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Hydro-Priming Effects on Seed Germination and Field Performance of Faba Bean in Spring Sowing

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Abstract: Seed priming has been used to advance germination and stand in several crops, but relevant research on faba bean (*Vicia faba* L.) is scarce. Laboratory and field trials were carried out for two years to study the effect of hydro-priming on faba bean germination and field performance in spring sowing. In laboratory trials, the effects of hydro-priming for 0, 8, 16, 24, 36, and 48 h on final germination percentage, germination speed, Timson's germination index, mean germination time, mean daily germination, synchronization index, and seedling vigor index were studied. All hydro-priming treatments improved germination parameters of faba bean seeds, except for final germination percentage and mean daily germination compared with non-primed seeds. Averaged over priming duration treatments, hydro-priming improved germination speed by 16.2%, germination synchrony by 20.7%, and seedling vigor index by 13.4%. All hydro-priming durations improved germination synchrony, while hydro-priming for 8, 16, and 24 h provided the highest values of germination speed (2.56, 2.58, and 2.37 seeds day^{-1}, respectively). Hydro-priming for 8 and 16 h provided the lowest values of mean germination time (5.81 and 5.96 days, respectively). In field trials, hydro-priming periods of 0, 8, 16, and 24 h were compared. On average, seed hydro-priming did not affect significantly seedling emergence 14 days after sowing in the first year, but significantly improved seedling emergence by 34.4% in the second year. No significant effect of seed hydro-priming was noted in the number of plants at 28 and 35 days after sowing. Seed priming for 8 h resulted in higher fresh weight at anthesis by 22.3% and 8.6% in the first and the second year, respectively, than the non-primed control. Similarly, seed priming for 8 h provided higher seed yield by 12.0% in the first year and by 5.9% in the second year compared with non-primed control. Overall, seed hydro-priming accelerated faba bean germination and seedling emergence, but the magnitude of the response was associated with the environment and was more evident under limited soil moisture after sowing, whereas the beneficial effect of priming was masked when rainfall followed sowing. Similarly, the beneficial effect of priming on seed yield was more pronounced with limited soil moisture after anthesis.

Keywords: crop yield; germination speed; seedling vigor; synchronization index

1. Introduction

Successful crop establishment and high seedling vigor are considered decisive factors for the success of most field crops, as these parameters contribute to uniform plant growth and maturity, better competition with weeds, and high productivity [1]. Therefore, improving seed vigor is a primary objective of the industry of seed production to enhance the critical and yield-defining stage of crop establishment. Nevertheless, low vigor of seeds or adverse environmental conditions after sowing may cause slow seed germination and unreliable seedling emergence under field conditions [2]. Seed quality will determine the possibility of producing healthy seedlings and sufficient plant population for

achieving high yield. In practice, seed quality loosely reflects the overall value of seed according to its intended purpose that must meet the expectations of the end user [3]. Under field conditions, poor seed quality can delay the onset of germination, adversely affect seedling vigor, and reduce the final crop stand. Establishment rate affects crop density and competitiveness of the crop stand [4], tillering or branching [5], and yield [6] and thus is of great importance. Vigorous plants have high ability to capture resources, can better tolerate pests and plant pathogens, compete with weeds, and are expected to be more tolerant to applied herbicides.

Priming is a simple procedure that partially hydrates seed in a controlled environment, followed by seed drying, so that germination processes begin, without radicle emergence [7]. This approach is useful for several crops where germination and emergence can be constrained by unfavorable soil conditions. Priming promotes germination causing a wide range of biochemical changes in the seed, the products of which normally persist after desiccation and are available once seeds absorb moisture after sowing. In addition, priming improves seed vigor, so that vigorous seeds result in early and uniform emergence as well as good stand establishment [8]. This pre-sowing technique can improve radicle emergence, germination rate, seedling vigor, and establishment as well as yield by making changes in metabolic activities in the seeds of several crops [9–13]. Different priming techniques have been examined to promote seed germination and seedling growth [14,15]. Priming promotes germination rate and uniformity due to some kind of metabolic repair of seeds during imbibition [16], build-up of germination-enhancing metabolites [17], osmotic adjustment [18], and a simple reduction in imbibition lag time [19]. The beneficial effects of priming have been observed in several field crops, such as wheat [20], maize [21], sugar beet [22], soybean [23], and sunflower [24].

