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1 **Nutri-priming as an efficient means to improve the agronomic performance of**
2 **molybdenum in common bean (*Phaseolus vulgaris* L.)**
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20 Abstract

21 Microelements play important roles in improving crop productivity and quality. Two
22 traditional methods of providing micronutrients are soil and foliar application, yet the cost
23 involved and phytotoxicity risks poses a major challenge in most global agricultural areas.
24 Nutri-priming represents thus a very promising and viable alternative to ensure that the
25 plants' requirements for micronutrients are met. This paper explores the Mo-priming effects
26 on the germination, growth and yield components of common bean (*Phaseolus vulgaris* L.).
27 In order to accomplish this research objective, our study is divided in two phases. The first
28 phase seeks to determine the effects of two durations (5 and 10 hours) of Mo-priming on the
29 germination parameters. Then, a greenhouse pot experiment was conducted with primed seeds
30 showing better germination performance to assess the effects of Mo-priming on certain
31 parameters related to biological nitrogen fixation and crop yield. Five seed pretreatments were
32 studied, namely, 0, 0.025, 0.05, 0.1 and 0.2% of ammonium heptamolybdate
33 $((\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O})$. Unprimed seeds treated with or without the recommended dose
34 through a soil application were used as controls. Hydropriming is sufficient to improve seed
35 germination performance. However, 0.1% Mo-priming for 10 hours improved the net CO_2
36 assimilation rate, chlorophyll content and biological nitrogen fixation. Six times more
37 nitrogen was fixed (65.8 kg ha^{-1}) in these plants than in the controls. Additionally, grain yield
38 increased by 115% compared to the negative control. Thus, compared to soil intake, nutri-
39 priming was a very efficient experimental method.

40 **Keywords:** *Phaseolus vulgaris*; Bean; Nutri-priming; Molybdenum; Biological nitrogen
41 fixation.

42 **1. Introduction**

43 Although agriculture is one of the main contributors to the economy of Morocco, fertilizer use
44 is low and only meets approximately 50% of the real needs (El Gharous and Boulal, 2016),
45 with nearly 618 500 tons year⁻¹ of nutrients, representing an average intake of 71.1 kg ha⁻¹
46 (World Bank, 2018). While many large, modern farms have largely integrated fertilizer into
47 their production, small farmers have not implemented this practice, but present an enormous
48 growth potential. In addition, the majority of Moroccan farmers use only the NPK trilogy.
49 This practice might be due to the high cost of microelements and farmer ignorance concerning
50 their existence and/or importance. Microelements, which are required in very small quantities,
51 play important roles in improving crop productivity and quality (Marschner, 2011). New
52 methods for microelement application must be implemented to 1) take advantage of the
53 improved productivity facilitated by the use of microelements, without negatively impacting
54 the quality of the environment, and 2) encourage farmers to use these elements. Two
55 traditional methods of providing micronutrients are soil and foliar application. However, in
56 the case of soil application, the availability of microelements may be reduced by the
57 complexity of controlling soil parameters like alkaline soil pH, calcareousness, low organic
58 matter and rapid adsorption on soil particles (Alloway, 2008). Moreover, the plant uptake was
59 restricted at low moisture (Yadegari, 2016). These constraints disappear or are limited when
60 the fertilizers are foliar-sprayed. However, multiple applications are often necessary
61 (increasing application costs) along with the risk of phytotoxicity (Marschner, 2011). As a
62 low-risk application, nutri-priming represents a good alternative to ensure that the plants'
63 requirements for micronutrients are met.

64 Priming is defined as a simple technique that involves soaking seeds in water or an
65 osmotic solution followed by redrying to its original weight (Lutts et al., 2016). Used
66 empirically to overcome dormancy, it includes the absorption of water at a sufficient level to
67 accomplish preparatory metabolic events preceding the radicle emergence. In the case of
68 nutri-priming, seeds are primed in nutrient solutions (Farooq et al., 2012), which combines
69 the biochemical effect of priming with water and the nutritional effect of the applied element.
70 It is an easy, inexpensive, cost-effective and environmentally friendly method (Ghassemi-
71 Golezani et al., 2008; Rehman et al., 2018) that may offer an attractive option to farmers.
72 Several microelements show good nutri-priming ability in various crops, including boron in
73 Stevia (Shahverdi et al., 2017), manganese and zinc in wheat (Rehman et al., 2018) and
74 soybean and zinc in maize (Imran et al., 2017b).

75 In addition to the problems associated with the micronutrient assimilation efficiency,
76 one of the major problems facing farmers in developing countries is the heterogeneity of
77 seedling emergence under inappropriate conditions for germination. This issue becomes more
78 noticeable in arid and semi-arid regions, and particularly in recent years with the impacts of
79 climate change (van der Esch et al., 2017, Porkka et al., 2017). In fact, seeds are sometimes
80 sown in seed beds with an unfavorable humidity because of insufficient rain, causing a
81 heterogeneous emergence. Nevertheless, accelerated and homogenous germination is required
82 for better crop establishment, efficient use of seed reserves, and ultimately, increased final
83 yields (Finch-Savage and Bassel, 2016). Priming is an effective method for improving
84 germination and its speed, as well as rapid emergence under favorable and stress conditions,
85 which, due to the induction of various biochemical changes, is essential for initiating
86 germination processes in seeds during the pretreatment (Farooq et al., 2012).

87 The nutrient content of seeds plays an important role in the germination process and
88 growth. During early stages of growth, plants use mineral nutrients derived partly from seed
89 reserves and partly from the soil (Rengel and Graham, 1995). During the germination process,
90 seed reserve are degraded by enzymes and transported to the growing embryo (Fincher,
91 1989). In this stage, external sources have less of an effect on plant development. Certainly,
92 sufficient mineral nutrient reserves in the seed are needed to ensure proper seedling growth
93 until the root system is functional. However, these nutrients can promote the growth of plants
94 in later stages (Imran et al., 2017a) and thus contribute to improving growth parameters, yield
95 and even the seed quality (Imran et al., 2015).

96 Molybdenum (Mo) plays an important role in nitrogen metabolism in plants,
97 particularly in legumes. It functions as a cofactor for nitrogenase enzymes, catalyzing the
98 redox reaction to convert atmospheric nitrogen to ammonium (NH_4^+) (Kaiser, 2005). It is also
99 part of the prosthetic group of nitrate reductase, the key enzyme required for nitrogen
100 assimilation in plants. Therefore, plant nitrogen metabolism is closely related to the
101 assimilable fraction of Mo in soil, particularly for legumes (Mendel and Hänsch, 2002).

102 This study explores the Mo-priming effects on the germination, growth and yield
103 components of common bean (*Phaseolus vulgaris* L.). Particular attention will be paid to
104 biological nitrogen fixation (BNF), because common beans have lower symbiotic fixation
105 efficiency (Toshiyuki and Shigekata, 1991; Peoples et al., 2009). In order to accomplish
106 this objective, we firstly evaluate the effects of two Mo-priming durations (5 and 10 hours) on
107 germination parameters. Then, a greenhouse pot experiment was conducted with primed seeds

108 showing better germination performance to assess the effects of Mo-priming on parameters
109 related to biological nitrogen fixation and crop yield

110 **2. Materials and methods**

111 **Nutri-priming treatment and germination assay**

112 Five treatments, 0 (distilled water) and 0.025, 0.05, 0.1 and 0.2% ammonium molybdate
113 $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$, were defined to study the effect of Mo-priming on germination and
114 growth traits of common bean (*Phaseolus vulgaris*). Healthy seeds with a similar shape and
115 size were selected and divided into batches (125 ± 2 g, approximately 300 seeds for each
116 treatment). Each batch was weighed and immersed in a 1% sodium hypochlorite solution for
117 5 min to remove pathogenic microorganisms. After three surface washes with distilled water,
118 seeds were primed at room temperature ($25 \pm 1^\circ\text{C}$) in an aerated treatment solution using an air
119 pump at a ratio of 1:5 (w:v). Each treatment was administered for two priming durations: 5
120 and 10 hours. At the end of priming process, the seeds were weighed (to determine the
121 amount of solution absorbed) and then dried to the initial moisture content at $25 \pm 3^\circ\text{C}$. The
122 hydration capacity (HC) and hydration index (HI) were then calculated using following
123 formulas (1) and (2), respectively:

$$124 \quad \text{HC} = \frac{\text{FW} - \text{IW}}{N} \quad (\text{g of solution seed}^{-1}) \quad (1)$$

