

Article

Potassium Nitrate Treatment Is Associated with Modulation of Seed Water Uptake, Antioxidative Metabolism and Phytohormone Levels of Pea Seedlings

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Abstract: (1) Background: Seed treatment with potassium nitrate (KNO_3) has been associated with dormancy breaking, improved germination and enhanced seedling growth and uniformity in a variety of plant species. However, the KNO_3 effect seems to be dependent on plant species and treatment conditions. (2) Methods: We describe the effect of incubation of dry pea seeds with different KNO_3 concentration on water uptake kinetic, early seedling growth, antioxidant metabolism and hormone profile in pea seedlings. (3) Results: Low (0.25 mM) KNO_3 levels increased seedling water uptake and growth, whereas high (40 mM) levels decreased seedling growth. KNO_3 treatment differentially affected the antioxidant defences. Low KNO_3 levels maintained the activity of antioxidant enzymes, while high levels reduced the activity of H_2O_2 -scavenging enzymes. KNO_3 induced a progressive decline in ascorbate levels and reduced (GSH) and oxidised (GSSG) glutathione. Low KNO_3 levels strongly increased GA_1 and decreased ABA in both seedlings and cotyledons, resulting in a decline in the ABA/GAs ratio. (4) Conclusions: Pea seed treatment with a low KNO_3 level promoted early seedling growth. In this process, an interaction among KNO_3 , antioxidant defences and ABA/GAs ratio is proposed.

Keywords: ABA; antioxidant defences; GAs; nitrate; seed germination; seedling growth; water uptake



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1. Introduction

Seed germination is the most critical stage in crop establishment, determining crop production [1]. Numerous methods have been used to promote seed germination and seedling establishment under normal and stressful conditions. In this sense, seed priming, defined as a pre-sowing treatment which involves controlled hydration of seeds during the first stage of germination, has been widely applied to improve the germination rate and seedling growth under different stress conditions [2–5]. Likewise, seed chemical treatment during imbibition has been successfully applied for both fundamental research purposes and for the stimulation of seed germination and seedling vigour [6–8].

Potassium nitrate (KNO_3) has been demonstrated to break seed dormancy, promote seed germination and enhance growth uniformity in a variety of plant species, including tomato, maize, *Arabidopsis* and pea [9–12]. However, the mechanism by which KNO_3 improves seed germination and seedling establishment remains unclear. The priming effect of KNO_3 seems to be dependent on the KNO_3 concentration and application method. For example, improved seedling establishment was observed when seeds of *Paspalum vaginatum* (cv. Sea-Spray) were imbibed in 20–50 mM KNO_3 for three days [13]. Conversely, other authors reported that water-imbibed pea seeds (*Pisum sativum* cv. Lincoln) displayed reduced germination and seedling growth when incubated for three days in the presence of 30 or 40 mM KNO_3 , whereas 10 mM KNO_3 stimulated early seedling growth. However, when the pea seeds were imbibed with KNO_3 (40 mM) for 24 h and then incubated in distilled water, seedling fresh weight was enhanced, whereas germination rate remained

unchanged [12]. Other authors [14] suggested that nitrates may enhance seed germination and early seedling growth due to the dual role of N as a plant essential element for growth and as a signalling molecule.

The effects of KNO_3 on plant hormones' regulation are unclear, with most studies focusing on abscisic acid (ABA) levels. KNO_3 has been reported to positively affect seed germination by modulating ABA metabolism or ABA signalling in developing seeds [15,16]. Gibberellins (GAs) are also important plant hormones for numerous physiological plant processes, including seed germination [17]. Other authors [18] reported that activation of ABA catabolism and GAs biosynthesis is required for seed germination. Low nitrate concentration decreases ABA content, leading to the induction of the *CYP707A2* gene, which encodes an ABA 8'-hydroxylase involved in ABA catabolism [15,19]. In addition, the up-regulation of the *CYP707A2* gene precedes the induction of the *GA3ox2* gene, related to GA biosynthesis [20]. Vidal et al. [12] described that exogenous KNO_3 enhanced GA_4 content and reduced ABA levels, resulting in a decrease in the ABA/GAs ratio. This effect was reversed by the action of the nitric oxide (NO)-scavenger 2-(4-carboxyphenyl)-4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO), suggesting an interplay between KNO_3 and NO metabolism [21]. This in turn would indicate the role of NO in seed germination and seed dormancy breaking [9,22].

