Industrial Hemp (Cannabis sativa L.) Varieties and Seed Pre-Treatments Affect Seed Germination and Early Growth of Seedlings

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Abstract: Seed germination and seedling growth are two essential early determinants of subsequent crop yield and quality. A high germination percentage of industrial hemp (Cannabis sativa L.) seed is required to import into Australia. The viability of hemp seed can decline rapidly depending on storage and other factors; hence, the quality of imported seed is not always reliable. Here, we aimed to investigate germination and early seedling growth responses of 14 industrial hemp varieties after being imported from various countries. Germination trials were conducted with 100 seeds of 14 varieties using a soil-less Petri dish assay and a compost growth medium under glasshouse conditions. We also assessed the effect of seed pre-treatments such as gibberellic acid (500 and 1000 mg L⁻¹), chlorine dioxide (500 and 1000 mg L⁻¹) and cold temperature (4 °C for 72 h) using 300 seeds of each of the three selected varieties in compost growth medium. Hemp varieties imported from China had higher germination and better seedling growth indices than those imported from Europe. All seed pre-treatments were associated with a decreasing trend in germination, but a positive effect on early growth responses was observed. Our findings indicate that the hemp variety Han FNQ performed better than many other varieties did regarding seed germination and seedling growth. Hemp seeds sanitising with 500 mg L⁻¹ of chlorine dioxide might improve the germination and early growth of seedlings.

Keywords: industrial hemp; gibberellic acid; chlorine dioxide; cold temperature; Western Australia

1. Introduction

Industrial hemp (Cannabis sativa L., family Cannabaceae) is a herbaceous, cross-pollinated annual crop that originated in central Asia [1]. It has had numerous uses for over a millennium, including food, fibre and medicine [1,2]. Hemp is a tall (up to 2.5 m), fast-growing, short-day plant with a deep, fibrous tap root system [3]. It grows on fertile and well-drained land with neutral to slightly alkaline clay loam or silt loam soils [3]. Industrial hemp (bio-types low in tetrahydrocannabinol (THC)) is cultivated for fibre and seeds, whereas cannabis biotypes with high cannabidiol (CBD) are used for medicinal purposes [4].

Industrial hemp has been grown as a fibre and seed crop in Western Australia for many years but has gained additional interest after it became legal to use the seeds and seed oil for food products [5]. Seed germination is a fundamental process that affects crop yield and quality [6]. Therefore, a high germination percentage is an Australian importation requirement for hemp seed, with a recent germination test (within six months) recommended for determining seed quality [7]. However, the viability of industrial hemp...
seeds can decline rapidly depending on storage conditions and other unknown factors; hence, the quality of local and imported sources is frequently unreliable. In addition to seed germination, early seedling growth is crucial for determining the seedling vigour of industrial hemp (i.e., seedling size, health, and growth rate). Newly germinated seedlings are dependent on nutrient reserves in seeds until they become photosynthetically competent and develop a relatively large root system to explore the soil. The mobilisation efficiency of nutrient reserves is associated with seedling vigour, a key determinant of seedling establishment, crop growth and yield [8,9].

Seed germination and early seedling growth depend on several factors, including varieties with different seed sizes [10–12], seed sources [13–15] and seed origins [16,17]. They also respond differently to pre-treatments with chemicals, such as plant growth regulators [18–20], seed disinfectants [21–28] and cold temperatures [29–33].

The effects of varieties and seed pre-treatments on germination and early establishment have been studied in industrial hemp, but only on a few varieties [34–39]. Therefore, studying the effects of varieties could serve as the basis for determining the adaptability and suitability of this crop species. Furthermore, studying the effects of different seed pre-treatments could identify a suitable surface disinfection method for hemp seed to improve germination and growth. Therefore, we investigated the impact of varieties and seed pre-treatments on germination and early seedling growth of 14 industrial hemp varieties, hypothesising that both varieties and seed pre-treatments influence germination and early seedling growth of industrial hemp.

2. Materials and Methods

2.1. Plant Material

Seeds of 14 varieties of industrial hemp (Cannabis sativa L.) were collected from different sources in Western Australia originally imported from overseas (Table 1). Seeds were kept in resealable aluminium foil bags and stored in a 15 °C cool-temperature room at The University of Western Australia (31°98′ S, 115°81′ E). Seeds of most French monoecious varieties were obtained from the Western Australian Hemp Growers’ Co-op Ltd. (Hemp-Gro). The Chinese dioecious varieties and one locally grown French variety (Felina 32) were obtained from Premium Hemp Australia and WA Department of Primary Industries and Regional Development (DPIRD). One widely adopted and locally grown Danish monoecious variety (Morpeth) was obtained from Food, Fibre and Land International Group Pty Ltd. (FFLI). All seeds came coated with the fungicide Thiram, except for the two locally available varieties (Felina 32 and Morpeth), pre-treated with RICHGRO® Mancozeb plus sulphur fungicide.