Faba bean (*Vicia faba* L.) is an important legume worldwide [25]. Under optimum growing conditions, germination of faba bean seeds takes about 10 to 14 days, while it may take longer in dry or very cold conditions. Sowing faba bean seeds early in the spring starting from the end of March until the end of April is often preferred in areas with cold winters to avoid the insufficient moisture necessary for seed germination in the soil. To sow faba bean seeds, the soil temperature must be at least 7 °C for satisfactory germination and high seedling vigor [26]. Lower temperatures may result in poor germination rates. Fields may be planted as soon as they can be worked on, but in rainfed areas, seed germination can be constrained by adverse conditions in the field. Therefore, priming could be useful in promoting crop stand under a different range of environmental conditions. Slow crop emergence and seedling growth increase the risk of pests and delay crop performance, which adversely affects seed yield. Previous research showed that seed priming with various substances improved faba bean performance under stress conditions [27–29]. However, the effect of hydro-priming on faba bean germination and emergence has not been examined, especially under field conditions. Additionally, most priming studies are confined to laboratory trials, while the assessment of seed priming effects under field conditions with variable weather conditions is scarce. Moreover, reducing the difference between percentages of germination under laboratory conditions and field emergence has become one of the important goals in addition to increasing crop yield.

The aim of this work was to study the effect of hydro-priming on faba bean germination and field performance in spring sowing and to identify the optimum period of seed hydro-priming. For this purpose, laboratory and field trials for two years were conducted.

2. Materials and Methods

2.1. Laboratory Trials

Laboratory experiments were carried out in the facilities of the Department of Agricultural Development, Democritus University of Thrace in Orestiada, Greece in 2013. Seeds of faba bean var. minor (cv. Chiaro di Torre Lama) were used. Chiaro di Torre Lama is an Italian, small grain faba bean cultivar, mainly used for animal feeding. The studied treatments included hydro-priming periods of faba bean seeds for 0, 8, 16, 24, 36, and 48 h. Because standard values of hydro-priming periods

were not available for faba bean, the studied periods were selected after preliminary trials. The seeds were immersed in distilled water according to the pre-determined durations. After hydro-priming, seeds were treated with sodium hypochlorite 0.05% for 5 min and placed in open air to dry to moisture content <10% of the dry weight of the seeds, i.e., equilibration conditions for safe storage [30]. Fifteen seeds were placed on paper media in plastic plates for each replication. The number of seeds per replication was deemed adequate after preliminary trials. All seeds of all treatments were placed for germination simultaneously at temperature 22–24 °C and 60% relative humidity. Seeds which did not receive any treatment, other than disinfection, were used as control. The germinated seeds were counted from day 1 to day 14. The number of normal seedlings was defined in accordance with ISTA criteria [31]. Each treatment was replicated four times.

The following germination parameters were determined:

(i) Final germination (FG) at the end of the experiment was calculated following Equation (1):

$$FG = \frac{N_g}{N_t} \times 100 \tag{1}$$

where N_g is the number of germinated seeds and N_t is the total number of seeds.

(ii) Germination speed (GS) (also known as germination rate or rate of Maguire) was calculated following Equation (2):

$$GS = \frac{\sum nt}{\sum n} \tag{2}$$

where n = number of normal germinated seeds at time t.

(iii) Timson's germination index (TGI) was calculated following Equation (3):

$$TGI = \sum G/t \tag{3}$$

where G = percentage of germinated seeds at two-day intervals and t = total germination period.

(iv) Mean germination time (MGT) was calculated following Equation (4):

$$MGT = \frac{\sum(n_1T_1 + n_2T_2 + \ldots + n_kT_k)}{\sum(n_1 + n_2 + \ldots + n_k)} \tag{4}$$

where n = number of newly germinated seeds and T = time from the beginning of the experiment.

(v) Mean daily germination (MDG) was calculated following Equation (5):

$$MDG = \frac{FG}{T} \tag{5}$$

where FG = final germination and T = number of days after the start of the test.

(vi) Synchronization index (SI), i.e., the degree of homogeneity of germination over time, was calculated following Equation (6):

$$SI = -\sum f_i \log_2 f_i \tag{6}$$

where f_i = relative frequency of germination.

Low values of synchronization index (SI) indicate more synchronized germination [32].