In addition, exogenous KNO_3 has been reported to lead to an increase in the expression of genes involved in N and C metabolism, as well as in energy production [15]. A role for nitrate reductase induction, related to N assimilation, and the antioxidative metabolism has also been described [11,12]. In that regard, the application of KNO_3 has been reported to increase antioxidant enzymes activity (superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate oxidase (AOX)) in seedlings from different plant species [11,12]. During imbibition, seeds take an increasing amount of oxygen, causing the accumulation of reactive oxygen species (ROS) and a shift in the redox state [23]. Although ROS were originally considered as toxic by-products, different studies revealed that they are used by most organisms as key signalling molecules [24]. In addition, it was recently described that a basal level of ROS is required to support life [25]. In fact, it was recently suggested that the type of oxidative modification integrated into different oxidative signalling pathways regulates many crucial aspects of plant biology [26]. Several works have described the implication of ROS and antioxidant metabolism in the germination process. The scientific literature contains plenty of information concerning the beneficial effects of ROS on germination and seedling growth processes [6–8,27–31]. In these works, the authors showed that a controlled ROS generation during seed imbibition may have a signalling function during germination as well as during the dormancy release process. In contrast, uncontrolled ROS accumulation could delay or even inhibit seed germination [31]. In addition, an interplay ROS/plant hormone has been described during the seed germination process [6–8,28,29].

In the present work, we attempted to explain if direct nitrate addition during imbibition may promote pea seed germination and seedling establishment and vigour. For this purpose, we treated dry pea seeds with different KNO_3 concentrations to study its effect on water uptake kinetic, early seedling growth and antioxidant metabolism-related enzyme activity in pea seedlings. In addition, the effects of KNO_3 on ABA and GAs levels in pea seedlings and cotyledons were also addressed.

2. Materials and Methods

2.1. Plant Material, Culture Conditions, Growth Measurements and Sampling

Pea (*Pisum sativum* cv. Lincoln) seeds were obtained from Ramiro Arnedo S.A, Murcia, Spain. In the first experiment, individual dry seeds were placed inside 3.5 cm diameter plastic cups, onto two discs of filtered paper moistened with 1 mL KNO_3 (0, 0.25, 0.5, 1, 5, 10, 20, 30, 40 or 80 mM). Twenty-five seeds per treatment were arranged in trays and placed inside plastic bags, containing some small holes, to avoid water evaporation, and incubated in darkness at 25 °C in an incubator (MIR-153, Sanyo, Osaka, Japan). The germination percentage and water absorption rate ($\mu\text{L H}_2\text{O/g}$ dry weight (DW), calculated

as the difference between the final and the initial weights, divided by the initial weight) were registered daily during KNO_3 treatment from days 0 to 4.

Subsequently, and based on the results of the preliminary assays, dry seeds were placed on 15 cm diameter Petri dishes, onto two discs of filtered paper moistened with 7 mL KNO_3 (0, 0.25 or 40 mM). Three to six Petri dishes per treatment containing 20 seeds per plate (biological replicate) were arranged and incubated at 25 °C for four days in an incubator (MIR 153, Sanyo) in darkness. Subsequently, seedling growth (length and mass) was recorded. Seedlings were separated from cotyledons and both were frozen in liquid nitrogen and stored at −80 °C for further analysis.

2.2. Enzyme Extraction and Assays

All operations were carried out at 4 °C. Four-day-old pea seedlings were homogenised and prepared for enzymatic analyses as described [12]. The activity of the antioxidant enzymes (ascorbate (ASC)-glutathione (GSH) cycle enzymes, SOD, POX, CAT and ascorbate oxidase (AOX)) was measured as previously reported [6,32]. The protein concentration was calculated according to Bradford [33]. The analyses were performed in a UV/Vis V-630 Bio spectrophotometer (Jasco, Tokyo, Japan).

2.3. Ascorbate and Glutathione Analyses

Frozen pea seedlings were ground into a fine powder in the presence of liquid nitrogen. Then, a 1M HClO_4 solution containing 1 mM EDTA and 1% (*w/v*) polyvinylpyrrolidone phosphate was added (1/3, *w/v*). The resulting extract was centrifuged at $12,000 \times g$ for 10 min at 4 °C. The pH of the supernatant was adjusted to 5.5–6 with 5 M K_2CO_3 . The new mixture was centrifuged at $12,000 \times g$ for 1 min to eliminate the precipitate of KClO_4 formed. The resulting supernatant was used to quantify the oxidised and reduced ascorbate and glutathione forms [32].

