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Chitosan Seed Priming Improves the Yield and the Root System of Faba Bean (*Vicia faba* L.)

Short Title: Chitosan Impact on Yield of Faba Bean

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Abstract: Faba bean is an important legume crop with food and feed applications. Since 2015, the culture of faba bean has decreased in France. Poor yield caused by climate change is proposed as one of the main reasons. Chitosan is a promising biopolymer with empirically proved impact on germination and disease tolerance of faba bean. However, the impact of chitosan seed priming on germination and yield of this plant is not experimentally studied. We followed chitosan primed faba bean seeds, from sowing until maturation, using germination, fruit and grain parameters. We observed that chitosan seed priming ameliorated several yield related traits. Germination rate, length of radicle and seedling, radicle dry matter, number of pods/plants, number of grains/pods were increased in chitosan primed faba beans. Total grain mass/plant increased up to 47% in treated plants. The probable underlying mechanisms, namely, root enhancement nitrogen metabolism, and elicitation of immune system are discussed. We conclude that, chitosan seed priming with compatible commercial preparations could be a potential solution to recompensate the loss of yield under variable climate conditions.

Key words: Faba bean, chitosan, seed priming, germination, yield.

1. Introduction

Faba bean (*Vicia faba* L.) is a high-quality crop, supplying well-balanced protein and carbohydrate together with numerous antioxidants and essential vitamins [1]. It is widely used as human food and animal feed, especially in Europe, Northern Africa, and China [1]. Equally important is the contribution of faba bean in maintaining the sustainability of agricultural systems, as it is highly efficient in the symbiotic fixation of atmospheric nitrogen [2]. Despite all these qualities cultivation of faba bean is still limited due to highly instable yield [3-5]. In addition, yield performance is often limited by sensitivity of the crop to environmental conditions (especially cold and drought) and the high susceptibility to diseases and pests [3]. Therefore, climate change derived increase in frequency and magnitude of heat waves and laten frosts [6] could be considered as important risk factors which threaten the yield performance of faba bean for European farmers. For example, experimental evidence relates considerable yield losses to abiotic stresses during crop floral development and anthesis [7]. Hence, finding ecological and sustainable solutions to increase faba bean tolerance to climate uncertainties would be of great interest to protect and maintain the yield of this agroecologically important plant.

Chitosan, deacetylated form of chitin, is a natural biopolymer which can be obtained from crustaceans' waste, different fungal sources and recently, from farmed insects. Chitosan is not only a biocompatible, and biodegradable polymer but, it is almost always produced by recycling of biowastes [8]. There is a growing body of evidence showing stimulating effects of chitosan on germination, growth, flowering and abiotic stress tolerance in a large variety of plant species [9-13]. Nevertheless, the application method

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and the impact of chitosan biostimulation on crops yield are not well studied.

Seed priming is a pre-sowing treatment which leads to a physiological state that enables seed to germinate more efficiently (see Ref. [14] for a deep review). Many seed priming techniques are based on seed imbibition [14]. The technique is known to improve germination and seedling growth and to increase seeds and seedlings tolerant to stressful conditions [15]. Previous studies suggest that priming Fabaceae (soybean) seeds with chitosan enhance germination rate and seedling tolerance to salinity stress [16]. There is little experimental evidence on the impact of chitosan seed treatment on the germination of faba bean. Moreover, whether priming faba bean seeds with chitosan have any outcome in terms of fructification and yield is not experimentally studied.

Here, we report the results of our experimental study on the impact of chitosan seed treatment on faba bean. Primed faba been seeds were followed from germination to fructification and we determined the impacts of chitosan on (1) germination rate; (2) seedling vigour; (3) podding success; and (4) yield.

2. Material and Methods

2.1 Chemicals and Preparations

We used a ready-to-use commercial chitosan preparation, FabaAxelerator® (specially designed for faba bean seed treatment). Chitosan was purchased from ChitosanLab (Brech 56400, France).

2.2 Plant Material and Experimental Design

Seeds of *Vicia faba* were provided by SARL Bresson Eric (Mantoche 70100, France).