2.2. Petri Dish Germination Assay (Experiment 1)

A germination experiment in a randomised complete block design with five replications was conducted at The University of Western Australia. Fifty seeds from each variety were used per germination test and placed onto filter paper soaked with 50 mL of Milli-Q® water in 5 Petri dishes (8.5 cm diameter). The Petri dishes were covered in aluminium foil and kept in the darkroom at 25 °C.

2.3. Glasshouse Germination Assay (Experiment 2)

The second germination experiment was carried out in a temperature-controlled glasshouse at The University of Western Australia (31°98′ S, 115°81′ E). The average temperature, light intensity and relative humidity inside the glasshouse were 19.9 °C, 0.90 W·m⁻² and 57.6%, respectively. The study used a randomised complete block design with five replications. Fifty seeds from each variety were used per germination test and sown about 2 cm deep in 5 plastic, mesh-bottom trays (29 cm × 34 cm), each filled with 2 kg of RICHGRO® UWA Plant Bio Mix M3 (Product Code: SSM2380). The moisture content of the potting mixture was maintained at 70% field capacity by weighing and watering on
alternate days. The plastic trays were re-randomised within the blocks every 5 d to reduce the effects of potential environmental gradients.

Table 1. List of 14 industrial hemp (Cannabis sativa L.) varieties locally available and imported into Western Australia.

| Variety | Sex Type | Country     | Seed Importer                                      |
|---------|----------|-------------|---------------------------------------------------|
| Ferimon | Monoecious | France      | WA Hemp Growers’ Co-op Ltd. (HempGro)             |
| Fedora 17 | Monoecious | France      | WA Hemp Growers’ Co-op Ltd. (HempGro)             |
| Santhica | Monoecious | France      | WA Hemp Growers’ Co-op Ltd. (HempGro)             |
| Felina 32 | Monoecious | France      | Premium Hemp Australia and DPIRD *                |
| Morpeth | Monoecious | Denmark     | Food, Fibre and Land International Group (FFLI)   |
| Bama 4  | Dioecious  | China       | Premium Hemp Australia and DPIRD                  |
| Han NE  | Dioecious  | China       | Premium Hemp Australia and DPIRD                  |
| Han FNH | Dioecious  | China       | Premium Hemp Australia and DPIRD                  |
| Yuma 1  | Dioecious  | China       | Premium Hemp Australia and DPIRD                  |
| Han FNQ | Dioecious  | China       | Premium Hemp Australia and DPIRD                  |
| Han NW  | Dioecious  | China       | Premium Hemp Australia and DPIRD                  |
| Puma 3  | Dioecious  | China       | Premium Hemp Australia and DPIRD                  |
| Han COLD | Dioecious | China       | Premium Hemp Australia and DPIRD                  |
| SI 1    | Dioecious  | China       | Premium Hemp Australia and DPIRD                  |

*DPIRD = Department of Primary Industries and Regional Development, WA.

2.4. Seed Pre-Treatments (Experiment 3)

This experiment was conducted in the same temperature-controlled glasshouse at The University of Western Australia as Experiment 2, with the same experimental design and growth conditions. Three hundred seeds of each of the three hemp varieties (Ferimon, Han NW and Morpeth) that were selected based on their poor results in the germination assays under both Petri dish and glasshouse conditions (germination < 50%) were used for seed pre-treatments. There were six treatments: (i) control (no seed pre-treatment), (ii) gibberellic acid (GA3) 500 mg·L⁻¹, (iii) GA3 1000 mg·L⁻¹, (iv) chlorine dioxide (ClO₂) 500 mg·L⁻¹, (v) ClO₂ 1000 mg·L⁻¹ and (vi) cold temperature (4 °C for 72 h). The doses for GA3, ClO₂ and cold-temperature treatments were determined based on the literature available on other crops as no research data were found on industrial hemp at the time of conducting experiments [18,26,31]. Seeds were rinsed with deionised H₂O and air-dried before the seed pre-treatments were applied. The commercial gibberellic acid powder was obtained from SIGMA-ALDRICH™ Pty Ltd., Castle Hill NSW 1765, Australia (Product No. G7645; CAS No. 77-06-5). For the ClO₂ treatments, commercial CleanOxide 4 g tablets were obtained from Natural Water SOLUTIONS®, Wangara WA 6065, Australia. Fifty seeds of each variety were soaked in 50 mL of GA3 (500 mg·L⁻¹ or 1000 mg·L⁻¹), 50 mL of ClO₂ (500 or 1000 mg·L⁻¹) or 50 mL of Milli-Q® water (control) in aluminium foil-wrapped plastic vials and kept at 25 °C for 24 h (with aeration for GA3 treatment) or 30 min for ClO₂ treatment. All chemically treated seeds were rinsed with flowing distilled water for 1 min before sowing. For the cold-temperature pre-treatment, fifty fresh seeds of each variety were placed in aluminium foil-wrapped plastic vials in a 4 °C cold room for 72 h before sowing. All seeds were sown in plastic trays, watered and re-randomised as described in Section 2.3 (Experiment 2).