2.4. Analysis of Plant Hormones

The analysis of ABA and GAs was performed in seedlings and cotyledons. Frozen samples were first lyophilised and then ground into a fine powder in a mortar. One 30-mg aliquot of each sample was sent to the Plant Hormones Quantification Service of the IBMCP (CSIC, Valencia) and quantified as described [34].

2.5. Statistical Analyses

Analyses of germination percentage, water uptake and seedling growth were performed on at least 20 biological replicates (individual seedlings). The remaining analysis was on three to six biological replicates (the pool of seedlings from a plate). Data were expressed as the mean \pm SE. The data were analysed by one-way ANOVA followed by a Tukey's multiple range test ($p \leq 0.05$) using the SPSS 26 software (IBM SPSS Statistics, Chicago, IL, USA). All experiments were repeated independently at least twice with similar results.

3. Results

In a preliminary experiment, we assayed the effect of different KNO_3 concentrations on the water uptake rate and germination rate of pea seeds. In general, higher KNO_3 concentrations (10–80 mM) delayed seed germination, although at days 3 and 4 the germination rate was similar to that of control seeds (Supplemental Table S1a). Lower KNO_3 concentrations (0.25–5 mM) showed similar germination rates when compared to the control treatment at every day of treatment (Supplemental Table S1b). Taking into account that the highest seedling fresh and dry weights were registered with 0.25 mM KNO_3 , this concentration, together with 40 mM KNO_3 as a representative of a high concentration, were used for further experiments.

Figure 1 shows the seedling water uptake for the control, 0.25 mM and 40 mM KNO_3 . The 40 mM KNO_3 treatment did not alter seedling water uptake compared to control seeds,

whereas 0.25 mM KNO₃ increased it significantly with respect to the control at days 1 and 3.

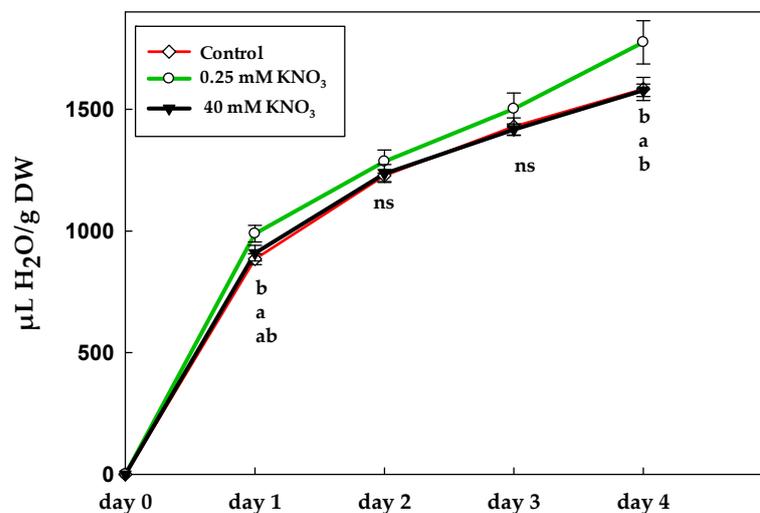


Figure 1. Effect of KNO₃ on the rate of water uptake of pea seedlings during 4 days of incubation. Each seed was weighed before being subjected to any treatment (initial dry weight (DW), day 0), and was then deposited in the bottom of a 3.5 cm diameter plastic cup, onto two discs of filter paper moistened with 0 (control), 0.25 and 40 mM KNO₃. The seeds were weighed daily and the rate of water absorption calculated as μL H₂O/g DW. Different letters (ordered from the top to the bottom for control, 0.25 mM and 40 mM KNO₃, respectively) within the same day indicate significant differences according to Tukey's test ($p \leq 0.05$).

A different effect for 0.25 and 40 mM KNO₃ was observed in the plant growth parameters (Figure 2). In general, in pea seedlings, the low KNO₃ concentration increased both FW and length by more than 15%, whereas 40 mM KNO₃ decreased the length and FW by 15% and 25%, respectively (Figure 2).