125 where:

126 FW: final weight of primed seeds

127 IW: initial weight of seeds

128 N: number of seeds

129

$$130 \quad \text{HI} = \frac{\text{HC}}{\text{Weight of one seed}} \quad (\text{g of solution g}^{-1} \text{ of seed}) \quad (2)$$

131 Twenty treated and nontreated seeds were allowed to germinate separately in 9 cm Petri
132 dishes containing Whatman filter paper N°1 moistened with 20 ml of distilled water. Three
133 replicates were used for each treatment. Petri dishes were then incubated in the dark at a
134 constant temperature of 25°C . The germinated seeds were counted, removed and the paper
135 moistened each day for seven days. The speed of germination (SG) and final germination
136 percentage (FGP) were calculated using formulas (3) and (4) (Maguire, 1962):

$$137 \quad \text{SG} = \sum \left(\frac{N_i}{D_i} \right) \quad (3)$$

138 where:

139 Ni: number of seeds germinated on the i^{th} day

140 Di: number of days to count the n^{th}

$$141 \quad \text{FGP} = \frac{Nt * 100}{N} \quad (4)$$

142 where:

143 Nt: total number of germinated seeds in each treatment

144 N: number of seeds used in the bioassay

145 **Determination of Mo contents in primed bean seeds**

146 Mo contents were determined in treated and untreated seeds. Approximately 2 g of
 147 ground seed samples were weighed in a porcelain capsule and placed in a muffle furnace for
 148 incineration at 550°C for 6 hours. The ash was dissolved in 5 ml of HNO₃ (5 N) and
 149 subsequently evaporated to dryness on a hot plate at 40°C for at least 2 hours. Then, samples
 150 were extracted with 30 ml of a 1:5 mixture of HNO₃ (1 N) and HCl (0.1 N). After adding 5 ml
 151 of LaCl₃ (0.1 N), the final solution was adjusted to 100 ml with HNO₃ (2%). Mo
 152 concentrations were measured using inductively coupled plasma spectrometry (ICP). All
 153 analyses were conducted in triplicate. For each treatment, three replicates were performed.
 154 The seed Mo content was calculated as follows (5) (Imran et al., 2017a):

$$155 \quad \text{Mo contents } (\mu\text{g seed}^{-1}) = \text{seed dry biomass (g)} * \text{Mo concentration } (\mu\text{g g}^{-1}) \quad (5)$$

156 **Plant material, growth conditions and experimental setup**

157 The soil used in this study was a sandy soil (87.8% sand) taken from within the top 30
 158 cm of the soil layer of an uncultivated agricultural plot in the rural commune of Ait Yaazem
 159 in the province of El Hajeb, Morocco (33°44'59" N latitude, 5°34'29"W longitude) (Fig. 1).
 160 The physico-chemical characteristics of the soil are detailed in Table 1. The experiment was
 161 performed under a shelter at the National School of Agriculture of Meknes (Meknes-Fes
 162 Region, Morocco). Certified seeds of the Michelet bean cultivar were purchased from a local
 163 seed dealer and planted in pots 30 cm in height with bottom and top diameters of 20 and 16
 164 cm, respectively. The pots were filled with a 5 cm layer of gravel at the bottom to improve
 165 drainage and 6 kg of dried soil.

166 According to the results of germination assays, seeds that had been primed for 10 hours
 167 were chosen for cultivation. On March 26, 2018, four seeds were planted in each pot. The
 168 plants were allowed to grow under natural conditions and were later thinned to one healthy
 169 plant per pot. Seedlings were irrigated with tap water to 70% of the field capacity three times
 170 a week or twice a day in warm weather. The experiment was established using a randomized

171 complete block design (RCBD) with 5 blocks and 7 treatments: M0, M1, M2, M3 and M4
 172 correspond to seeds primed in 0, 0.025, 0.05, 0.1 and 0.2% ammonium molybdate,
 173 respectively. Unprimed seeds without (NC: negative control) or with (PC: positive control)
 174 the recommended dose provided by a soil application were used as controls. For PC, the
 175 recommended dose applied was 1 kg ha⁻¹ of Mo (approximately 0.25 ppm) in the form of
 176 ammonium molybdate at the V4 stage (third trifoliolate leaf) (Bhuiyan et al., 2008). A
 177 schematic illustrating the experimental setup is summarized in Figure 2. As the bean is a
 178 legume capable of fixing atmospheric nitrogen, a small amount of nitrogen fertilizer (starter-
 179 N) was applied. Thus, NPK (20-60-80 kg ha⁻¹) were provided as a basic fertilizer before
 180 sowing. At the flowering stage, all treated plants received 30 kg ha⁻¹ of K₂O in order to correct
 181 the K deficiency.

182 During cultivation, growth parameters (plant height, stem diameter and leaf number)
 183 were measured every 10 days from emergence to harvest. The relative growth rate (RGR) was
 184 calculated using equation (6) to quantify the rate at which the plant height increased at
 185 specific time points (James and Richards, 2006):

$$186 \quad \text{RGR (cm cm}^{-1} \text{ day}^{-1}) = \frac{\text{Ln } x_{t_2} - \text{Ln } x_{t_1}}{t_2 - t_1} \quad (6)$$

187 where:

188 x_{t_1} is plant height measured at time t_1

189 x_{t_2} is plant height measured at time t_2

190 $t_2 - t_1$ is the number of days between x_{t_1} and x_{t_2}

191 **Measurements of photosynthesis and chlorophyll contents**

192 At the seed filling stage, leaf gas exchange measurements were recorded *in situ* with a
 193 Li-COR6400 portable photosynthesis system (LI-COR-6400, LI-COR Biosciences, Inc.,
 194 Lincoln, Nebraska, USA), connected to a 2 x 3 cm leaf chamber and a red-blue diode
 195 illuminator. Measures of the CO₂ assimilation rate (A_n) in relation to calculated stomatal
 196 conductance (g_{sw}) and transpiration (T_r) were measured from 9:00 to 15:00 on the middle leaf
 197 of each plant. Leaf chamber conditions during measurements were: air flow rate 400 $\mu\text{mol s}^{-1}$,
 198 leaf temperature 25°C, CO₂ concentration 400 $\mu\text{mol s}^{-1}$, and photon flux density 200 $\mu\text{mol m}^{-2}$
 199 s^{-1} . After a 5-minute period to allow the leaves to acclimate to the chamber conditions, ten
 200 data logs were recorded at 1 min intervals.

201 After measuring leaf gas exchange, leaves were harvested and the contents of
 202 photosynthetic pigments (chlorophyll a and b) were analyzed using a spectrophotometer
 203 (Jenway 6315 UV/Visible) in 100% acetone extracts prepared using the method reported by

204 Shabala and colleagues (Shabala et al., 1998). Chlorophyll concentrations are reported as μg
 205 g^{-1} of fresh weight (FW).

206 **Measurements of other growth, yield and biological nitrogen fixation parameters**

207 At harvest, the aerial part of each plant was cut and the root portion was carefully
 208 removed from the pot, separated from the adhering soil with a low pressure water jet and
 209 washed with distilled water. After wiping the roots with absorbent paper, the root length was
 210 measured. Then, the fresh weight of each part was determined. All samples were placed in a
 211 60°C drying oven for 48 hours. The dried samples were weighed and then ground into a fine
 212 powder for Kjeldahl nitrogen determination. All yield components were calculated per plant
 213 to determine the grain yield per plant, and subsequently the grain yield per hectare, including
 214 the pod number, grain number and 1000-grain weight.

215 At harvest, the total nodule number was determined and three groups of ten nodules
 216 were randomly removed from each plant to estimate the specific nodular weight and the total
 217 nodular weight. The amount of biologically fixed nitrogen (BFN) was calculated based on the
 218 N supply, the soil richness and the total amount of N fixed by the plant using equation (7)
 219 (Vadez and Drevon, 2001; Aydi et al., 2008; Bargaz et al., 2011, modified)

$$220 \quad \text{BFN (mg plant}^{-1}\text{)} = (PN_t + SN_f) - (SN_i + N_s) \quad (7)$$

221 where:

222 PN_t : total plant nitrogen

223 SN_f : final soil nitrogen content in each pot

224 SN_i : initial soil nitrogen content in each pot

225 N_s : nitrogen supply provided before sowing

226 **Statistical analysis**

227 Values are presented as the means \pm SD (n=5). Statistical analyses were performed with
 228 the SPSS software (version 20). Data were first tested for normality and homogeneity of
 229 variance to assess the statistical demands. The data were then analyzed using one-way
 230 analysis of variance (ANOVA) followed by the Student-Newman-Keuls (SNK) multiple
 231 comparison test. A p value of <5% (p<0.05) was considered statistically significant.