2.5. Data Collection

Germination data were recorded every 24 h for 7 d for the Petri dish germination assay and 20 d for the glasshouse germination assay. The cumulative data were used to calculate the final germination percent (GP). Seedlings were harvested 20 days after sowing and measured for the proportion of normal seedlings (NS) and abnormal seedlings (AS), shoot length (SL), shoot dry weight (SDW), root dry weight (RDW), seedling growth rate (SGR), and seedling vigour index (SVI). Measurements of NS, AS and SVI are described in Gupta and Solanki [40] where NS were termed as "seedling showed the continued devel-
opment, noninfected and intact seedlings” and AS were defined as “damaged, deformed, unbalanced or decayed seedlings”. SL was measured as the average height of the shoot per plant. SDW and RDW were measured as the average weights of dried (oven-dried at 70 °C for 72 h) shoot and root biomass per plant, respectively. SGR was measured as the average increment in SL in millimetres per day and the SVI was calculated as percent germination multiplied by the shoot length (SL) in cm. Root growth indices (average root length (ARL), average root diameter (ARD), average fine root length (AFRL) and average coarse root length (ACRL)) were calculated after root scanning using an Epson Perfection V800 Photo Scanner® to generate image files. The images were cropped and analysed in “batch mode” using WinRHIZO™ software version 2009 (Regent Instruments Inc., Quebec City, QC, Canada), with the cut-off threshold values set to ≤0.2 mm for fine roots and >0.2 mm for coarse roots.

2.6. Statistical Analysis

Analysis of variance (ANOVA) was performed using the Genstat® software version 19.1 (VSN International Ltd., Hemel Hempstead, UK). One-way ANOVA was used to determine seed origin differences in the 14 industrial hemp varieties for germination and early growth indices (Experiments 1 and 2). Two-way ANOVA was employed to assess the effect of three hemp varieties, six seed pre-treatments and the interaction among them (Experiment 3). The post hoc Tukey’s test was used for multiple comparisons and to estimate significant differences among treatment means \((p \leq 0.05)\).

3. Results

3.1. Germination Response in Petri Dish Assay (Experiment 1)

Significant variations in the seed germination percent (GP) were observed among the 14 industrial hemp varieties in the Petri dish assay (Table 2), ranging from 17.0 to 70.0% (mean, 43.0%). Overall, the tested hemp varieties imported from China had a higher GP (47.0%) than the tested varieties imported from Europe (36.0%). The hemp varieties Han FNQ (70.0%), Han FNH (66.0%) and Han NE (62.0%) had higher GPs, whereas the varieties SI 1 (17.0%), Ferimon (18.0%) and Han NW (26.0%) had lower GPs.

Table 2. Seed weight and germination percent of 14 industrial hemp \((Cannabis sativa L.)\) varieties imported into Western Australia. One-way ANOVA showed the varieties differing significantly \((p \leq 0.05)\) for seed weight and percent of seed germination in Petri dish and glasshouse. Means within the same column followed by different letters differ significantly at \(p \leq 0.05\). *** = \(p < 0.001\).

| Seed Origin | Variety | TSW | GP (Petri Dish) | GP (Glasshouse) |
|-------------|---------|-----|----------------|-----------------|
| Europe      | Ferimon | 18.5 ef | 18.0 e | 46.0 cd |
|             | Fedora 17 | 21.1 de | 52.0 abcd | 74.0 abc |
|             | Santhica | 19.2 def | 28.0 de | 62.0 abcd |
|             | Felina 32 | 15.1 f | 42.0 abcd | 48.0 cd |
|             | Morpeth | 21.2 de | 38.0 bede | 48.0 cd |
|             | Mean     | 19.0 | 36.0 | 56.0 |
| China       | Bama 4 | 34.6 b | 40.0 bcde | 60.0 bcde |
|             | Han NE | 28.8 c | 62.0 ab | 64.0 abcd |
|             | Han FNH | 23.7 cde | 60.0 ab | 82.0 ab |
|             | Yuma 1 | 35.3 b | 54.0 abcd | 70.0 abc |
|             | Han FNQ | 24.0 cd | 70.0 a | 92.0 a |
|             | Han NW | 26.8 c | 26.0 de | 38.0 de |
|             | Puma 3 | 35.6 b | 32.0 cde | 66.0 abcd |
|             | Han GOLD | 35.5 b | 58.0 abc | 66.0 abcd |
|             | SI 1 | 62.3 a | 17.0 e | 13.0 e |
|             | Mean | 34.1 | 47.0 | 61.0 |
|             | Minimum | 15.1 | 17.0 | 13.0 |
|             | Maximum | 21.2 | 70.0 | 92.0 |
|             | Mean | 28.7 | 43.0 | 59.0 |
|             | \(p\)-value | <0.001 *** | <0.001 *** | <0.001 *** |
3.2. Germination Response in the Glasshouse (Experiment 2)