According to the ANOVA analysis, the KNO₃ treatments significantly affected all the enzymes of the ASC-GSH cycle as well as POX activity, measured at day 4 of treatment. At 40 mM KNO₃, a near 2-fold decrease in APX and POX activity was observed (Table 1). Conversely, with the same treatment, DHAR and GR significantly increased by about 60%. An increase in MDHAR, DHAR and GR (ascorbate and glutathione recycling activities) was also observed for the 0.25 mM KNO₃ treatment (Table 1). Moreover, POX activity significantly increased (near 2.5-fold) in 0.25 mM KNO₃ with respect to the 40 mM KNO₃ treatment, though the increase with respect to the control (27%) was non-significant (Table 1).

Table 1. Effect of KNO₃ on the activity of different antioxidant enzymes on four-day-old pea seedlings. Ascorbate oxidase (AOX), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and peroxidase (POX) activity are expressed in nmol × min⁻¹ × mg⁻¹ protein. Superoxide dismutase (SOD) activity is expressed in Units (U) × mg⁻¹ protein.

KNO ₃ (mM)	AOX	APX	MDHAR	DHAR	GR	POX	SOD
0	60.5 ± 4.1	39.9 ± 4.2 a	198 ± 12.9 b	0.52 ± 0.05 b	50.8 ± 3.0 b	1383 ± 172 ab	34.8 ± 2.5
0.25	51.9 ± 1.7	34.8 ± 2.2 a	316 ± 15.9 a	0.80 ± 0.08 a	85.8 ± 3.1 a	1765 ± 243 a	37.5 ± 3.8
40	53.1 ± 3.8	18.2 ± 2.2 b	165 ± 19.9 b	0.85 ± 0.04 a	80.6 ± 3.9 a	713 ± 43 b	27.7 ± 2.8
^a F	2.39 ns	13.7 **	23.4 ***	23.4 ***	31.7 ***	8.46 **	2.71 ns

^a Significant F values from one-way ANOVA are denoted at 99.9% (***) or 99% (**) levels of probability (ns, not significant). Data represent the mean ± SE from at least six measurements. Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

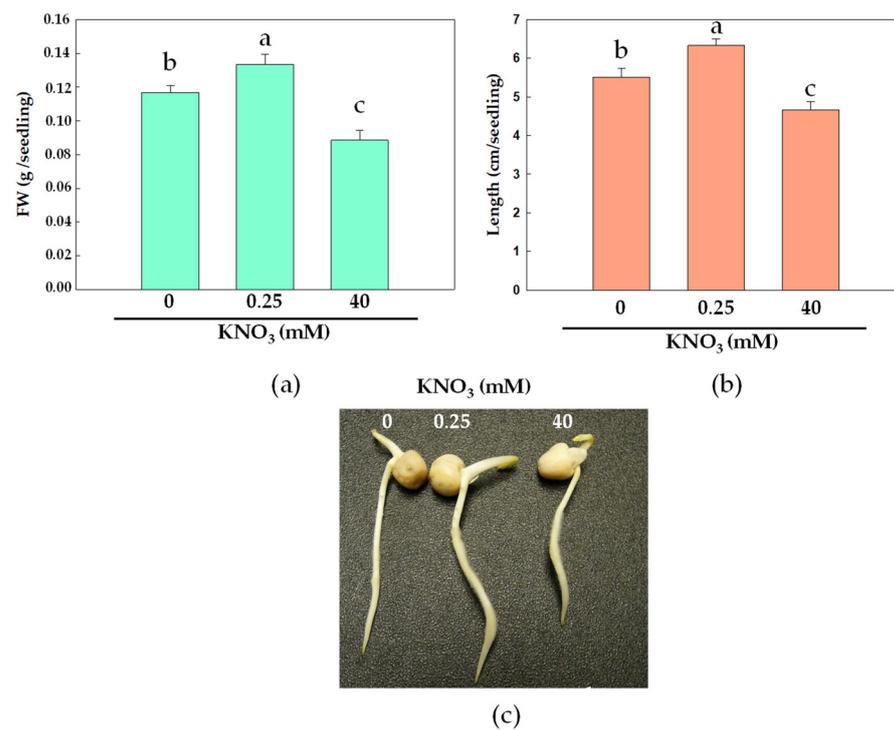


Figure 2. Effect of KNO₃ on the (a) fresh weight (FW) and (b) length of pea seedlings after four days of incubation. (c) Picture showing the visual effects of KNO₃ on seedling growth. Data represent the mean \pm SE from at least 30 measurements. Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

The KNO₃ treatments also affected the ascorbate and glutathione content (Table 2). In that sense, KNO₃ strongly decreased the reduced ascorbate (ASC) content in a concentration-dependent manner (Table 2). The oxidised ascorbate (DHA) was only detected in those samples treated with the highest KNO₃ level (Table 2). At 0.25 mM KNO₃, a decrease in both reduced (GSH) and oxidised (GSSG) glutathione levels, and thus in the total concentration of glutathione, was observed. However, an increase in the redox state of the glutathione pool was observed (Table 2). In contrast, at 40 mM KNO₃, GSH and total glutathione levels were not affected, though a decrease in GSSG was observed and, consequently, an increase in the redox state of the glutathione pool was registered (Table 2).