(vii) Seedling vigor index (SVI) was estimated as per Equation (7):

$$SVI = [\text{Seedling length}] \times [\text{Final germination}] \tag{7}$$

Vigor testing does not only measure the percentage of viable seed in a sample, but also reflects the ability of those seeds to produce normal seedlings under less than optimum growing conditions similar to those which may occur in the field. High values of seedling vigor index (SVI) indicate a vigorous seed lot [33].

2.2. Field Trials

Field experiments were conducted in 2013 and 2016 at the Farm of Democritus University of Thrace in Orestiada, Greece (41°30′07.0″ N latitude, 26°32′24.8″ E, 22 m above sea level). The experiments were established on a silty clay loam soil (8.0% sand, 52.4% silt, and 39.6% clay) with pH (1:1 H_2O) 6.7 and organic matter content 1.01%, and were situated at nearby areas of the same field. The previous crop in the field before the initiation of the experiments was oilseed rape (*Brassica napus* L.) in the first year and winter wheat (*Triticum aestivum* L.) in the second year. Seedbed preparation consisted of conventional tillage in the fall (moldboard plow and disc harrow) followed by rotary plow before sowing.

Faba bean (cv. Chiaro di Torre Lama) was sown by hand on 15 March 2013 and 8 March 2016 at a sowing depth of 3–4 cm. A quantity of 50 kg P_2O_5 ha^{-1} as superphosphate was broadcast applied and immediately incorporated into the soil just before sowing as a usual fertilization practice for legume cultivation in the area. Potassium fertilization was not deemed necessary as per soil analysis. The experiments were performed under rainfed conditions. Manual weed control was applied as required during the vegetative stage. The basic weather data for each growing season are shown in Table 1.

Table 1. Basic weather conditions during the entire growing season and selected growth periods in each year of the experimentation.

Growth Period	Variable	2013	2016
Entire growing season	Mean temperature (°C)	18.1	16.6
	Total rainfall (mm)	259.5	241.7
Two weeks after sowing	Mean temperature (°C)	8.5	8.9
	Total rainfall (mm)	64.3	7.2
Two weeks before anthesis	Mean temperature (°C)	22.2	22.4
	Total rainfall (mm)	3.4	14.6
Two weeks after anthesis	Maximum temperature (°C)	27.7	22.5
	Total rainfall (mm)	4.2	24.6

The experiments were set in a randomized complete block design with four replications. Plots were 4 m long and consisted of 10 rows with a distance of 25 cm, while the plant to plant distance in the row was 5–6 cm. Blocks were separated by a 2-m alley. The studied treatments included hydro-priming periods of faba bean seeds for 0, 8, 16, and 24 h, conducted in the same way as in the laboratory trials. The hydro-priming periods for the field experiments were based on findings of the laboratory trials. Percentages of seedling emergence were assessed at 14, 28, and 35 days after sowing, targeting vegetative growth of faba bean (i.e., leaf development, formation of side shoots, and stem elongation), which is critical for establishing adequate crop stand. At anthesis, plants from 1 m of row were cut at ground level and weighed to determine fresh weight. At maturity, all pods from plants in two central rows from each plot were hand-harvested and the seeds were weighed to determine seed yield (moisture content 12%).

2.3. Data Analysis

Data from laboratory trials were subjected to one-way analysis of variance (ANOVA) with four replications. Differences between means were tested with Tukey's honestly significant difference (HSD) test at $p < 0.05$. In addition, simple regression analysis was conducted to explore significant associations between germination parameters and priming durations. Data from field trials were analyzed using two-way ANOVA with four priming durations and two years. Before the ANOVA, Bartlett's test was used to check the homogeneity of variances of the data set to ensure that the assumptions of homogeneity of variances were met. Due to significant interaction between treatments and years for some variables, means of all variables are presented separately for each year. Means are compared at $p < 0.05$ using Fisher's protected least significant difference (LSD) test.

3. Results

3.1. Laboratory Trials

Averaged over treatments, hydro-priming did not affect significantly final seed germination percentage, with germination percentages reaching 91.1% and 92.4% in control and primed seeds, respectively (Table 2). On the contrary, hydro-priming improved significantly germination speed and Timson's index. On average, germination speed in primed seeds was 16.2% higher than in non-primed seeds (2.37 vs. 2.04, respectively). In addition, hydro-priming lowered germination time and improved germination synchrony (lower value of synchronization index), while mean daily germination did not differ significantly between non-primed and primed seeds. Germination in primed seeds was 20.7% more synchronized compared with that in non-primed seeds. Moreover, primed seeds produced more vigorous seedlings than non-primed seeds, in terms of seedling vigor index.