232 **3. Results**

233 The culture cycle extends from March 26 to June 19, 2018. The emergence coincided
 234 with a period marked by an average temperature of 16.1°C , while the flowering-pod
 235 formation stage (from May 20) was characterized by an average temperature of 26.5°C .

236 Generally, the interval fluctuations were [7.9 - 11°C], [16.1 - 26.7°C] and [23.6 - 42.8°C] for
237 the minimum, average and maximum temperatures respectively.

238 **Seed hydration and germination indices and Mo contents of primed seeds**

239 The data recorded for the hydration indices (HC and HI), germination indices (SG and
240 FGP) and Mo contents are presented in Table 2. HC and HI fluctuated between 0.32 and 0.51
241 g of solution per seed and between 0.73 and 1.16 g of solution per g of seeds, respectively.
242 These two indices are strongly influenced by the priming duration. In fact, the seed absorption
243 capacity of the solution was 43% higher when the priming duration doubled.

244 All treatments resulted in a higher FGP than the control, with the exception of the 0.2%
245 treatment for 10 hours, which produced a similar value to the control (82%). In addition, the
246 highest germination percentage (100%) was obtained when seeds were soaked in 0.1%
247 ammonium molybdate for 10 hours. However, no significant differences were observed
248 between these treatments. With the exception of 0.2% treatment, the statistical analysis
249 revealed a significant increase in SG in response to all treatments compared to the control
250 (untreated seeds). The highest value (18.9) was recorded for the 0.1% treatment for 10 hours,
251 which represents an improvement of 47% compared with the control. For both priming
252 durations, the 0.2% treatment had no effect on this parameter. Figure 3 highlights the priming
253 effects on germination kinetics. A ten hour priming duration allowed us to obtain the highest
254 germination rate during the first 12 hours, particularly with water, whose germination rate
255 was 49% compared with oscillating values ranging from 25 to 30% for other treatments. For
256 the 5 hour priming duration, the germination rate did not exceed 13%, while the untreated
257 seeds did not germinate for up to 12 hours. After 24 hours and with the exception of the 0.2%
258 treatment, all primed seeds were germinated at rate greater than 50% for both durations and at
259 a rate greater than 80% for the 10 h priming duration, in contrast to the control whose first
260 day germination rate did not exceed 20%.

261 Compared to the natural seed reserves, primed bean seeds showed
262 significantly increased Mo contents in proportion to the priming concentration, whereas the
263 priming duration only positively correlated with the highest concentration of priming solution
264 (0.1 and 0.2%). In fact, seed enrichment was 16-, 27-, 65- and 110-fold higher after Mo-
265 priming for 5 hours in 0.025, 0.05, 0.1 and 0.2% solutions, respectively, than the initial
266 content ($2.1 \mu\text{g seed}^{-1}$). The Mo contents increased to 179.9 and $372.4 \mu\text{g seed}^{-1}$ after 10 hours
267 of priming in 0.1 and 0.2% ammonium molybdate, respectively. Thus, a large amount of Mo

268 accumulated during the first 5 hours, representing 91, 83, 76 and 62% of the seed contents
269 primed for 10 hours in 0.025, 0.05, 0.1 and 0.2% treatments, respectively.

270 **Growth parameters**

271 Figure 4 shows the evolution of the increase in the bean plant height (stem height and
272 RGR). No significant differences were observed between treatments during all stages. In
273 addition, the same growth evolution pattern was observed in response to all treatments. At
274 harvest, the minimum height growth was recorded in M4 plants, with an average of 43.9 cm,
275 and the highest value (51.4 cm) was observed for M2 plants (Table 3). For all treatments, the
276 highest RGR was recorded during the 10-20 day interval after emergence and then decreased
277 until the end of the study. Likewise, the leaf number, stem diameter, total fresh biomass and
278 total dry biomass did not show any significant difference between treatments. Nevertheless, a
279 significantly greater root length was measured in M1 and M2 plants, which showed an
280 increase of approximately 66.6 and 50% compared to the NC, respectively. However, few
281 differences were observed between plants subjected to these treatments and plants derived
282 from seeds primed in water (M0) and those that received molybdc fertilization by soil
283 application (PC). Additionally, a regressive effect of Mo-priming was noted in M4 plants, but
284 the reported value was similar to the NC plants.

285 **Gas exchange and chlorophyll content**

286 The effects of Mo-priming on photosynthetic parameters are shown in Figure 5. A_n
287 increased significantly in M2 and M3 plants and the values obtained from M2 plants were
288 similar to PC plants. The highest average, which was calculated for M3 plants, was 113 and
289 48% greater than NC and PC, respectively. In contrast, this parameter was reduced in plants
290 treated with the highest concentration of priming solution compared to M2 and M3, but a
291 statistically significant difference was not observed compared with NC. Moreover, A_n
292 measured in M0 and M1 plants remained similar to NC values. T_r and g_{sw} also showed the
293 same trend as A_n . In contrast, C_i was inversely correlated with A_n . Thus, C_i decreased
294 significantly in PC, M2 and M3 plants compared to the NC plants. The highest average value
295 ($248 \mu\text{mol CO}_2 \text{ mol}^{-1}$) was observed in M4 plants. Significantly increased chlorophyll
296 contents were only observed in the leaves of M3 plants (Fig. 6). Furthermore, no significant
297 differences were noted between all other treatments.

298 **Biological nitrogen fixation**

299 Table 4 shows a significant increase in the total nodule number of M1, M2 and M3
300 plants compared to both controls. However, no differences were observed between these three
301 treatments, with values ranging from 335 to 439 nodules per plant. In M4 plants, this

parameter was reduced but the values were the same as those recorded in plants from unprimed (NC and PC) and hydroprimed seeds (M0). Similar to the number of nodules per plant, the specific and total nodular weights exhibited the same trends. Significant increases in the amount of fixed nitrogen per plant were observed in the M3 plants (328.9 mg plant⁻¹), followed by the M1 and M2 plants, with an average of 202.7 mg plant⁻¹. However, the quantity fixed by M4 plants remained significantly lower (101.4 mg plant⁻¹) than in plants that received the other Mo-priming treatments but was significantly superior to the quantities fixed by both controls (55.6 mg plant⁻¹). In contrast, BNF was reduced by 38% in plants that germinated from hydroprimed seeds compared to controls.

311 **Yield components**

Both Mo fertilization methods induced slight increases in all yield components (Table 5). However, the increases were only significant in the M3 plants, which produced the highest grain number per pod (4.4), an increase of 41.2% compared with NC plants. Additionally, the maximum yield was found in M3 plants, with an average of 13.8 q ha⁻¹, representing 115 and 49% increases compared to NC and TP plants, respectively. The lowest yield was recorded for the M4 plants, with an average of 6.2 q ha⁻¹.

318 **4. Discussion**

319 **Mo-priming effect on germination**

The priming solution concentration did not affect the seed absorption capacity for the same priming duration, potentially due to the low osmotic potential of the priming solutions, which did not slow the absorption. In contrast, seed hydration was improved by increasing the treatment duration to 10 hours, and thus we concluded that the first phase of early germination (imbibition) had not yet been completed after soaking for 5 hours.

The speed of germination is important for successful crop establishment (Finch-Savage and Bassel, 2016). All treatments, except for seeds primed in the highest Mo concentration, resulted in faster germination. However, no difference was detected between the seeds primed in water and those primed with ammonium molybdate solutions. Thus, this effect is due to the priming technique. The acceleration of primed seed germination is due to 1) a reduced imbibition time (Brocklehurst and Dearman, 2008), which is explained by a better water absorption efficiency due to aquaporin activation (Boursiac et al., 2005), and 2) the rapid stimulation of many metabolic processes involved in the first two phases of germination (imbibition and germination *sensu stricto*) (Wahid et al., 2008). In fact, the seeds shortened the first two phases and rapidly entered the growth phase by protruding the radicle.