Table 2. Effect of KNO₃ on ascorbate and glutathione concentration in pea seedlings. Reduced (ASC) and oxidised (DHA) ascorbate and reduced (GSH), oxidised (GSSG) and total glutathione are expressed in nmol g⁻¹ fresh weight (FW). (nd: not detected.)

KNO ₃ (mM)	ASC	DHA	GSH	GSSG	GSH/ GSH + GSSG	Total Glutathione
0	1401 \pm 73 a	nd	390 \pm 19 a	20.16 \pm 3.12 a	0.951	425 \pm 32 a
0.25	535 \pm 34 b	nd	263 \pm 14 b	9.27 \pm 2.06 c	0.966	281 \pm 11 b
40	375 \pm 85 b	7.60 \pm 4.39	447 \pm 26 a	14.10 \pm 1.81 b	0.969	452 \pm 29 a
^a F	60.9 ***		32.9 ***	21.7 ***		15.2 ***

^a Significant F values from one-way ANOVA are denoted at 99.9% (***) levels of probability. Data represent the mean \pm SE from at least six measurements. Different letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

Potassium nitrate affected the ABA and GAs levels in both the seedlings and the cotyledons. GA₄ was detected in both tissues, while GA₁ was only detected in the seedlings. In seedlings treated with 0.25 mM KNO₃, a 6-fold increase in GA₁ as well as a slight decrease in GA₄ was observed (Figure 3a). In cotyledons, the effect of KNO₃ on GA₄ levels was not statistically significant (Figure 3b).

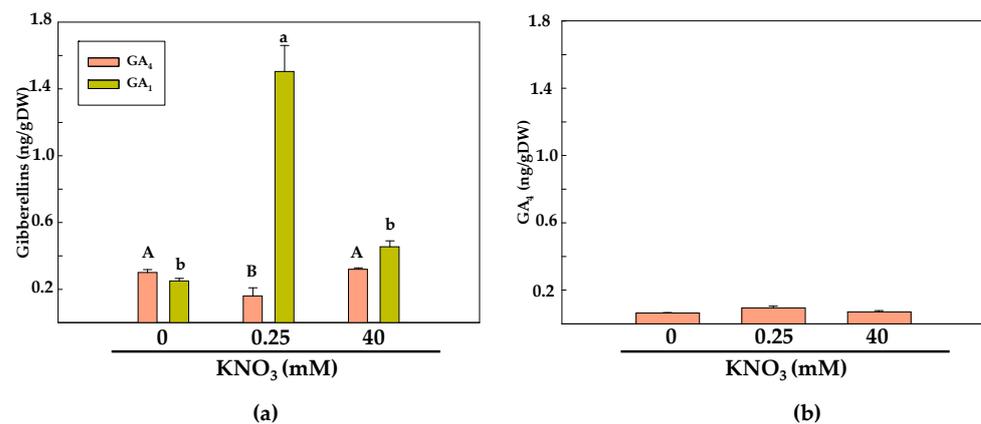


Figure 3. Effect of KNO₃ on gibberellins (GAs) levels in (a) pea seedlings and (b) cotyledons after four days of incubation. Data represent the mean \pm SE from at least three measurements. Different letters (uppercase and lowercase letters for GA₄ and GA₁, respectively) indicate significant differences according to Tukey's test ($p \leq 0.05$). (DW, dry weight.)

The effect on ABA content varied depending on KNO₃ concentration and the type of tissue (seedling or cotyledon). In seedlings, at 0.25 mM KNO₃, a 54% decrease in the ABA level was observed, while 40 mM KNO₃ concentration produced a 1.7-fold increase in ABA, compared to control seedlings (Figure 4a). ABA levels in the cotyledons were six times lower than in seedlings under control conditions. At 0.25 mM KNO₃, ABA levels declined in the cotyledon, though no effect was observed at 40 mM KNO₃ (Figure 4a). As a consequence of the KNO₃-induced changes in ABA and GAs in pea seedlings and cotyledons, an important decrease in the ABA/total GAs ratio occurred in the cotyledons and, especially, in the seedlings (Figure 4b).