2.3 Germination Test

In total 100 randomly selected seeds were divided into two experimental groups. First group (50 seeds) was soaked in 1 μL FabaAxelerator®/g of seed and the second group (control) was treated with the same amount of distilled water. After manual shaking for 60 s, seeds were air-dried in ambient temperature for 24 h. They were then wrapped in humid paper towels and were incubated at 25 ± 1 °C. On day 6 of incubation the seedlings were separated and were photographed on similar black backgrounds. The process was repeated 3 times. Measurements were done on photos, using ImageJ (ImageJ bundled with 64-bit Java 1.8.0_172). Following measurements were done: (i) the germination percentage, (ii) shoot length and (iii) radicle length. We calculated SVI (seedlings vigour index) as suggested by Abdul-Baki and Anderson [17].

SVI (seedling vigour index) = (mean root length in mm + mean shoot length in mm) × percentage of seed germination.

Germinated seeds were then dried at 70 °C for 72 h and dry mass of seedlings and the radicle dry mass were measured to the nearest 0.001 g.

2.4 Plantation Test

The pot experiment was carried out at the pot-yard of Amiroy, Arc-lès-Gray 70100, France, during an 8-month period between October 2020 and June 2021. The soil of the experiment was sandy loam with pH 6.8 and was collected from a nearby organic farm. No fertilisation and irrigation were done before or during the experiment.

Hundred seeds were treated as previously described. Twenty-four hours after treatment in October 2020 (28/10/2020), they were planted on 1-m rows. Each row contained 18 seeds. In June 2021 (22/06/2021) we harvested all visible pods. Number of plants/rows, number of pods/plants, weight of pods/plant, number of grains /pods, mean weight of grains/treatment, total weight of grains per plant and mean of 1,000 kernel weight were measured.

2.5 Statistical Analysis

All data were analysed in R 4.1.0 for windows. All means are expressed as mean ± SEM (standard error of the mean). Significant differences among experimental groups were determined by Welch Two Sample *t*-test analysis using "R" [18]. For count data, we performed Wilcoxon signed-rank test.

3. Results

3.1 Germination Test

After 6 days of incubation, there was no significant difference in the rate of germination between experimental groups (92% in treated vs. 82% in control; Figs. 1a and 1b). Nonetheless, radicles were 26% longer in chitosan treated seeds (56.63 ± 2.92 mm vs. 41.7 ± 2.55 mm in control; t-test, $p < 0.01$, df = 70) (Fig. 1c). Radicles in chitosan treated seedlings had 41% more dry mass than controls (0.017 ± 0.002 g in chitosan treated vs. 0.011 ± 0.001 g in control; t-test, $p < 0.05$, df = 42) (Fig. 1d). Chitosan priming had also increased the size of shoot up to 20% (39.72 ± 2.31 mm in chitosan treated vs. 31.63 ± 1.29 in controls; t-test, $p < 0.01$, df = 43) (Fig. 1e). Consequently, the SVI was respectively 1.13 and 0.83 in chitosan primed and control seeds. Chitosan treatment was also associated with 20% increase in seedling dry weight (0.388 ± 0.011 g in chitosan treated vs. 0.351 ± 0.012 g in control; t-test, $p < 0.05$, df = 77) (Fig. 1f).

3.2 Plantation Test

To study impact of chitosan on the yield of faba bean, we followed them during an 8-month period under identical conditions and identical number of starter seeds in each group. In June 2021 the rate of success (seed to mature plant) was 50% and 30% (Fig. 2a), respectively in chitosan primed and control group. Visual comparisons showed chitosan primed plants had more developed roots (Figs. 2b and 2e) and were taller than controls (Figs. 2c and 2d). Chitosan treated plants tended to produce more tillers (i.e. lateral shoots) than controls (an average of 1.9 tillers/plant in treated seeds and 1.5 tillers/plant in control) (Fig. 2c).

We found no significant difference in average grain weight 0.75 ± 0.26 g/treated vs. 0.71 ± 0.23 g/control; t-test, $p < 0.32$, df = 21) (Fig. 3a). However, the 1,000 kernel weight tended to increase with chitosan priming (720.8 ± 30.3 g in treated plants vs. 616.4 ± 48.4 g in control; t-test, $p < 0.086$, df = 29).