The germination percent varied significantly among the 14 hemp varieties in the glasshouse assay, ranging from 13.0 to 92.0% (mean 59.0%) (Table 2). The tested varieties from China had a higher GP (61.0%) than the tested varieties from Europe (56.0%). The varieties Han FNQ (92.0%), Han FNH (82.0%) and Fedora 17 (74.0%) had higher GPs, whereas the varieties SI 1 (13.0%), Han NW (38.0%) and Ferimon (46.0%) had lower GPs.

3.3. Germination Response after Seed Pre-Treatments (Experiment 3)

The pre-treatment effect on GP was significant, whereas the main effect of variety and the interaction effect of variety × treatment did not significantly influence GP. Compared with the control (CTRL, 56.0%), the GP was significantly lower in seeds treated with 1000 mg·L⁻¹ of GA₃ (GA2, 28.0%). There was a decreasing nonsignificant trend in GP in all other seed pre-treatments compared to the control (Figure 1a).

![Figure 1. Cont.](image-url)
Figure 1. The main effect of seed pre-treatment on (a) germination, (b) proportions of normal and abnormal seedlings and (c) seedling vigour index of industrial hemp (*Cannabis sativa* L.). Means followed by different letters indicate significant differences in seed germination, normal and abnormal seedlings, and seedling vigour index among six seed pre-treatments. Error bars in each column represent the standard error of mean (±SE) for the corresponding seed pre-treatment. Two-way ANOVA showed significant main effects of seed pre-treatments (on percent of seed germination, normal and abnormal seedlings, and seedling vigour index), but the main effect of variety and interaction were insignificant. The data on individual seed pre-treatments were averaged across three varieties (n = 18). CTRL = control (no seed pre-treatment); GA1 = 500 mg L\(^{-1}\) GA3; GA2 = 1000 mg L\(^{-1}\) GA3; CLO1 = 500 mg L\(^{-1}\) ClO\(_2\); CLO2 = 1000 mg L\(^{-1}\) ClO\(_2\); COLD = cold temperature (4 \(^\circ\)C for 72 h).

3.4. Seedling Growth Response in the Glasshouse (Experiment 2)

The seedling growth indices—NS, AS, RDW, SL, SGR and SVI—varied significantly among the 14 hemp varieties (Table 3). The varieties Han FNQ, Morpeth and Felina 32 produced 100% NS followed by Han FNH (97.0%), Han NE (97.0%), Fedora 17 (95.0%), Yuma 1 (94.0%), Santhica (91.0%) and Puma 3 (90.0%). Bama 4 (32.0%), SI 1 (25.0%), Han NW (21.0%), Han COLD (19.0%) and Ferimon (12.0%) produced higher ASs. SI 1 had the highest RDW (25.4 mg plant\(^{-1}\)), while the other varieties were statistically indistinguishable. SL and SGR were found higher for the varieties, Han NE (10.5 cm and 5.2 mm d\(^{-1}\)), Han FNQ (9.3 cm and 4.6 mm d\(^{-1}\)) and Puma 3 (8.8 cm and 4.4 mm d\(^{-1}\)), while Han NW (5.1 cm and 2.5 mm d\(^{-1}\)), Morpeth (5.1 cm and 2.6 mm d\(^{-1}\)) and Ferimon (5.9 cm and 3.0 mm d\(^{-1}\)) had the lower values. Han FNQ (853.8), Han NE (666.6), Puma 3 (602.2) and Fedora 17 (598.6) had higher SVIs, while SI 1 (83.0), Han NW (206.6), Morpeth (252.2) and Ferimon (269.4) had the lower values.

