulation of seed water relations via osmotic priming to improve germination under stress condition. HortScience (USA) 1989, 24, 1105–1112.

15. Khan, A.A. Preplant physiological seed conditioning. Hort. Rev. 1992, 13, 131–172.

16. Ashraf, M.; Foolad, M.R. Pre-sowing seed treatment –A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Adv. Agron. 2005, 88, 223–271.

17. Paparella, S.; Araujo, S.S.; Rossi, G.; Wijayasinghe, M.; Carbonera, D.; Balestrazzi, A. Seed priming: State of the art and new perspectives. Plant Cell Rep. 2015, 34, 1281–1293. [CrossRef] [PubMed]

18. Ehrenshaft, M.; Brambl, R. Respiration and mitochondrial biogenesis in germinating embryos of maize. Plant Physiol. 1990, 93, 295–304. [CrossRef] [PubMed]

19. Attucci, S.; Carde, J.P.; Raymond, P.; Saint Ges, V.; Spiteri, A.; Pradet, A. Oxidative phosphorylation by mitochondria extracted from dry sunflower seeds. Plant Physiol. 1991, 95, 390–398. [CrossRef] [PubMed]

20. Thornton, J.M.; Collins, A.R.S.; Powell, A.A. The effect of aerated hydration on DNA synthesis in embryos of Brassica oleracea L. Seed Sci. Res. 1993, 3, 195–199. [CrossRef]

21. Farooq, M.; Basra, S.M.A.; Rehman, H.; Saleem, B.A. Seed priming enhances the performance of late sown wheat (Triticum aestivum L.) by improving chilling tolerance. J. Agron. Crop Sci. 2008, 194, 55–60. [CrossRef]

22. Giri, G.S.; Schillinger, W.F. Seed priming winter wheat for germination, emergence and yield. Crop Sci. 2003, 43, 2135–2141. [CrossRef]

23. Mazor, L.; Perl, M.; Negbi, M. Changes in some ATP-dependent activities in seeds during treatment with polyethylene glycol and during the redrying process. J. Exp. Bot. 1984, 35, 1119–1127. [CrossRef]

24. Bujałoński, W.; Nienow, A.W. Large-scale osmotic priming of onion seeds: A comparison of different strategies for oxygenation. Sci. Hortic. 1991, 46, 13–24. [CrossRef]

25. Michel, B.E.; Kaufmann, M.R. The osmotic potential of polyethylene glycol 6000. Plant Physiol. 1973, 51, 914–916. [CrossRef]

26. Demir, I.; Mavi, K. Effect of salt and osmotic stresses on the germination of Pepper seeds of different maturation stages. Braz. Arch. Biol. Techn. 2008, 51, 897–902. [CrossRef]

27. Maguire, J.D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 1962, 2, 176–177. [CrossRef]

28. IBM Corp. IBM SPSS Statistics for Windows, Version 25.0; IBM Corp: Armonk, NY, USA, 2017.

29. Roberts, E.; Smith, R.D. Dormancy and the pentose phosphate pathway. In The Physiology and Biochemistry of Seed Dormancy and Germination; Khan, A., Ed.; North-Holland Publishing Co: Amsterdam, The Netherlands, 1977; pp. 385–411.
30. Pérez-Fernández, M.A.; Calvo-Magro, E.; Montanero-Fernández, J.; Oyola-elascio, J.A. Seed germination in response to chemicals: Effect of nitrogen and pH in the media. J. Environ. Biol. 2006, 27, 13–20.

31. Blandino, C.; Fernández-Pascual, E.; Marin, M.; Vernet, A.; Pritchard, H.W. Seed ecology of the geophyte Conopodium majus (Apiaceae), indicator species of ancient woodland understoreys and oligotrophic meadows. Plant Biol. 2019, 21, 487–497. [CrossRef] [PubMed]

32. Tahaei, A.; Soleymani, A.; Shams, M. Seed germination of medicinal plant, fennel (Foeniculum vulgare Mill), as affected by different priming techniques. Appl. Biochem. Biotech. 2016, 180, 26–40. [CrossRef] [PubMed]

33. Ahmadvand, G.; Soleiman, F.; Saadatian, B.; Pouya, M. Effect of seed priming with potassium nitrate on germination and emergence traits of two soybean cultivars under salinity stress conditions. Am. Eurasian J. Agric. Environ. Sci. 2012, 12, 769–774.

34. Espanany, A.; Fallah, S.; Tadayyon, A. Seed priming improves seed germination and reduces oxidative stress in black cumin (Nigella sativa) in presence of cadmium. Ind. Crops Prod. 2016, 79, 195–204. [CrossRef]

35. Kumar, S.; Hemantaranjan, A.; Mondal, S.; Bose, B. Impact of KNO3 Primed seeds on the performance of late sown sesame (Sesamum indicum L.). IJBSM 2016, 7, 950–954. [CrossRef]

36. Patil, K.; Ravat Anilkumar, L.; Trivedi, V.; Hirpara, A.; Sasidharan, N. Effect of seed priming treatment in chickpea (Cicer arietinum L.). IJCS 2018, 6, 1064–1069.

37. Alizadeh, M.A.; Sajjadi Jaghargh, S.S.; Sharifi, R.S.; Calagari, M.; Sedghi, M. Effect of seed priming and moist chilling on emergence traits of six populations (Anthemis haussknechtii Boiss. & Reut. and Anthemis pseudocotula Boiss.) in greenhouse condition. J. Med. Plants Prod. 2019, 8, 41–51.

38. Damalas, C.A.; Koutroubas, S.D.; Fotiadis, S. Hydro-priming effects on seed germination and field performance of faba bean in spring sowing. Agriculture 2019, 9, 201. [CrossRef]

39. Karssen, C.M.; Haigh, A.; Toorn, P.; Eges, R. Physiological mechanisms involved in seed priming. In Recent Advances in the Development and Germination of Seeds; Taylorson, R.B., Ed.; Plenum Press: New York, NY, USA; NLondon, UK, 1990; pp. 269–280.

40. Gray, D.; Steckel, J.R.A.; Hands, L.J. Responses of vegetable seeds to controlled hydration. Ann. Bot. 1990, 66, 227–235. [CrossRef]

41. Vandelook, F.; Van Assche, J.A. Deep complex morphophysiological dormancy in Sanicula europaea (Apiaceae) fits a recurring pattern of dormancy types in genera with an Arcto-Tertiary distribution. Botany 2008, 86, 1370–1377. [CrossRef]

42. Willis, C.G.; Baskin, C.C.; Baskin, J.M.; Auld, J.R.; Venable, D.L.; Cavender-Bares, J.; Donohue, K.; de Casas, R.R.; Bradford, K.; Burghardt, L.; et al. The evolution of seed dormancy: Environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytol. 2014, 203, 300–309. [CrossRef] [PubMed]

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