335 Additionally, seed priming in water or osmotic solutions has been reported to trigger
336 processes related to germination by activating ATPase and other enzymes promoting ATP
337 availability (Nasri et al., 2011). During this phase, the acceleration of germination is also
338 associated with the increased respiration contributing to rapid ATP production. This energy
339 source then allows the mobilization of seed reserves and macromolecules, resulting in embryo
340 elongation. Thus, the increased speed of germination is attributed to early mobilization of
341 reserves due to the activation of α and β amylases (Lee and Kim, 2000). For example,
342 according to Kata et al. (2014), hydropriming increases α -amylase activity, resulting in better
343 mobilization of stored carbohydrates.

344 The negative effect of the highest concentration on speed germination might be
345 explained by ionic toxicity, either by Mo ions and/or excess nitrogen. Indeed, the seeds
346 primed in 0.2% ammonium molybdate displayed a greenish color, regardless of the priming
347 duration. The toxic effect of Mo might also be related to the increased levels of abscisic acid
348 (ABA) in seeds, which is not only responsible for the desiccation and maturation of seeds but
349 also for the inhibition of germination in dormant seeds. Mo is an aldehyde oxidase cofactor
350 that catalyzes the final step of abscisic aldehyde oxidation to ABA (Mendel and Haensch,
351 2002). Thus, the highest concentration of Mo probably increased the ABA level to a sufficient
352 concentration that would delay but not inhibit germination.

353 Our germination test results are consistent with several studies confirming this positive
354 effect of priming, including hydropriming, on improving the seed germination performance of
355 *Cicer arietinum*, *Lens culinaris* (Johnson et al., 2005), *Vigna radiata* (Umair et al., 2011) and
356 *Phaseolus vulgaris* (Ghassemi-Golezani et al., 2010).

357 According to the hydration indices, germination indices and Mo contents of primed
358 seeds, a 10 hour priming duration allowed seeds to enter the activation phase. At priming
359 durations ranging from 5 to 10 hours, we noticed a slowing of solution absorption and a slight
360 increase in the speed and rate of germination.

361 **Effect of Mo-priming on growth parameters**

362 Common bean (*Phaseolus vulgaris* L.) has a lower efficiency of BNF (15 kg ha^{-1}) than other
363 legumes, such as soybean (137 kg ha^{-1}) (Martinez-Romero, 2003; Peoples et al., 2009).
364 Moreover, Mo deficiency negatively affects the nitrogenase enzyme system present in
365 bacteroids (Kaiser et al., 2005) and subsequently limits BNF under field conditions (Campo et
366 al., 2009). Thus, the application of Mo fertilizers can enhance BNF. Bean fertilization with
367 Mo is accomplished either by soil application or by foliar feeding. With the first method, the

368 Mo availability may be reduced due to the complexity of controlling soil parameters like
369 alkaline soil pH, calcareousness, low organic matter and rapid adsorption on soil particles
370 (Alloway, 2008). Moreover, the plant uptake was restricted at low moisture (Yadegari, 2016).
371 These constraints disappear or are limited when the fertilizers are foliar-sprayed. However, in
372 most of the global agricultural areas, this method increases the farmer's cost of cultivation
373 while crop prices have not kept pace. Based on the results of the present study, Mo-priming
374 exerted a positive, significant effect on BNF compared to soil application. In fact, seeds
375 primed in 0.1% ammonium molybdate for 10 hours accumulated 180 μg of Mo per seed,
376 corresponding to approximately 36 g of Mo per hectare, allowing plants to fix 65.8 kg of
377 nitrogen per hectare. The greater level of BNF may be due to the high specific nodular weight
378 and total nodule number, which are substantially influenced by Mo-priming. A moderately
379 positive correlation ($r = 0.63$) was established between these two parameters. However, the
380 highest values were observed in the M1 plants, which were slightly decreased as the Mo
381 concentration increased. Thus, the active nodules were probably more abundant in the roots of
382 M3 plants. Additionally, both control plants (NC and PC) fixed approximately 11.1 kg ha^{-1} ,
383 similar to the reported BNF of 15 kg ha^{-1} for common bean (Peoples et al., 2009). As shown
384 in the study by Umair et al. (2011), the priming of *Vigna radiata* seeds in 0.02 and 0.04%
385 sodium molybdate dihydrate for 5 hours significantly increases the BNF by 47 and 36%, with
386 38.6 and 35.7 kg ha^{-1} of fixed nitrogen, respectively. The second treatment approximately
387 corresponds to M1 in our study, which allowed us to fix an estimated amount of nitrogen at
388 40.5 kg ha^{-1} . These interesting results are attributed to the primordial role of Mo in BNF as
389 cofactor of nitrogenase. Moreover, *Rhizobium* is very effective when sufficient Mo
390 concentrations are provided to plants (Pessoa et al., 2001).

391 Mo-primed seeds ensure that plants have an early source of this micronutrient compared
392 to the soil application. In fact, the Mo bioavailability to the plant and thus to bacteroids in the
393 early stages increases the activities of nitrogenase and nitrate reductase (González et al.,
394 2008), and subsequently increases nitrogen assimilation. These changes increase chlorophyll
395 biosynthesis (Hopkins, 2003). However, the Mo provided by soil application does not
396 influence the chlorophyll content, probably due to insufficient intake or the application at late
397 stage, because the most important factor affecting the effectiveness of Mo fertilization is the
398 application period (Masi and Boselli, 2011). Our study revealed a positive effect of Mo-
399 priming in 0.1% ammonium molybdate (M3) on the chlorophyll content, which strongly and
400 positively correlated with the leaf photosynthetic capacity. A_n , g_{sw} and T_r were much greater

401 in M3 plants. Meanwhile, C_i inversely correlated with A_n . An increase in g_{sw} increased T_r and
402 improved A_n (Moualeu-Ngangue et al., 2016). The role of Mo in maintaining an adequate
403 chlorophyll content and therefore high photosynthetic efficiency of plants has been reported
404 (Chen et al., 2004).

405 Despite the positive effects of Mo-priming on BNF, the chlorophyll content and A_n , it
406 did no alter the growth parameters (stem height, leaf number, stem diameter and biomass). A
407 potential explanation for this difference is that the initial nitrogen and Mo content in the soil
408 used in this study were sufficient to ensure the normal vegetative growth of the negative
409 control plants, without inducing symptoms of Mo deficiency. The present results are
410 consistent with the findings reported by Alkhamisi et al. (2017), who showed that Mo-
411 priming of wheat seeds did not affect plant height. However, El-Hersh et al. (2011), Singh et
412 al. (2014) and Arun et al. (2017) reported that Mo-priming significantly improved growth
413 parameters in lentils, chickpea and cowpea, respectively.

414 In contrast to all growth parameters, root length was influenced by molybdic
415 fertilization. Seed treatment has been shown to promote root development and subsequently
416 the absorption efficiency of the elements from the soil solution. According to Harris (2001),
417 seed priming with ammonium molybdate results in significantly higher uptake of N, P, and K
418 in cowpea compared to hydropriming and control conditions. The influence of Mo on root
419 elongation is probably associated with the stimulation of ABA production. In fact, Mo
420 catalyzes the last step in the ABA biosynthesis pathway (Mendel and Hänsch, 2002), resulting
421 in its accumulation in the roots and the subsequent induction of root cell elongation. Notably,
422 Mo exerted a toxic effect on two plants from seeds treated with 0.2% ammonium molybdate.
423 Approximately 50 days after emergence, black spots were observed on bean stems, leading to
424 plant death.