Table 2. Germination parameters of faba bean seeds in different priming duration treatments.

Duration	FG (%)	GS (Seed Day^{-1})	TGI (Seed Day^{-1})	MGT (Day)	MDG (Seed Day^{-1})	SI (Unitless)
0 h	91.1 ab	2.04 c	13.59 d	7.03 a	6.51 ab	2.36 a
8 h	95.6 a	2.56 a	17.08 ab	5.81 d	6.83 a	1.99 ab
16 h	97.8 a	2.58 a	17.17 ab	5.96 d	6.98 a	1.96 b
24 h	95.6 a	2.37 ab	15.83 bc	6.28 bcd	6.83 a	1.87 b
36 h	88.9 ab	2.22 bc	14.79 c	6.27 bcd	6.35 ab	1.79 b
48 h	84.4 b	2.10 bc	14.01 c	6.32 bcd	6.03 b	1.76 b

Different letters within each variable indicate significant differences according to Tukey's HSD test at $p < 0.05$ (FG: final germination; GS: germination speed; TGI: Timson's germination index; MGT: mean germination time; MDG: mean daily germination; SI: synchronization index).

Germination parameters of faba bean seeds were significantly affected by priming duration (Table 2). Final germination showed a curvilinear response to priming duration (Figure 1a) with hydro-priming for 16 h providing the highest germination percentage. Hydro-priming for 8 and 16 h provided the highest values of germination speed and Timson's germination index, while showed the lowest values of mean germination time. However, the values of these parameters (germination speed and Timson's index) declined with increasing priming durations above 24 h (i.e., 36 h and 48 h). Mean daily germination did not differ considerably among treatments, except from priming for 48 h, which showed significantly lower values than priming for 8, 16, and 24 h. Synchronization index was reduced linearly with increasing priming duration (Figure 1b) indicating that longer hydro-priming maximized germination synchrony (lowest values of synchronization index).

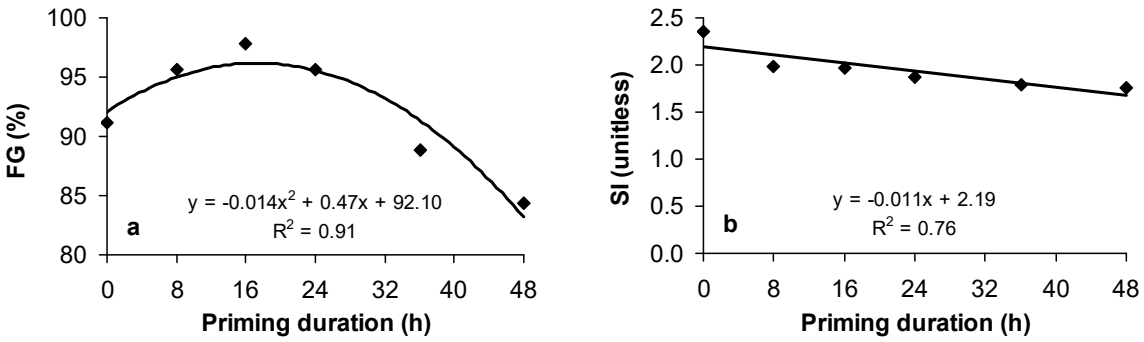

Figure 1. Regression equations (**a**) between priming duration and final germination (FG) and (**b**) between priming duration and synchronization index (SI).

3.2. Field Trials

Overall, mean temperature and total rainfall were quite similar in the two growing seasons (Table 1). However, total rainfall two weeks after sowing was higher in 2013 than in 2016 (64.3 mm

vs. 7.2 mm). On the other hand, total rainfall two weeks before and two weeks after anthesis was lower in 2013 (3.4 mm and 4.2 mm, respectively) than in 2016 (14.6 mm and 24.6 mm, respectively). Moreover, higher temperatures prevailed two weeks after anthesis in 2013 (27.7 °C) than in 2016 (22.5 °C). In field trials, hydro-priming periods of 0, 8, 16, and 24 h were compared. Seed hydro-priming did not affect plant number 14 days after sowing in the first year, while it significantly improved seedling emergence in the second year, on average by 34.4%, compared with the non-primed control (Figure 2). No significant effect of seed hydro-priming was noted in the number of plants at 28 and 35 days after sowing.