Chitosan primed plants produced 46% more pods (16.70 ± 2.96 pods/plant vs. 9.00 ± 1.36 in control; Wilcoxon test $p < 0.05$, df = 70) (Fig. 3d). Seeds of chitosan primed plants were 51% more numerous than controls (49.17 ± 9.04 grain/plant vs. 24.18 ± 4.53 grain/plant in controls; Wilcoxon test $p < 0.05$, df = 16) (Fig. 3b). Finally, the mean weight of pods/plant (67.23 ± 12.2 g vs. 35.11 ± 6.37 g; t-test, $p < 0.05$, df = 18) and total weight of grain/plant (33.05 ± 6.00 g vs. 17.52 ± 3.2 g; t-test, $p < 0.05$, df = 18) were also 47% higher in chitosan primed plants (Figs. 3c, 3e and 3f).

4. Discussion

We followed chitosan primed faba bean seeds, from sowing to maturation. Our results suggest that chitosan seed priming increases the yield of faba bean up to 47%. It seems that chitosan seed priming can increase the yield of faba bean by enhancing multiple traits.

The effects of seed priming with chitosan could be observed from early stages of seed germination. As such, primed seeds germinated more rapidly, produced more vigorous seedlings and more dry matter during the first days of germination. High seedling vigour is considered a decisive factor for the success of most field crops, as these parameters contribute to uniform plant growth and maturity, better competition with weeds, and high productivity [19]. We also considered a more developed root system and more numerous root nodules in chitosan primed plants (Fig. 2). Similar stimulating effect of chitosan on the root development was observed in the germination test. For legume crops, root traits have been one of the most important attributes enabling the plant to mine water efficiently and to tolerate the drought stress [20]. Drought stress, is considered as one of the prominent consequences of the climate change [21] and a major constraint with a non-negligible negative impact on