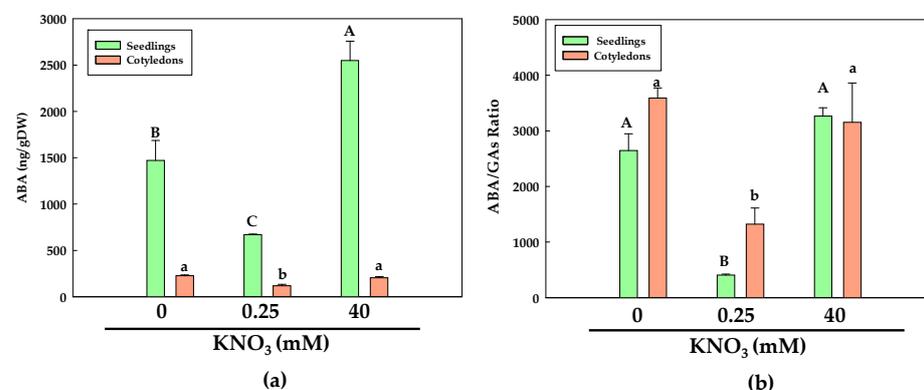


Figure 4. Effect of KNO₃ on (a) abscisic acid (ABA) levels and (b) ABA/GAs ratio in pea seedlings and cotyledons after four days of incubation. Data represent the mean \pm SE from at least three measurements. Different letters (uppercase and lowercase letters for seedlings and cotyledons, respectively) indicate significant differences according to Tukey's test ($p \leq 0.05$). (DW, dry weight.)

4. Discussion

Nitrogen is a macronutrient that may be a limiting factor for plant growth. At low concentrations, nitrate can stimulate seed germination in a variety of different plant species [14]. The mechanism of action of KNO₃ on the improvement of seed germination and/or early growth is far from being completely understood. The complexity about nitrate effects on seed germination and early seedling growth could be due to its dual role as a nutrient and a signalling molecule [14]. Nitrate stimulation of seed germination is often associated with plant species whose seeds require light for germination [35,36].

Potassium nitrate has been reported to improve pea seed germination and plant performance, but this effect is dependent on the concentration and the mode of application [1–4].

In the present work, we applied KNO_3 directly to dry seeds; under these conditions a low KNO_3 concentration, such as 0.25 mM, increased the early seedling growth of peas, but there was no effect on the germination rate. This effect was parallel with an increased water uptake by the seed. In that regard, water uptake is an integral requirement for the initiation and completion of the germination process [37]. In contrast, when 40 mM KNO_3 was applied directly to the dry seed, there was a significant decrease in water uptake. However, when the application of low KNO_3 followed imbibition in distilled H_2O for 24 h, a contrasting effect on seedling growth was observed [12]. A minor effect was found at a low concentration (1 mM), whereas increased seedling FW and length were observed at a high concentration (30 mM). On the other hand, direct imbibition in 30 mM KNO_3 reduced both the fresh mass and length of seedlings [12].

4.1. Antioxidant Metabolism

There is limited information on the effect of KNO_3 priming on the antioxidant metabolism of plant seedlings. Only a few papers have reported some connection with POX, SOD, CAT and AOX enzymes [11,12]. In the present study, pea seedlings were found to contain very low DHAR activity, suggesting they mainly use MDHAR activity for ascorbate recycling, which utilises NADH as an electron donor. From an energy point of view, this is much more efficient than the DHAR pathway, which uses GSH as a source of reducing power [38]. Similarly, using the same pea cultivar, a higher MDHAR activity than that of DHAR was also reported at subcellular level [39,40]. Moreover, in pea seeds (cv. Alaska) imbibed in 20 mM H_2O_2 , no DHAR activity was recorded, leading to a DHA accumulation in pea seedlings [6]. In addition, the seedlings subjected to 0.25 mM KNO_3 treatment displayed 2-fold higher MDHAR activity than seedlings treated with 40 mM KNO_3 , suggesting a higher capacity to recycle ascorbate. A similar response was observed with APX and POX activity, indicating a reduced ability to control H_2O_2 levels in 40 mM KNO_3 -treated seedlings when compared to controls and seedlings treated with a low KNO_3 concentration. MDHAR activity showed a different response to KNO_3 treatments in comparison to that which occurred with APX and POX activity. In this sense, low KNO_3 treatment increased both MDHAR activity and the H_2O_2 -scavenging enzymes APX and POX, whereas high KNO_3 treatment produced a decrease in the activity of these enzymatic antioxidants. Both APX and some type of POXs can use ASC as an electron donor. These types of POXs can thus oxidise ASC and organic phenols at comparable rates [41]. Therefore, if APX and POX decreased, a lower level of ASC is oxidised and thus lower MDHA can be generated. This could explain the decline in MDHAR recorded by 40 mM KNO_3 treatment.