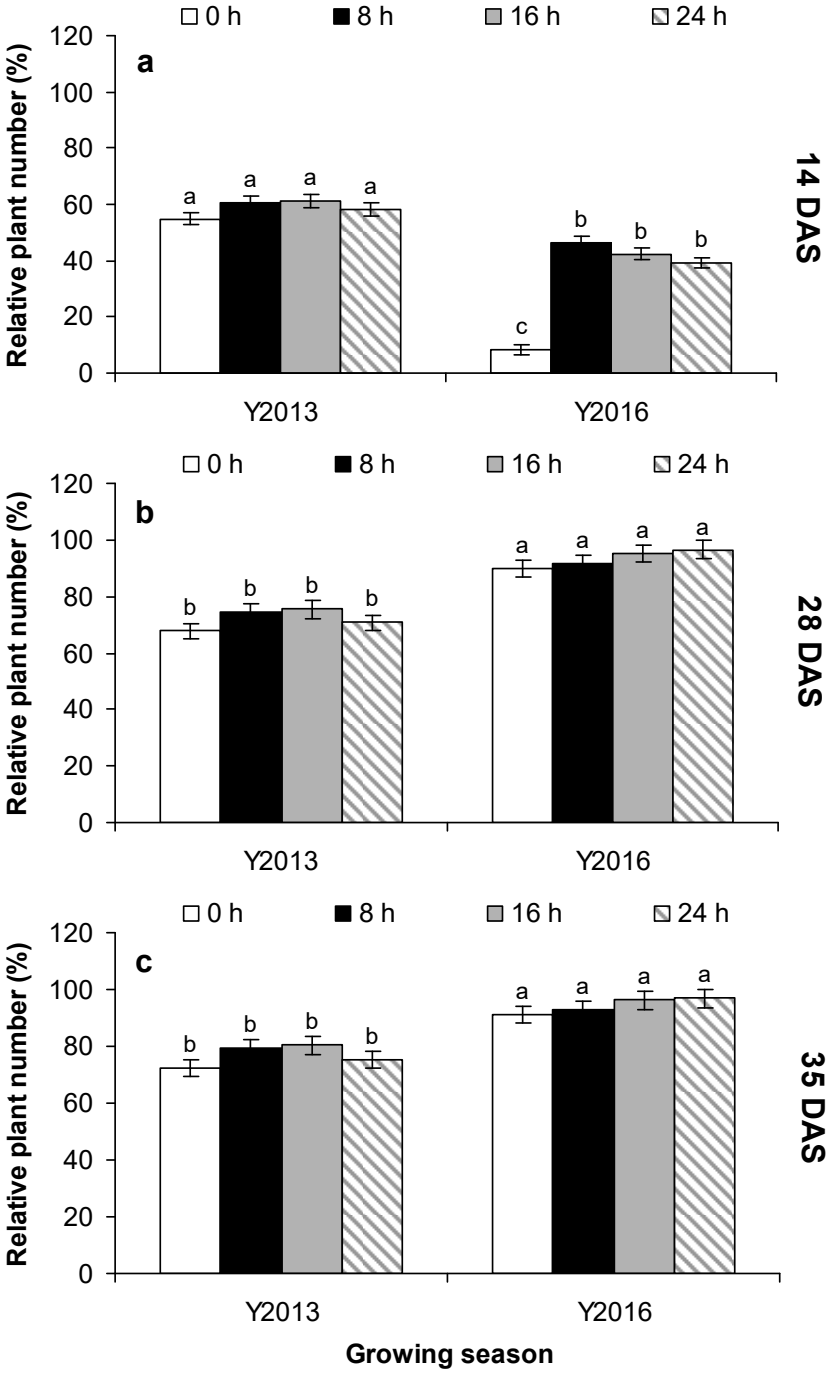

Figure 2. Plant number per m of row (%) at 14 (**a**), 28 (**b**), and 35 (**c**) days after sowing (DAS) (different letters show statistically significant differences according to least significant difference (LSD) test at $p < 0.05$; vertical lines denote standard errors of means).

Fresh weight at anthesis was the highest in plants from primed seeds for 8 h in the first year and a similar trend was obtained in the second year (Figure 3). Compared with the non-primed control, seed priming for 8 h provided higher grain seed yield by 12.0% in the first year and by 5.9% in the second year.