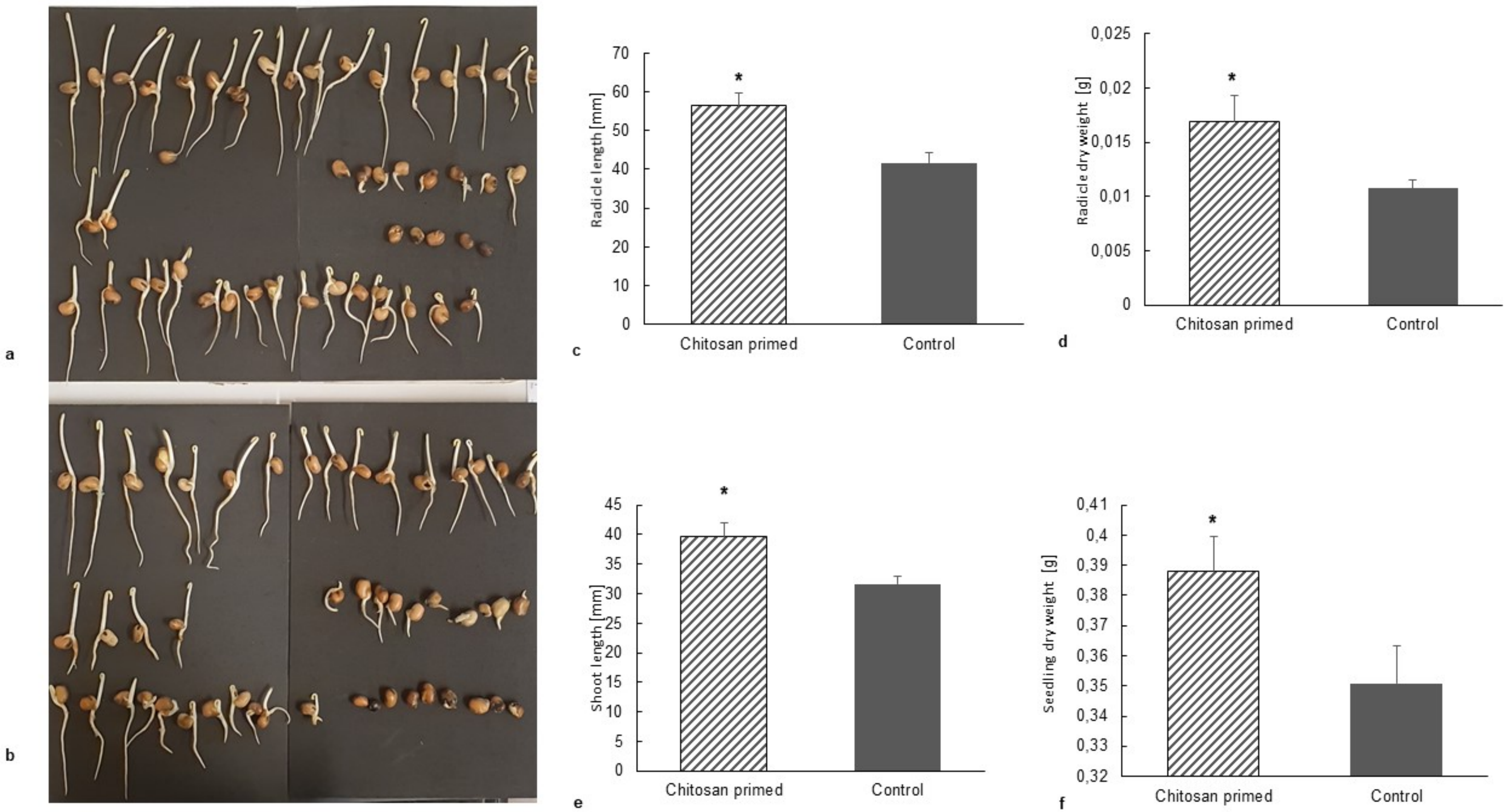

Fig. 1 Effect of priming faba bean seeds with chitosan on germination traits under laboratory conditions.
Although germination percent did not change with chitosan priming, (a) primed seeds tended to germinate more harmoniously than (b) controls. (c) The size and (d) the dry weight of radicle, and (e) the size of shoots were increased by chitosan treatment. Chitosan treatment was also associated with higher seedling dry weight (f).
* Statistically significant differences ($p < 0.05$).

Fig. 2 Impact of chitosan seed priming on faba bean cultured under natural climatic conditions (October 2020 to July 2021).

(a, c & d) Chitosan seed primed plants (right) were grown taller in a more harmonious manner than controls (left).

(b & e) Treated plants (right) have more developed roots (left).