3.5. Seedling Growth Response after Seed Pre-Treatments (Experiment 3)

The interaction of variety × treatment had a significant effect only on SL and SGR. In contrast, the main effects of variety and seed pre-treatments on NS and AS were significant, and the pre-treatments effect also significantly influenced SVI. Treatments did not have a significant impact on NS and AS compared to the control (Figure 1b). Compared among treatments, seeds treated with 500 mg L\(^{-1}\) of ClO\(_2\) (CLO1) had higher NSs and lower ASs (97.0% and 3.0%, respectively) than the seeds treated with 500 mg L\(^{-1}\) (GA1, 66.0% and 34.0%, respectively) and 1000 mg L\(^{-1}\) of GA3 (GA2, 64.0% and 36.0%, respectively) (Figure 1b). Seeds treated with 500 mg L\(^{-1}\) of GA3 (GA1) had significantly higher SVIs (587.8) than seeds treated with cold temperature (COLD, 290.2), 500 mg L\(^{-1}\) (CLO1, 264.9) and 1000 mg L\(^{-1}\) (CLO2, 259.3) of ClO\(_2\) (Figure 1c). The variety Morpeth had a significantly higher NS and lower AS (88.0% and 12.0%, respectively) than the variety Ferimon (66.0% and 34.0%, respectively) (Figure 2). SL and SGR significantly differed among the treatments within the Morpeth and Han NW varieties, but not in Ferimon. The SL and SGR were
higher for Morpeth treated with 1000 mg·L\(^{-1}\) of GA\(_3\) (GA2, 20.1 cm and 10.1 mm·d\(^{-1}\), respectively), Han NW treated with 1000 mg·L\(^{-1}\) of GA\(_3\) (GA2, 17.7 cm and 8.9 mm·d\(^{-1}\), respectively) and Morpeth treated with 500 mg·L\(^{-1}\) of GA\(_3\) (GA1, 17.4 cm and 8.7 mm·d\(^{-1}\), respectively) (Figure 3).

Table 3. Seedling growth responses of 14 industrial hemp (\textit{Cannabis sativa} L.) varieties in glasshouse (Experiment 2). One-way ANOVA showed the varieties differing significantly (\(p \leq 0.05\)) for different seedling growth attributes. Means within the same column followed by different letters differ significantly at \(p \leq 0.05\). * = \(p < 0.05\); ** = \(p < 0.01\); *** = \(p < 0.001\); ns = not significant (\(p \geq 0.05\)).

| Varieties   | NS   | AS    | SDW  | RDW  | SL    | SGR  | SVI     |
|------------|------|-------|------|------|-------|------|---------|
| Ferimon    | 88.0 | abcd  | 12.0 | abc  | 69.0  | 6.4 b | 5.9 def  |
| Fedora 17  | 95.0 | abc   | 5.0  | bc   | 76.8  | 6.6 b | 81 abc d |
| Santhica   | 91.0 | abc   | 9.0  | bc   | 86.7  | 8.8 b | 8.6 abc |
| Felina 32   | 100.0| a     | 0.0  | c    | 89.4  | 10.8 b| 7.8 abc d|
| Morpeth     | 100.0| a     | 0.0  | c    | 64.3  | 11.9 b| 5.1 ef |
| Bama 4      | 68.0 | d     | 32.0 | a    | 79.1  | 6.3 b | 6.1 cdef |
| Han NE      | 97.0 | abc   | 3.0  | bc   | 97.0  | 11.6 b| 10.5 a  |
| Han FNH     | 97.0 | ab    | 3.0  | bc   | 62.1  | 7.9 b | 7.2 bcdef|
| Yuma 1      | 94.0 | abc   | 6.0  | bc   | 87.0  | 9.6 b | 7.7 bcde |
| Han FNQ     | 100.0| a     | 0.0  | c    | 71.7  | 7.7 b | 9.3 ab  |
| Han NW      | 79.0 | abcd  | 21.0 | abc  | 59.3  | 7.8 b | 5.1 f  |
| Puma 3      | 90.0 | abc   | 10.0 | bc   | 117.8 | 10.0 b| 8.8 ab  |
| Han COLD    | 77.0 | bcd   | 19.0 | abc  | 90.9  | 9.0 b | 7.3 bcdef|
| SI 1        | 75.0 | cd    | 25.0 | ab   | 117.9 | 25.4 a| 6.1 cdef|

Minimum  | 68.0 | 0.0  | 59.3 | 6.3  | 5.1   | 2.5  | 83.0    |
Maximum  | 100.0| 32.0 | 117.9| 25.4 | 10.5  | 5.2  | 853.8   |
Mean     | 89.0 | 10.0 | 83.5 | 10.0 | 7.4   | 3.7  | 462.4   |
P-value   | 0.008** | 0.017* | 0.121ns | <0.001*** | <0.001*** | <0.001*** | <0.001*** |