425 **Effect of Mo-priming on grain yield**

426 The priming of bean seeds in 0.1% ammonium molybdate resulted in the highest yield
427 (estimated at 13.8 q ha^{-1}), representing a 115% increase compared with the NC, in the present
428 study. However, greater than 45% increases in yield were recorded in plants fertilized through
429 the soil application method. Thus, the positive effect of Mo-priming on grain yield was
430 attributed to both the Mo nutritional effect and the biochemical advantages induced during the
431 priming process. This assumption was supported by an examination of the hydropriming
432 effect on yield, which was 55% greater than the NC. Yield, per plant or per hectare, was
433 highly positively correlated with the number of grains per pod compared to other yield

434 components ($r=0.96$). Therefore, we concluded that the increased yield induced by molybdc
435 fertilization was due to the grain number improvement. The results of these all yield
436 components were consistent with other studies examining different legume species, but the
437 magnitude of the response depends on the crop species. Ghassemi-Golezani et al. (2008) and
438 Arun et al. (2017) highlighted this positive effect of Mo-priming on the yield of chickpea and
439 in cowpea, respectively. The authors reported that this technique significantly improved
440 number of pods per plant, number of seeds per plant, pod length and 1000-grain weight.
441 Regarding hydropriming, some studies reported a positive effect on yield (Rashid et al., 2004;
442 Arif et al., 2007). Furthermore, Mo-priming increases the yield attributes to certain levels and
443 then gradually decreases, highlighting the toxic effect on M4 plants for all parameters studied.

444 **Conclusion**

445 Bean seeds primed with a solution enriched with Mo exhibited significantly increased
446 levels of nitrogen fixed per plant compared to the soil application, which, partially through
447 ecological processes, restore nitrogen to land areas that have extensively been used for
448 agriculture. In fact, bean seeds, which are known to have a low BNF efficiency (15 kg ha^{-1}),
449 subjected to Mo-priming in 0.1% ammonium molybdate for 10 hours allowed the plant to fix
450 65.8 kg ha^{-1} and increase the grain yield by 115%. Thus, nutri-priming has emerged as a
451 promising approach to increase crop performance because of the small amount of Mo, the
452 more uniform application and the absorption efficiency. Nevertheless, the optimal treatment
453 differs according to the concentrations and durations of priming and drying, which is
454 considered a major limitation of this method. Therefore, many trials are needed to identify the
455 most appropriate strategy for each situation.

456

457

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462

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- 603

604 **Figure legends**

605 **Fig. 1**

606 Location of the soil sampling site (prepared using ArcGIS software 10.3.1.).

607 **Fig. 2**

608 Schematic illustrating the experimental set up.

609 **Fig. 3**

610 Germination kinetics of unprimed (control: -) and primed (M0: 0 ×, M1: 0.025 ●, M2: 0.05
611 ▲, M3: 0.1 ■ and M4: 0.2 ◆ % of ammonium molybdate) bean seeds (*Phaseolus vulgaris*)
612 with two priming durations (a: 5 hours, b: 10 hours).

613 **Fig. 4**

614 Changes in the stem height (a) and relative growth rate (RGR) (b) of bean plants (*Phaseolus*
615 *vulgaris*) that germinated from unprimed seeds (NC -: without Mo fertilization, PC +: with
616 recommended dose of Mo provided by soil application) and primed seeds (M0: 0 ×, M1•:
617 0.025, M2▲: 0.05, M3■: 0.1 and M4◆: 0.2% of ammonium molybdate). Each symbol
618 represents the mean of 5 replicates.

619 **Fig. 5**

620 Changes in (a) A_n : CO₂ assimilation rate, (b) g_{sw} : stomatal conductance, (c) C_i : intercellular
621 CO₂ concentration and (d) T_r : transpiration rate of bean plants (*Phaseolus vulgaris*) that
622 germinated from unprimed seeds (NC: without Mo fertilization, PC: soil application of the
623 recommended Mo dose) and primed seeds (M0: 0, M1: 0.025, M2: 0.05, M3: 0.1 and M4:
624 0.2% ammonium molybdate). Data are presented in standard box plots. The lines at the top
625 and bottom of the box delimit the lower and upper quartiles, respectively; the thick middle
626 line represents the median. The lower and upper whiskers represent the minimum and
627 maximum values for the sample, respectively. The different letters above the boxes indicate
628 significant differences according to the SNK test at $p < 0.05$.

629 **Fig. 6**

630 Leaf chlorophyll contents (■: chlorophyll a, ■: chlorophyll b and ■: total chlorophyll)
631 reported as $\mu\text{g g}^{-1}$ fresh weight of bean plants (*Phaseolus vulgaris*) that germinated from
632 unprimed seeds (NC: without Mo fertilization, PC: soil application of the recommended Mo
633 dose) and primed seeds (M0: 0, M1: 0.025, M2: 0.05, M3: 0.1 and M4: 0.2% ammonium
634 molybdate). Data are presented as the means of 5 replicates; the vertical bars indicate standard
635 deviations. Different letters indicate significant differences according to the SNK test at $p <$
636 0.05.