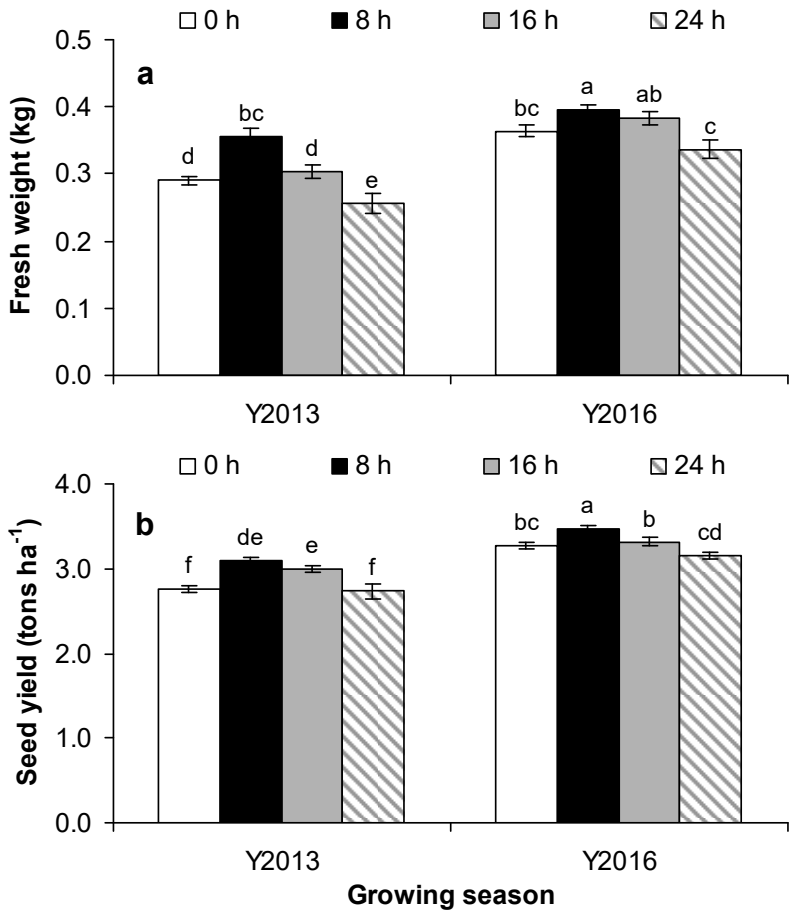

Figure 3. Fresh weight per m of row (kg) (**a**) and seed yield (tons ha^{-1}) (**b**) (different letters show statistically significant differences according to LSD test at $p < 0.05$, vertical lines denote standard errors of means).

4. Discussion

This study provides useful information concerning faba bean seed germination and field performance under hydro-priming. Hydro-priming of seeds under laboratory conditions generally improved germination parameters of faba bean, in agreement with previous studies on other crop species. However, the effectiveness of seed hydro-priming in the field differed depending on growth conditions. Hydro-priming promoted faba bean germination and seedling emergence in the field, but the magnitude of the response was associated with the environment and was more evident under limited soil moisture after sowing, whereas the beneficial effect of seed hydro-priming was masked when rainfall followed sowing. Similarly, the beneficial effect of priming on seed yield was more pronounced with limited soil moisture after anthesis. Data on improving germination of faba bean with hydro-priming are scarce in the literature. Therefore, information from this work could be of practical importance for enhancing faba bean establishment and possibly yield of this crop. Several studies have confirmed that hydro-priming advanced germination of different crop species resulting in higher values of germination parameters and seedling growth, such as germination index [34], germination time [35], seedling dry weight [36], and seedling vigor index [37]. Moreover, hydro-priming can promote germination, particularly under disadvantageous growth conditions, increasing germination

rate in many crop species [38–42]. In the present study, primed seeds produced more vigorous seedlings than non-primed seeds, in terms of seedling vigor index. Seedling vigor determines the potential for rapid germination, uniform emergence, and development of normal seedlings [43]. Seedling early vigor describes the establishment of strong seedlings in any environmental condition [44] and it is usually associated with seed vigor. Although genetic factors, such as hard-seediness and seed chemical composition, influence the expression of seed vigor, the conditions of seed development, maturation, storage, and aging also influence seed vigor. Additionally, adverse pre-harvest environment (e.g., high humidity and warm temperature) or seed mechanical damage can also cause loss in seed viability and vigor. In any case, hydro priming can help in producing high vigor seedlings.