Figure 2. Main effect of variety on the proportions of normal and abnormal seedlings of industrial hemp (\textit{Cannabis sativa} L.). Means followed by different letters indicate significant differences for percent of normal and abnormal seedlings among the three varieties. Error bars in each column represent the standard error of mean (±SE) for the percent of normal/abnormal seedlings. Two-way ANOVA showed significant main effects of variety and seed pre-treatments, but the interaction was not significant. The data on individual varieties were averaged across six seed pre-treatments (n = 18).
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17 (83.5 cm) had the lower values. SI 1 (0.31 mm), Han NW and Han COLD (0.28 mm), and 14 hemp varieties (Table 4). ARL was higher for the varieties, SI 1 (255.5 cm), Morpeth (246.3 cm) and Puma 3 (200.1 cm), while Bama 4 (43.2 cm), Ferimon (82.6 cm) and Fedora 18). 3.7. Root Growth Response after Seed Pre-Treatments (Experiment 3) 3.6. Root Growth Response in the Glasshouse (Experiment 2) 3.6. Root Growth Response in the Glasshouse (Experiment 2) Figure 3. Interaction effect of variety × seed pre-treatment on (a) shoot length (cm) and (b) shoot growth rate (mm d−1) of industrial hemp (Cannabis sativa L.). Means followed by different letters indicate significant differences for shoot length and shoot growth rate among six seed pre-treatments within and across three varieties. Error bars in each column represent the standard error of mean (±SE) for the corresponding seed pre-treatment. CTRL = control (no seed pre-treatment); GA1 = 500 mg L−1 of GA3; GA2 = 1000 mg L−1 GA3; CLO1 = 500 mg L−1 ClO2; CLO2 = 1000 mg L−1 ClO2; COLD = cold temperature (4 °C for 72 h). 3.6. Root Growth Response in the Glasshouse (Experiment 2) The root growth indices—ARL, ARD and ACRL—varied significantly among the 14 hemp varieties (Table 4). ARL was higher for the varieties, SI 1 (255.5 cm), Morpeth (246.3 cm) and Puma 3 (200.1 cm), while Bama 4 (43.2 cm), Ferimon (82.6 cm) and Fedora 17 (83.5 cm) had the lower values. SI 1 (0.31 mm), Han NW and Han COLD (0.28 mm), and Ferimon (0.27 mm) had the higher ARD, while Felina 32 (0.19 mm), Han FNH (0.20 mm), Han NE and Santhica (0.22 mm) had the lower ARD. ACRL was higher for the varieties Morpeth (79.4 cm), SI 1 (78.9 cm) and Puma 3 (57.4 cm), while it was lower for Bama 4 (10.0 cm), Felina 32 (15.3 cm), Ferimon (17.3 cm) and Fedora 17 (19.4 cm).
Table 4. Root growth responses of 14 industrial hemp (*Cannabis sativa* L.) varieties in glasshouse (Experiment 2). One-way ANOVA showed the varieties differing significantly (*p* ≤ 0.05) for different root growth attributes. Means within the same column followed by different letters differ significantly at *p* ≤ 0.05. ** = *p* < 0.01; *** = *p* < 0.001; ns = not significant (*p* ≥ 0.05). ARL = average root length (cm); ARD = average root diameter (mm); AFRL = average fine root length (cm); ACRL = average coarse root length (cm).

| Varieties   | ARL    | ARD    | AFRL  | ACRL  |
|-------------|--------|--------|-------|-------|
| Ferimon     | 82.6 bc| 0.27 abc| 65.3  | 17.3 bc |
| Fedora 17   | 83.5 bc| 0.24 abc| 64.1  | 19.4 bc |
| Santhica    | 129.3 abc| 0.22 bc| 99.1  | 30.2 bc |
| Felina 32   | 102.3 bc| 0.19 c  | 87.0  | 15.3 bc |
| Morphet     | 246.3 a| 0.26 abc| 166.9 | 79.4 a  |
| Bama 4      | 43.2 c | 0.26 abc| 33.2  | 10.0 c  |
| Han NE      | 128.5 abc| 0.22 bc| 98.1  | 30.4 bc |
| Han FNH     | 131.6 abc| 0.20 bc| 107.1 | 24.5 bc |
| Han NW      | 147.9 ab | 0.24 abc| 106.7 | 41.2 ab |
| Han COLD    | 153.2 abc| 0.28 ab | 110.9 | 42.3 abc|
| SI 1        | 255.5 a| 0.31 a  | 176.6 | 78.9 a  |

Minimum 43.2 0.19 33.19 10.0
Maximum 255.5 0.31 176.62 79.4
Mean 140.6 0.25 102.97 37.7
*p*-value 0.037 ** <0.001 *** 0.077 ns 0.006 **

3.7. Root Growth Response after Seed Pre-Treatments (Experiment 3)

The main effect of variety and interaction effect of variety × treatment on root growth indices (ARL, ARD, AFRL and ACRL) were nonsignificant. In contrast, the pre-treatment effects on ARD and ACRL were significant. Seeds treated with 1000 mg L⁻¹ of GA₃ had significantly higher ARDs (GA2, 0.46 mm) than seeds treated with cold temperature (COLD, 0.33 mm), 500 mg L⁻¹ of GA₃ (GA1, 0.30 mm) and the control (CTRL, 0.25 mm) (Figure 4a). Seeds treated with 500 mg L⁻¹ of ClO₂ (CLO₁) had higher ACRLs (122.3 cm) than seeds treated with 500 mg L⁻¹ of GA₃ (GA1, 72.7 cm) and the control (CTRL, 67.1 cm) (Figure 4b).
4. Discussion