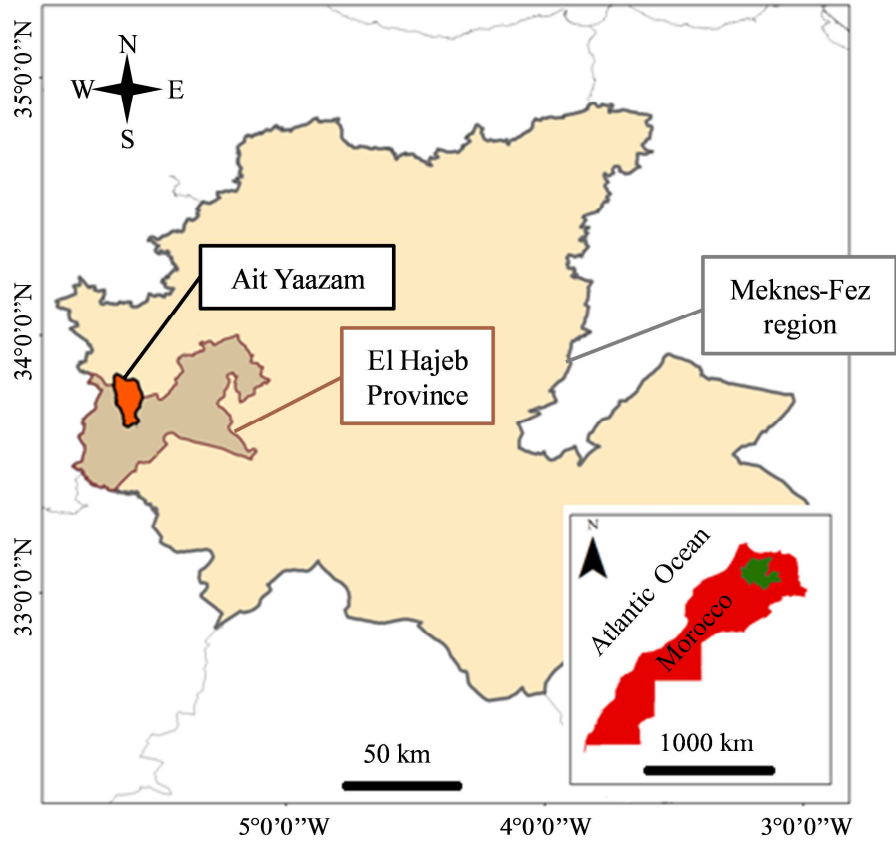


Fig. 1

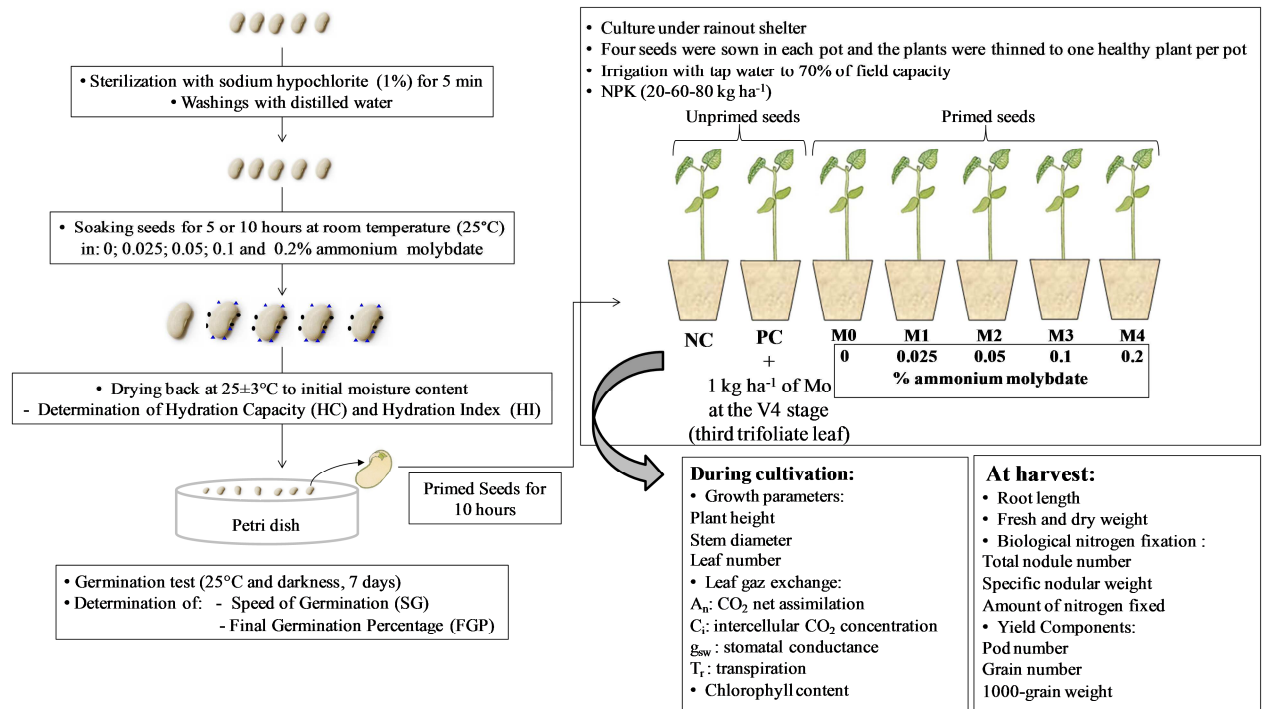


Fig. 2

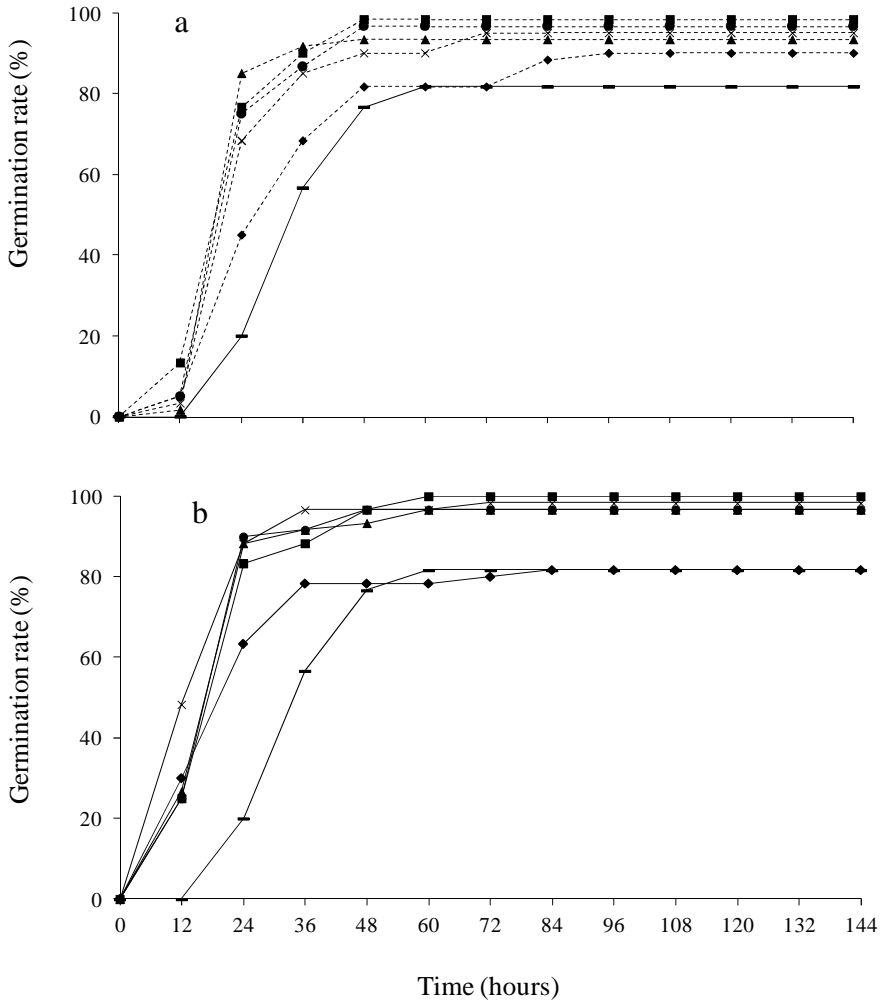


Fig. 3

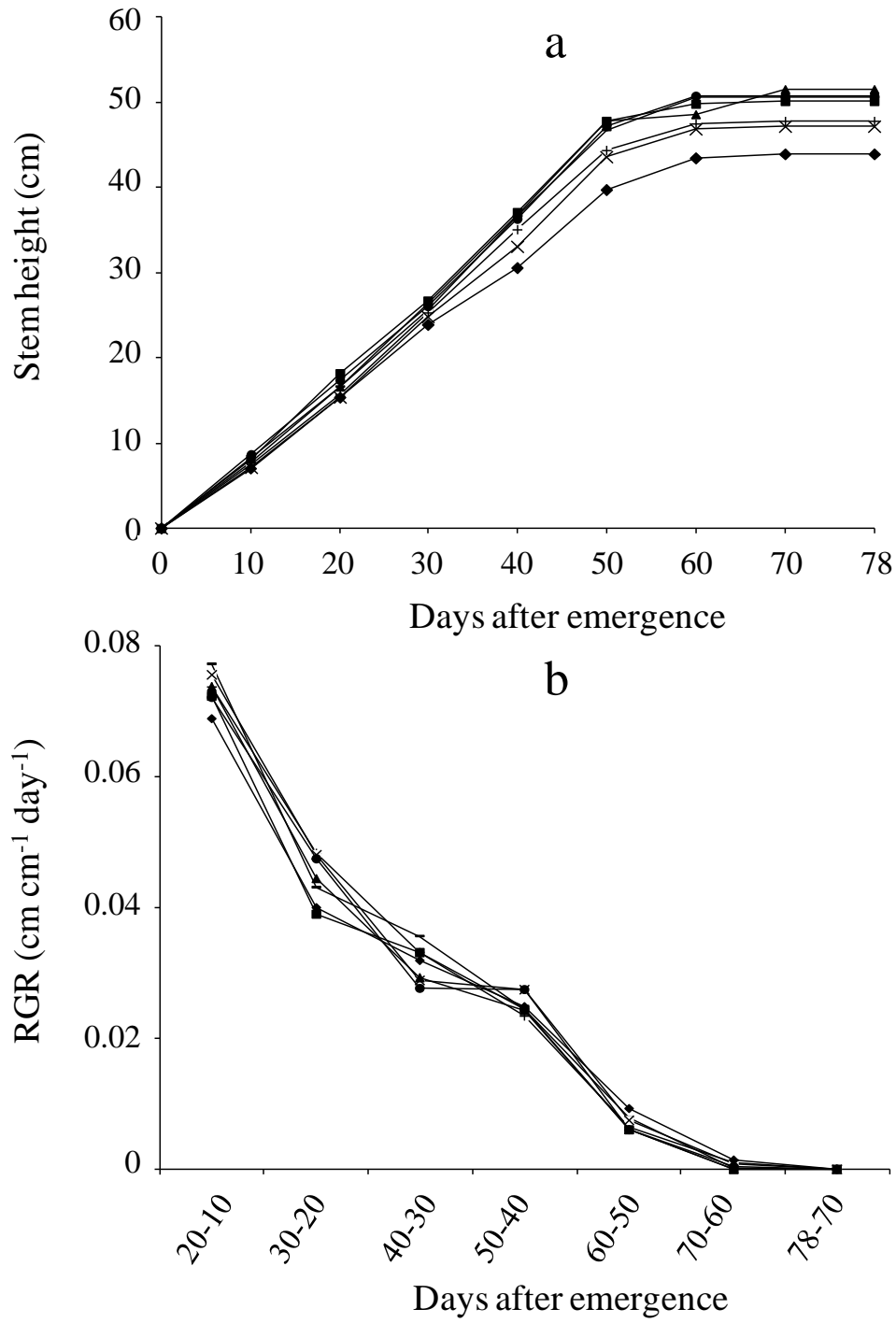


Fig. 4

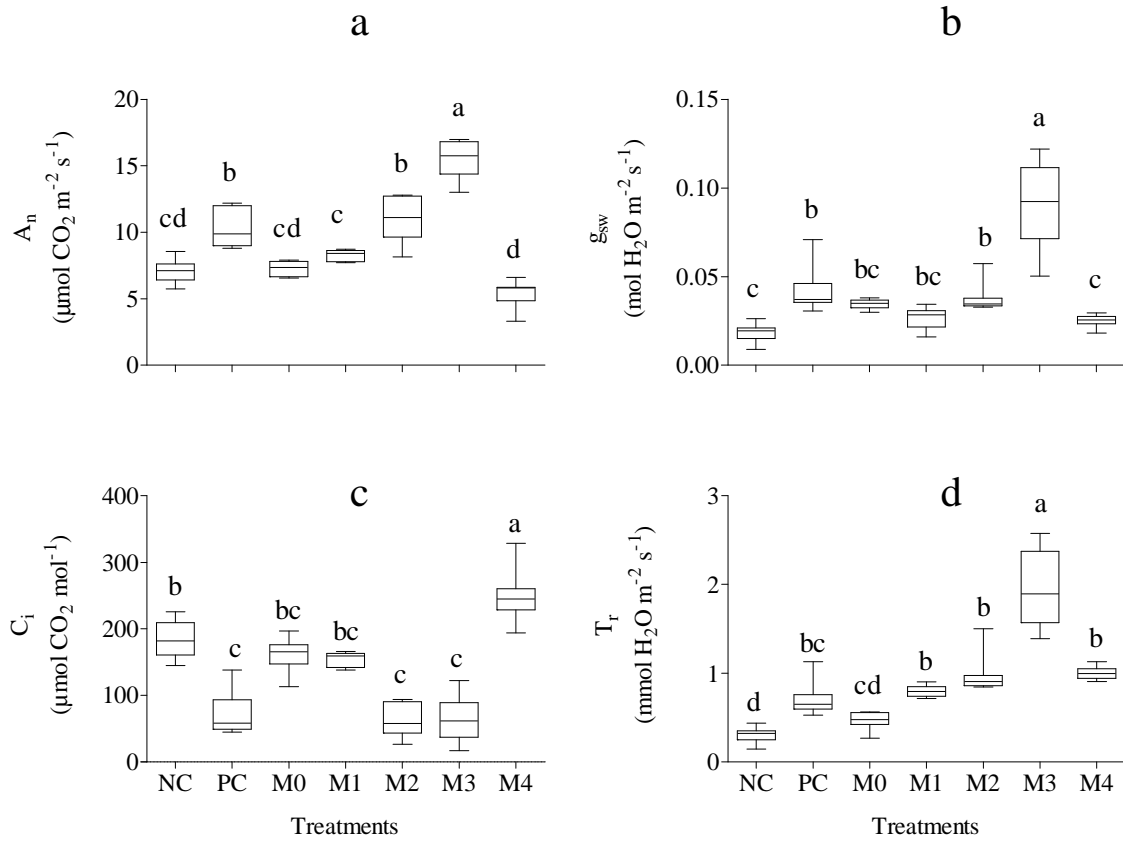


Fig. 5

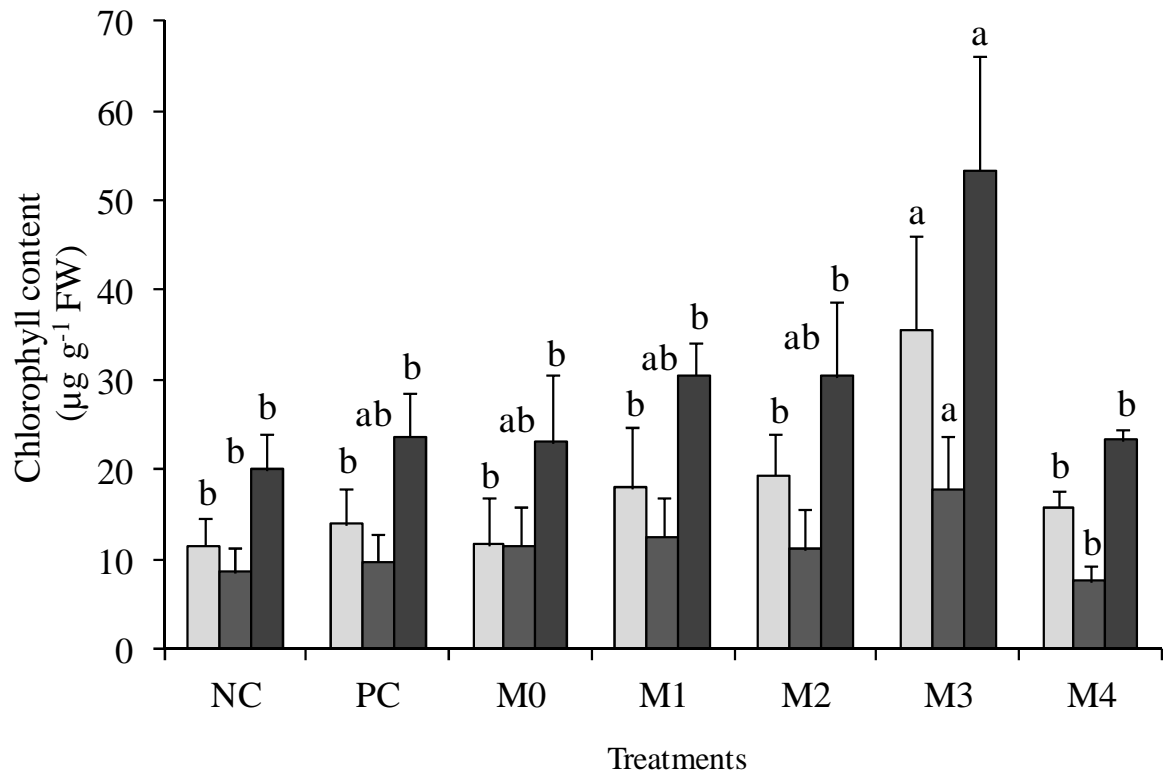


Fig. 6

Table 1: Physico-chemical characterization of the soil used in the experiment

Parameters (unit)	Soil property
Clay %	7.5
Fine-loam %	2.9
Coarse-loam %	1.8
Total sand %	87.8
pH	8.31
Electrical conductivity (dS m ⁻¹)	0.045
Total limestone (mg kg ⁻¹)	0
Organic matter (%)	0.58
Mineral nitrogen (mg kg ⁻¹)	10.5
Olsen P ₂ O ₅ (mg kg ⁻¹)	26.34
Extractable K ₂ O (mg kg ⁻¹) ^a	115.2
Extractable Zn (mg kg ⁻¹) ^b	12.86
Extractable Mo (mg kg ⁻¹) ^b	0.83

Extractants: ^aAmmonium Acetate; ^bDiethylene Triamine Penta-Acetic Acid (DTPA)

Table 2: Changes in hydration indices, germination indices and Mo content of bean seeds (*Phaseolus vulgaris*) unprimed (control) and primed (0, 0.025, 0.05, 0.1 and 0.2% ammonium molybdate) with two durations (5 and 10 hours). The data represent Mean \pm SD, n=3; the different letters indicate significant differences according to the SNK test at $p < 0.05$.