Under field conditions, hydro-priming treatments did not always translate into a significant advantage in terms of seedling emergence. These findings could be associated with different soil moisture between growing seasons due to different levels of rainfall (64.3 mm in 2013 vs. 7.2 mm in 2016) after sowing (Table 1). In fact, seed hydro-priming accelerated faba bean germination and seedling emergence in the field, particularly under limited soil moisture after sowing (2016), whereas the beneficial effect of hydro-priming was masked when rainfall followed sowing (2013). In rainfed areas, seed germination and plant growth are often constrained by adverse conditions in the field. Specifically, soil seedbed is a complex environment that poses multiple stresses to seeds and seedlings [1]. Therefore, seedlings are vulnerable to this complex environment, particularly to the physical stresses induced by soil during germination and seedling expansion (i.e., mechanical impedance, available water, temperature, and oxygen). These attributes often interact with each other and vary considerably with available water content. Such interactions can reduce the time between sowing and seedling emergence when the seedbed deteriorates. In this regard, seed priming can promote crop establishment across a wide range of seedbed conditions. For example, inhibition of germination due to deficit of water was alleviated by using primed lentil seeds [42]. In addition, early seedling emergence from primed seeds allowed efficient and longer use of light and soil resources by plants during growth and development [45]. By this point of view, early emergence and high crop density resulting from vigorous seeds can increase competition of individual plants for water under limited irrigation conditions. Therefore, priming may be helpful in reducing the risk of poor stand establishment under conditions of water deficit and permit more uniform growth under conditions of irregular rainfall.

Regarding priming duration, hydro-priming for 8 h translated into a significant advantage in terms of plant fresh weight in both years. It has been reported that plants from primed seeds emerge faster, produce more vigorous seedlings, and yield better than plants from non-primed seeds [18]. In addition, hydro-priming promoted dry matter accumulation in upland rice, accelerating physiological activities up to maturity [46]. Similar findings have been reported for wheat [47], maize [48], and basil [49]. The beneficial effect of hydro-priming on plant growth could be associated with a better developed root system of the plants from primed seeds, which probably contributed to increased nutrient uptake and, hence, dry matter accumulation and yield compared with plants from non-primed seeds. Moreover, faster seedling emergence from primed seeds would result in better resource acquisition and utilization and more time for optimal growth and seed filling, resulting in higher yield [50]. Apart from the novelty regarding species, the present study supplies additional information on the effects of priming duration with respect to growth conditions. Despite the fact that the hydro-priming procedure is a well-known technique of seed invigoration, previous studies stress that more research is needed to determine the importance of hydro-priming in different plant species [51,52]. Considering this practical necessity, the present study provides new information in seed hydro-priming topic, apart from practical information for enhancing seed germination and field performance of faba bean.

5. Conclusions

This study provides practical information on the role of hydro-priming in faba bean germination and field performance in spring sowing as well as on the optimum period of seed hydro-priming.

In laboratory trials, the effects of hydro-priming for 0, 8, 16, 24, 36, and 48 h on final germination percentage, germination speed, Timson's germination index, mean germination time, mean daily germination, synchronization index, and seedling vigor index were studied. Averaged over priming duration treatments, hydro-priming improved germination speed by 16.2%, germination synchrony by 20.7%, and seedling vigor index by 13.4%, but did not affect significantly final germination percentage and mean daily germination compared with non-primed seeds. In field trials, seed priming for 8 h resulted in higher fresh weight at anthesis by 22.3% and 8.6% in the first and the second year than the non-primed control. Similarly, seed priming for 8 h provided higher seed yield by 12.0% in the first year and by 5.9% in the second year compared with non-primed control. Overall, seed hydro-priming accelerated faba bean germination and seedling emergence in the field, but the magnitude of the response was associated with the environment and was more evident under limited soil moisture after sowing, whereas the beneficial effect of priming was masked when rainfall followed sowing. Similarly, the beneficial effect of priming on seed yield was more pronounced with limited soil moisture after anthesis. As hydro-priming is a simple technique, evaluating the efficacy of this priming method in different environmental conditions is essential to optimize our chosen priming technique. While seed priming is indeed a promising technology to mitigate the adverse effects of climate change on crop production, future work targeting other priming agents and the usability of various priming options for varied agro-ecosystems and different crops is needed.

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