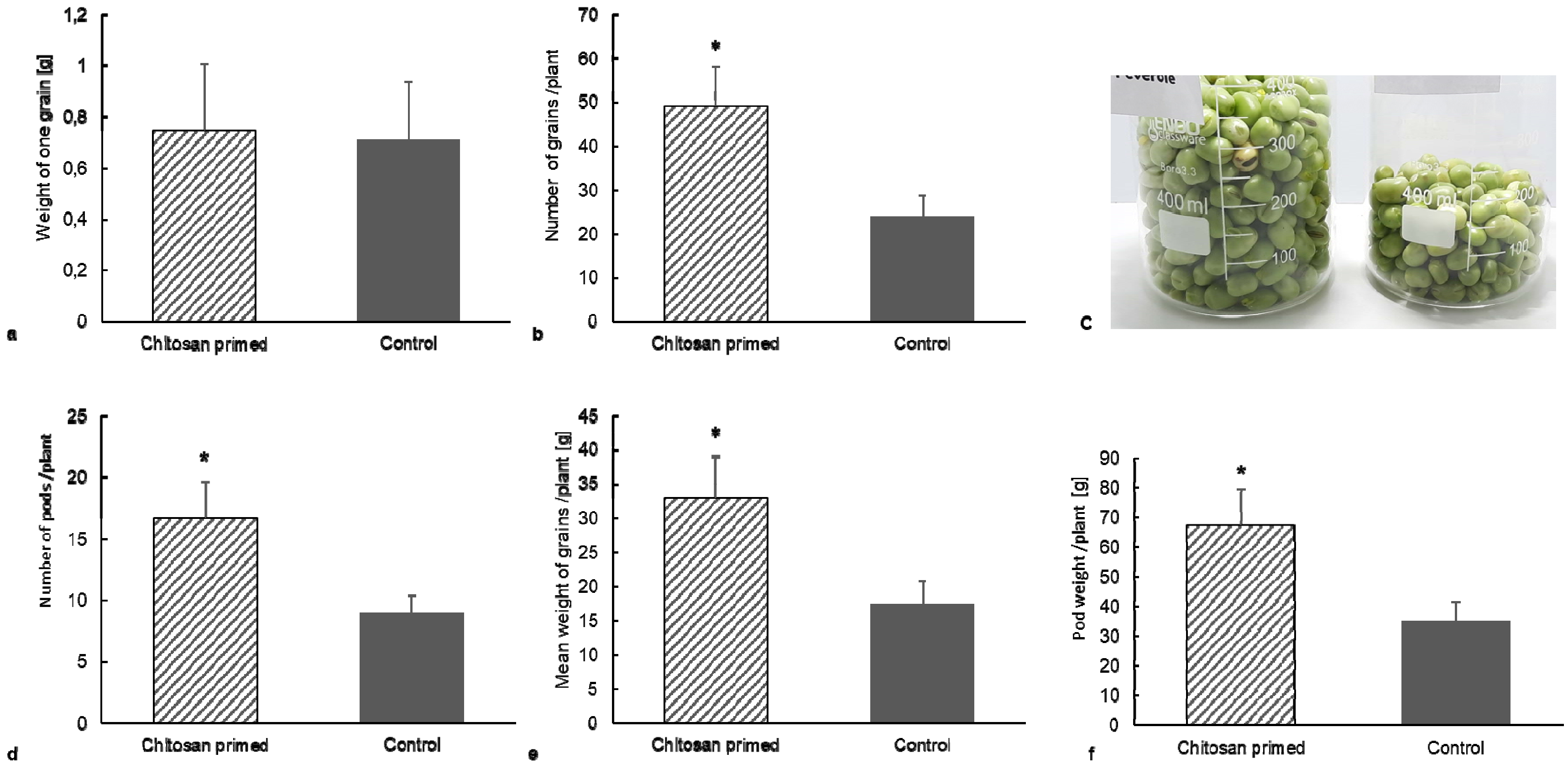

Fig. 3 Chitosan seed priming increases several grain and fruit traits of faba bean.

(a) Average grain (kernel size) does not change with chitosan treatment. (b, c & e) Chitosan primed plants produce more grain, more pods (d & f), than controls.

* Statistically significant differences ($p < 0.05$).

crops yield [22, 23]. Our plantation test corroborates this hypothesis. The chance of reaching to adult stage was increased about 20% with chitosan seed priming. Chitosan primed plant also tended to produce more tillers. In this way, chitosan priming can increase the yield on surface unite by increasing the number of productive plants. In addition, while the average grain weight and the 1,000 kernel weight did not change between controls and treated plants, we observed that chitosan priming was associated with an increase in the number of pods/plants, weight of pods/plant and a considerable increase in the yield/plant.

The mechanisms underlying germination, vigour and yield improvement are rarely studied. It has been proposed that application of chitosan increases key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and improves the transportation of nitrogen in the functional leaves, which enhances plant growth and development [24]. Together with root system improvement, more efficient absorption and transformation of nitrogen could play a key role in yield increase of faba bean. Al-Ahmadi [25] showed that chitosan in different concentration and time of exposure could affect miotic index of faba bean cells. Therefore, stimulation of cell proliferation can be speculated as one of the underlying mechanisms of more developed roots and taller plants. Other studies, showed chitosan elicits the immune responses of faba bean towards infectious diseases [26, 27] by activating guard cells and narrowing stomatal pores following perception of MAMPs (microbe-associated molecular patterns) [28]. The elicitation of immune defence against invasive microbes can also be considered as a potential mechanism which increases the chance of reaching to adult stage and increases the yield of faba bean.

5. Conclusion

We provided experimental evidence that chitosan seed priming can improve germination, growth, and the yield of faba bean. This increase in the yield was maintained in chitosan primed plants and recompensates the negative impact of the late spring frost stresses (our plots were exposed to two late spring frosts (< -6 °C)) and the drought stress (almost no rain during March and April 2021), which has been proposed as the main risk factors for faba bean culture in Europe [3].

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