Parameters	Duration (h)	Unprimed	Primed seeds in ammonium molybdate				
		control	0	0.025%	0.05%	0.1%	0.2%
Hydration Capacity (HC) (g of solution seed ⁻¹)	5	-	0.32	0.35	0.39	0.38	0.33
	10	-	0.51	0.49	0.50	0.50	0.51
Hydration Index (HI) (g of solution g ⁻¹ of seed)	5	-	0.73	0.80	0.89	0.85	0.74
	10	-	1.16	1.12	1.13	1.13	1.16
Final Germination Percentage (FGP) (%)	5		95 \pm 3 ^a	97 \pm 2 ^a	93 \pm 2 ^a	98 \pm 2 ^a	90 \pm 7 ^a
	10	82 \pm 8 ^a	98 \pm 2 ^a	97 \pm 2 ^a	97 \pm 2 ^a	100 \pm 0 ^a	82 \pm 4 ^a
Speed of Germination (SG) (% d ⁻¹)	5		16.2 \pm 1.0 ^a	17.2 \pm 0.6 ^a	17.8 \pm 0.6 ^a	18.2 \pm 0.2 ^a	13.0 \pm 3.2 ^b
	10	10 \pm 0.8 ^b	18.6 \pm 0.7 ^a	18.7 \pm 0.6 ^a	18.4 \pm 0.4 ^a	18.9 \pm 0.1 ^a	14.4 \pm 0.81 ^b
Mo content (μ g seed ⁻¹)	5		2.8 \pm 0.0 ^g	33.7 \pm 1.0 ^f	57.4 \pm 9.8 ^e	137.1 \pm 7.1 ^d	230.5 \pm 7.6 ^b
	10	2.1 \pm 0.1 ^g	5.1 \pm 0.3 ^g	36.9 \pm 1.1 ^f	68.8 \pm 6.5 ^e	179.9 \pm 11.5 ^c	372.4 \pm 44.0 ^a

Table 3: Growth parameters of bean plants (*Phaseolus vulgaris*) from unprimed seeds (NC: without Mo fertilization, PC: with recommended dose of Mo provided by soil application) and primed seeds (M0: 0, M1: 0.025, M2: 0.05, M3: 0.1 and M4: 0.2 % of ammonium molybdate). The data represent Mean \pm SD, n=5; the different letters indicate significant differences according to the SNK test at $p < 0.05$.

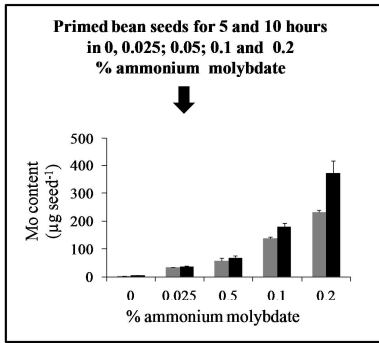
Parameters	NC	PC	M0	M1	M2	M3	M4
Stem height (cm)	50.6 \pm 1.5 ^a	47.8 \pm 2.4 ^a	47.1 \pm 3.6 ^a	50.7 \pm 2.5 ^a	51.4 \pm 1.3 ^a	50.1 \pm 3.3 ^a	43.9 \pm 8.8 ^a
Root length (cm)	22.6 \pm 2.3 ^c	30.1 \pm 1.9 ^{abc}	33.1 \pm 5.4 ^{ab}	37.4 \pm 3.5 ^a	36.1 \pm 4.6 ^a	34 \pm 4.8 ^{ab}	25.4 \pm 3.7 ^{bc}
Leaf number	12.6 \pm 0.7 ^a	13.8 \pm 1.0 ^a	13.40 \pm 1.5 ^a	13.8 \pm 1.8 ^a	13.4 \pm 2.1 ^a	14.2 \pm 0.7 ^a	11.8 \pm 2.9 ^a
Stem diameter (mm)	6.5 \pm 0.2 ^a	5.8 \pm 0.4 ^a	6.6 \pm 0.3 ^a	6.3 \pm 0.2 ^a	6.7 \pm 0.4 ^a	6.4 \pm 0.3 ^a	6.5 \pm 0.7 ^a
Total fresh biomass (g)	51.9 \pm 5.2 ^a	53.9 \pm 4.4 ^a	52.7 \pm 5.0 ^a	61.6 \pm 7.1 ^a	58.6 \pm 9.4 ^a	64.0 \pm 6.8 ^a	50.8 \pm 9.7 ^a
Total dry biomass (g)	7.8 \pm 0.6 ^a	8.4 \pm 0.8 ^a	7.9 \pm 0.9 ^a	9.4 \pm 0.9 ^a	8.6 \pm 1.4 ^a	9.4 \pm 1.1 ^a	7.6 \pm 1.3 ^a

Table 4: Biological nitrogen fixation parameters of bean plants (*Phaseolus vulgaris*) from unprimed seeds (NC: without Mo fertilization, PC: with recommended dose of Mo provided by soil application) and primed seeds (M0: 0, M1: 0.025, M2: 0.05, M3: 0.1 and M4: 0.2 % of ammonium molybdate). The data represent Mean \pm SD, n=5; the different letters indicate significant differences according to the SNK test at $p < 0.05$.

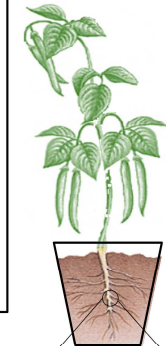
Parameters	NC	PC	M0	M1	M2	M3	M4
Total nodule number per plant	181 \pm 8.4 ^c	237 \pm 82.2 ^{bc}	189 \pm 40.7 ^c	434 \pm 62.9 ^a	439 \pm 46.0 ^a	335 \pm 26.7 ^{ab}	232 \pm 15.6 ^{bc}
Specific nodular weight (mg)	3.4 \pm 0.4 ^b	6.2 \pm 0.9 ^b	5.7 \pm 2.6 ^b	16.7 \pm 3.6 ^a	14 \pm 1.1 ^a	13.4 \pm 2.4 ^a	7.8 \pm 5.2 ^b
Total nodular weight (g)	0.6 \pm 0.1 ^c	1.5 \pm 0.2 ^c	1.1 \pm 0.5 ^c	7.3 \pm 1.6 ^a	6.1 \pm 0.5 ^{ab}	4.5 \pm 0.8 ^b	1.8 \pm 1.2 ^c
Amount of fixed nitrogen (mg plant ⁻¹)	55.6 \pm 0.1 ^d	55.5 \pm 0.0 ^d	34.4 \pm 0.0 ^e	202.7 \pm 0.4 ^b	202.8 \pm 0.0 ^b	328.9 \pm 0.2 ^a	101.4 \pm 0.2 ^c
Amount of fixed nitrogen (kg ha ⁻¹)	11.1 \pm 0.0 ^d	11.1 \pm 0.0 ^d	6.9 \pm 0.0 ^e	40.5 \pm 0.1 ^b	40.6 \pm 0.0 ^b	65.8 \pm 0.0 ^a	20.9 \pm 0.0 ^c

Table 5: Yield components of bean plants (*Phaseolus vulgaris*) from unprimed seeds (NC: without Mo fertilization, PC: with recommended dose of Mo provided by soil application) and primed seeds (M0: 0, M1: 0.025, M2: 0.05, M3: 0.1 and M4: 0.2 % of ammonium molybdate). The data represent Mean \pm SD, n=5; the different letters indicate significant differences according to the SNK test at $p < 0.05$.

Parameters	NC	PC	M0	M1	M2	M3	M4
Pod number per plant	5.4 \pm 0.8 ^{ab}	5.2 \pm 0.6 ^{ab}	4.4 \pm 0.5 ^{ab}	4.4 \pm 0.5 ^{ab}	4.6 \pm 0.7 ^{ab}	5.8 \pm 0.6 ^a	3.0 \pm 0.9 ^b
Grain number per pod	3.1 \pm 0.4 ^b	3.7 \pm 0.5 ^{ab}	3.5 \pm 0.4 ^{ab}	3.9 \pm 0.2 ^{ab}	3.8 \pm 0.3 ^{ab}	4.4 \pm 0.5 ^a	3.1 \pm 0.6 ^b
1000-grain weight (g)	332.9 \pm 12.3 ^{ab}	328.5 \pm 9.4 ^{ab}	344.5 \pm 16.7 ^{ab}	363.8 \pm 18.8 ^a	342.0 \pm 14.6 ^{ab}	339.1 \pm 17.7 ^{ab}	304.5 \pm 28.1 ^b
Grain yield (g plant ⁻¹)	3.2 \pm 0.6 ^b	4.6 \pm 1.3 ^{ab}	4.2 \pm 1.0 ^b	5.5 \pm 0.7 ^{ab}	5.0 \pm 1.1 ^{ab}	6.9 \pm 1.3 ^a	3.1 \pm 1.0 ^b
Grain yield (quintals ha ⁻¹)	6.4 \pm 1.2 ^b	9.3 \pm 2.7 ^{ab}	8.4 \pm 2.1 ^b	10.9 \pm 1.3 ^{ab}	10.0 \pm 1.6 ^{ab}	13.8 \pm 2.7 ^a	6.2 \pm 2.1 ^b



Proportional increase in Mo content in seeds



Primed seeds for 10 hours



Significant increase in nodule weight and nodule number