Similarly, H_2O_2 -primed pea seeds also showed an increase in APX and POX activity [6]. It may be that pea seedlings treated with 0.25 mM KNO_3 displayed an efficient regulation of ROS generation when compared to those treated with 40 mM KNO_3 , which in turn correlated with an enhanced seedling growth. The increase of POX and the maintenance of APX activity by KNO_3 treatment were also reported by Vidal et al. [12]. In tomato seeds, KNO_3 increased germination rates and enhanced SOD and catalase activities but had no effect on APX activity [11].

Information about the effect of KNO_3 treatments on the ascorbate pool is very scarce. Under the assayed conditions, KNO_3 progressively decreased ASC content. However, DHA only accumulated in seedlings treated with 40 mM KNO_3 . According to our results, this response suggests that an effect on the biosynthetic pathway of ascorbate could take place. These results are in contrast to those reported by Vidal et al. [12], who observed a 3-fold increase in ASC in response to 10 mM KNO_3 or 50 μM sodium nitroprusside (SNP, a NO-donor). This may reflect the differences in the experimental procedures between the present study and the one reported previously [12]. Regarding glutathione levels, 0.25 mM KNO_3 produced a decrease in both GSH and GSSG, leading to a slight increase in the redox state of the glutathione pool. Likewise, 20 mM H_2O_2 -treated pea seeds showed a decline in GSH and GSSG [6]. In this study, 40 mM KNO_3 had no effect on GSH but

reduced GSSG levels, thus increasing the redox state of the glutathione pool. The reduced accumulation in GSSG in both KNO₃ treatments can be linked to the observed increase in GR activity. Again, these results are in contrast to those reported by Vidal et al. [12], where no significant differences in GSH content with 10 mM KNO₃ were reported. In addition, these authors reported an accumulation of GSSG, which resulted in a decrease in the redox state of the glutathione pool. This highlights that the effect of KNO₃ treatment is highly dependent on the mode of application. Alternatively, decreased ASC and GSH levels in seed treated with 0.25 mM KNO₃ can be related to altered metabolism rather than reduced recycling of both molecules. In this sense, MDHAR, as well as DHAR and GR, activity was nearly 60% higher than in the control seedlings.

4.2. Plant Hormones

Potassium nitrate has been reported to affect GAs and ABA metabolism, though there is limited information available in this regard. It has been reported that exogenous KNO₃ or NO accumulation can modulate ABA and GAs content by increasing ABA catabolism as well as the up-regulation of GAs biosynthesis, respectively [15,18,20]. Vidal et al. [12] observed that KNO₃ or SNP treatments increased GAs levels and decreased ABA concentration in pea seedlings. These effects were reversed by incubation with cPTIO (a NO-scavenger), suggesting that, in part, the effect of KNO₃ in seedling growth and plant hormone levels may be due to partial generation of NO from KNO₃ [21]. In that sense, NO has a significant role in seed germination and the breaking of seed dormancy [9,22,27].

It is well known that GAs, together with ABA, are involved in seed dormancy and germination, promoting germination in many plant species [21,42]. In this study, a major proportion of GAs were present in the seedlings (GA₁ and GA₄), with only a small amount of GA₄ reported in the cotyledon. This suggests that during the growth and development of the seedling, GAs could be mobilised from the cotyledon to the rest of the plant, although an increase in GAs biosynthesis cannot be ruled out [20]. Hormone levels in particular organelles would seem to be dependent on metabolism and transportation [21]. GAS biosynthesis genes are expressed in different tissues in the embryonic axes of Arabidopsis seeds [42]. Therefore, it is likely that GAs and their precursors are actively transported inside the embryo and/or from the cotyledon to the embryo by specific transporters.