4.1. Varieties Have Impacts on Germination and Early Growth of Industrial Hemp

Overall, the varieties imported from China had 33.0% and 10.3% higher GPs under Petri dish and glasshouse conditions, respectively, than the varieties imported from Europe. Gallagher and Wagenius [14] reported that seeds collected from different provenances impact the germination and seedling survival of some dominant C₄ grass species. In coneflowers (Echinacea spp.), germination was higher for seeds collected from commercial organic seed sources than public germplasm sources [15]. Guavira (Campomanesia adamantium (Cambess.) O. Berg) seeds collected from the local market had a lower germination than fresh seeds immediately sown after harvesting [17].

The hemp varieties differed in seed size, with the dioecious varieties imported from China having 79.5% larger seeds than the monoecious varieties imported from Europe. The impact of seed size on germination was reported by Manonmani and Vanangamudi [12] in Indian sandalwood (Santalum album) seeds, who recommended selecting large seeds for higher GP and seedling vigour. Seed size also influences seedling growth parameters. Cicek and Tilkı [11] reported that larger seeds of sweet chestnut (Castanea sativa Mill.) had seedlings with a higher survival rate, seedling diameter, height and dry weight. In Cryptocarya alba (Mol.) Looser (a common tree in the Chilean Matorral), seedlings from larger seeds had a larger shoot and higher dry biomass (root, shoots and leaves) [10].

The hemp varieties also significantly differed in seedling growth indices (except SDW). The effect of seed source on seedling growth attributes has been reported in Heinsia crinita (Afzel.) G. Taylor (a common vegetable in south-eastern Nigeria) where seedlings from a specific seed source (i.e., provenance) had the highest shoot and root length, collar diameter and seedling dry weight [13]. The hemp varieties from China had a higher SL and SGR (6.4%), and higher SVI (20.0%) and RDW (19.4%) than the hemp varieties from Europe. Therefore, growers might include the varieties imported from China in adaptation trials in Western Australia alongside other locally grown and imported varieties from Europe. In parallel, variety specific trials should also be considered.
4.2. Gibberellic Acid May Decrease Seed Germination but May Promote Early Growth of Industrial Hemp

The effect of gibberellic acid (GA$_3$), a well-known phytohormone that regulates plant growth, has been rarely studied in industrial hemp, though it has been well studied in other crops [18,20,41–44].

Hemp seeds treated with 1000 mg L$^{-1}$ of GA$_3$ had a 50.5% lower GP than the control. The occurrence of a lower GP due to GA$_3$ treatment was also reported by Zhu, et al. [45] in sweet sorghum (Sorghum bicolor [L.] Moench), with the lower GP attributed to lower water uptake by the seeds. Seeds treated with either 500 or 1000 mg L$^{-1}$ of GA$_3$ had 942.4% and 978.8% higher ASs than those treated with 500 mg L$^{-1}$ of ClO$_2$. In Guavira (Campomanesia adamantium) (Cambess.) O. Berg), a higher AS is attributed to the harvest time and storage conditions of the source seeds [17].

GA$_3$ significantly increased SL and SGR by approximately 250.0% and 150.0% than the control when Han NW seeds were treated with 1000 mg L$^{-1}$ of GA$_3$ and Morpeth seeds treated with 500 mg L$^{-1}$ of GA$_3$, respectively. Cornea-Cipcigan, et al. [46] reported a higher plant height with increased seedling vigour in Cyclamen species (swinebread spp.) when plants were treated with foliar application of GA$_3$.

GA$_3$ also improved the early root growth of industrial hemp, particularly an 84.0% higher ARD than the control when seeds were treated with 1000 mg L$^{-1}$ of GA$_3$. In tomato (Solanum lycopersicum L.), soaking seeds in 900 mg L$^{-1}$ of GA$_3$ produced the highest germination, root length and plant height than in 0, 300 and 600 mg L$^{-1}$ of GA$_3$ [18]. This might indicate that higher concentrations of GA$_3$ can be harmful for hemp seed germination, while it may promote early seedling growth in some varieties. However, variety-specific studies should be conducted further with lower concentrations of GA$_3$.

4.3. Chlorine Dioxide Enhances Root Growth of Industrial Hemp Seedlings

Chlorine dioxide (ClO$_2$), a chlorine-based disinfectant, is known for its fungicidal, bactericidal and viricidal properties and is effective in decontaminating seeds and sprouts from a wide range of microorganisms and human pathogens [22–24]. However, the effect of ClO$_2$ on seed germination and seedling growth of industrial hemp has not been studied so far but has recently been explored in other crops, including vegetables [23], cucurbits [24] and barley [26].