Decreased ABA levels in seedlings treated with 0.25 mM KNO₃ were correlated with enhanced seedling biomass. In addition, seeds treated with 40 mM KNO₃, which achieved the lowest seedling growth, also had the highest ABA levels in their seedlings. During seed dormancy breaking and germination in Arabidopsis, both a decline in ABA content and an accumulation of NO was reported [18]. This response was parallel to the induction of the *ABA-8-hydroxylase* gene, involved in ABA catabolism [15,18]. A decline in ABA levels has also been described in H₂O₂-treated pea seeds [6,8,43]. These authors suggested that this response can result from either the stimulation of ABA catabolism or the inhibition or slowdown of its biosynthesis.

As a result of the effect of KNO₃ on GAs and ABA content, a decrease in the ABA/GAs ratio was observed by 0.25 mM KNO₃, mainly in the seedlings, when compared to controls and samples treated with 40 mM KNO₃. It has been demonstrated that the ABA/GAs balance is crucial during the early stages of germination [44]. It has also been suggested that the key role of phytohormones such as ABA and GA during seed germination is interdependent with ROS metabolism [27]. A tight control of ROS production appears to be crucial for seed germination [45]. In fact, an accumulation of ROS and NO in germinating seeds, as well as an enhanced seed germination by the exogenous application of H₂O₂, has been reported [6,32,43]. Thus, KNO₃ can trigger a higher seedling growth by regulating ABA and GAs metabolisms, likely associated with an over-generation of NO from KNO₃.

5. Conclusions

The application of a low KNO₃ concentration to dry pea seeds promoted early seedling growth, which was linked to the maintenance (APX, SOD, POX) and/or the increase

(MDHAR, DHAR, GR) of antioxidant defences, leading to an efficient regulation of the ROS generation. In addition, and although 0.25 mM KNO₃ decreased GSH levels, a slight increase in the redox state of glutathione pool, which could be associated with an increase in GR, was observed. In general, a low KNO₃ concentration was associated with an increased GA₁ and decreased ABA in seedlings, which resulted in a decline in the ABA/GAs ratio. Data also suggest a modulation of GAs and ABA metabolism by KNO₃, in which a partial role of NO could not be ruled out. Furthermore, we suggest an interaction among KNO₃, antioxidant defences and the modulation of the ABA/GA ratio during the early growth of pea seedlings (Figure 5).

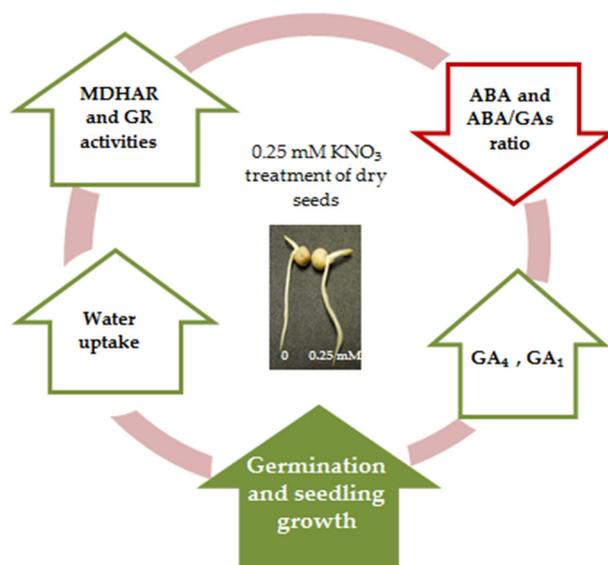


Figure 5. Simplified model summarising the effect of low KNO₃ concentration on promoting pea seed germination and seedling growth.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/seeds1010002/s1>, Table S1a: Effect of different KNO₃ treatments on the germination rate of pea seeds, Table S1b: Effect of different KNO₃ concentrations on some growth parameters in pea seedling after 4 days of treatments.

Author Contributions: Conceptualization, J.A.H. and P.D.-V.; methodology, J.A.H. and P.D.-V.; validation, J.A.H., P.D.-V., G.B.-E. and J.R.A.-M.; formal analysis, J.A.H. and J.R.A.-M.; investigation, J.A.H. and P.D.-V.; resources, J.A.H. and P.D.-V.; data curation, J.A.H., P.D.-V., G.B.-E. and J.R.A.-M.; writing—original draft preparation, J.A.H. and P.D.-V.; writing—review and editing, J.A.H., P.D.-V., G.B.-E. and J.R.A.-M. All authors have read and agreed to the published version of the manuscript.

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