Chlorine dioxide (ClO$_2$) did not significantly decrease GP in hemp seeds compared with the control. In barley (Hordeum vulgare L.), GP decreased by 94.5% and 100% compared to the control (0 mg L$^{-1}$ of ClO$_2$) when seeds were treated with 1000 and 2000 mg L$^{-1}$ of ClO$_2$, respectively [26]. However, no significant differences were observed in GP between barley seeds treated with 500 mg L$^{-1}$ of ClO$_2$ and the control. In addition, the germination of lucerne seeds and other sprouts pre-soaked with 200 mg L$^{-1}$ of ClO$_2$ did not significantly differ from untreated seeds [23].

The ClO$_2$ treatments increased the root growth of industrial hemp, with an 82.3% higher ACRL and 64.0% higher ARD than the control when seeds were treated with 500 and 1000 mg L$^{-1}$ of ClO$_2$, respectively. In addition, barley seeds treated with 500 and 1000 mg L$^{-1}$ of ClO$_2$ significantly increased the growth of barley roots (fresh weight, total length and root number) [26]. The mechanisms by which ClO$_2$ promoted root growth were by regulating antioxidant enzymes (catalase and peroxidase), cell membrane H$^+$-ATPase, root activity and lignin content [26]. Overall, lower concentrations of ClO$_2$ (<500 mg L$^{-1}$) do not affect seed germination and promote root growth, whereas higher concentrations (≥1000 mg L$^{-1}$ ClO$_2$) can inhibit seed germination with improved root growth.

4.4. Cold Pre-Treatment Does Not Influence Germination and Early Growth of Industrial Hemp

Pre-treating seeds with cold temperature (usually, 0–10 °C) breaks physiological dormancy in water-permeable seeds and combinational dormancy (occurrence of physiological and physical dormancy) in some water-impermeable seeds [29,30]. Cold pre-treatment influences seed germination in many halophyte species and species grown in temperate conditions.
or Mediterranean areas [29,31,32]. However, the impact of cold pre-treatments on seed germination of industrial hemp had not been directly studied.

Cold pre-treatment had no significant effect on germination and early seedling growth of industrial hemp. The GP, NS, AS, SVI, SL, SGR, ARD and ACRL did not differ significantly from the control when seeds were treated with 4 °C for 72 h. In dimorphic seeds of *Atriplex centralasiatica* (a herbaceous salt-secreting halophyte), cold stratification (4 °C for 4 d) increased germination, but did not improve the final GP [31]. Cold stratification at 5 °C for 3 months decreased the germination of *Clinopodium sandalioticum* (wild aromatic half-shrub endemic in Sardinia Island, Italy) seeds [32]. In addition, the root growth of sugarcane seedlings was inhibited by cold stress, and the average root length and root volume were decreased after cold treatment (4 °C for 7 d) as compared to the control [33]. Thus, a short-duration cold pre-treatment might not be effective for hemp seed germination and early growth of seedlings, as observed; rather, a prolonged period of cold pre-treatment or cold-moist stratification should be tested further to measure the effects.

5. Conclusions

Overall, the 14 hemp varieties differed significantly in seed germination and early seedling growth indices. Of them, the variety Han FNQ had a significantly higher seed germination under both Petri dish and glasshouse conditions, as well as a higher seedling length, shoot growth rate and seedling vigour index than the varieties, Ferimon, Morpeth, Bama, Han NW and SI 1. All seed pre-treatments (500 and 1000 mg L⁻¹ of GA₃; 500 and 1000 mg L⁻¹ of ClO₂ and cold pre-treatment at 4 °C for 72 h) were associated with a decreasing trend in germination, but a positive effect on early growth responses such as shoot and root growth was observed. However, the application of a lower concentration of ClO₂ (≤500 mg L⁻¹) might be beneficial for hemp seed germination and seedling growth.

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**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| TSW          | thousand seed weight (g) |
| GP           | % germination |
| NS           | % normal seedling |
| AS           | % abnormal seedling |
| SDW          | shoot dry weight plant⁻¹ (mg) |
| RDW          | root dry weight plant⁻¹ (mg) |
| SL           | shoot length (cm) |
| SGR          | shoot growth rate (mm·d⁻¹) |
| SVI          | seedling vigour index = % germination × seedling length (cm) |
| ARL          | average root length (cm) |
| ARD          | average root diameter (mm) |
| AFRL         | average fine root length (cm) |
ACRL average coarse root length (cm)
CTRL control (no seed pre-treatment)
GA1 500 mg L⁻¹ GA₃
GA2 1000 mg L⁻¹ GA₃
CLO1 500 mg L⁻¹ ClO₂
CLO2 1000 mg L⁻¹ ClO₂
COLD cold temperature (4 °C for 72 h).

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