

Seed Soaking Times and Irrigation Frequencies Affected the Nutrient Quality and Growth Parameters of *Hordeum vulgare* L. Cultivated in Hydroponics

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Abstract

The choice of hydroponic systems for fodder production is of great importance to Sub-Saharan Africa and specifically South Africa, considering the current water crisis. This study investigated the impacts of seed soaking times and irrigation frequency on the vegetative and nutritive properties of *Hordeum vulgare* grown in a hydroponic room. *H. vulgare* seeds were weighed and soaked in sterile containers filled with 500 mL solution of 20% solution of sodium hypochlorite for 1, 3, 8, 16 and 24 h at room temperature. Once soaked, the seeds were transferred to a hydroponic system and irrigated using flood irrigation. After the 8-day growing period, growth parameters were measured, and samples were oven-dried, pulverized and then subjected to nitrogen and protein analysis. It was observed that shorter soaking time with varied irrigation frequencies had the highest impact on the weight, and nutrient yield of *H. vulgare* although other growth parameters investigated such as leaf length and root map expansion deviated from this trend under different soaking times and irrigation frequencies. This study revealed that a 1-h pre-soaked treatment was the best for cultivating barley hydroponically. This treatment is recommended for the cultivation of barley as it proved to be beneficial to the farmer in terms of quality yield.

Keywords: barley, fodder feed, forage crop, organic fodder, subsistence farming

1. Introduction

Hordeum vulgare (barley) was one of the first crops to be domesticated for human consumption and is still one of the most important cereal crops grown worldwide [1, 2]. Due to its high nutritional value and palatability, barley is the fourth-largest cereal in terms of grain production worldwide, with nearly 60% used as animal feed, around 30% used for malt production, 7% used for seed production, and only 3%

used for human food [3]. The South African Department of Agriculture, Forestry, and Fisheries reported 421,800 tons of barley production in 2018. According to the report, the country's high maize production hampers the feed market for barley, so barley is grown specifically for malting. Furthermore, because barley production is limited to winter rainfall areas, there is a need to optimize cultivation techniques so that it can be produced year-round [4].

Globally, the demand for feeds and forage has increased due to the increase in the livestock population [5]. Hydroponically sprouted cultivars are used as a dietary supplement for animals in South Africa, the United Kingdom, the United States of America, Australia, and other parts of the world due to their ease of germination and growth [6, 7]. Besides, various authors have reported different harvesting and growth cycles for a barley fodder mat, ranging from a 6-day harvest cycle to a 10-day harvest cycle, implying that hydroponically produced fodder has a short growth period [8, 9]. The quality and quantity of fodder produced by hydroponics are also of interest. Farmers in India, for example, discovered that feeding their dairy cattle hydroponically grown barley increased milk yield from 0.5 to 2.5 l/day. They discovered an improvement in animal health as well as the fat content of their cow's milk in addition to the increased yield [10].

Climate change is affecting agriculture and natural water resources all over the world, which has an impact on the sustainability of food and water resources [11]. Because water and agriculture are inextricably linked and vital to most societies' economies and security, hydroponic cultivation ensures year-round production while consuming less water [9, 12], as opposed to run-to-waste systems in field production [13]. It was reported earlier that hydroponic production used only about 2% of the water required for field production of the same crop [14] thus, the introduction of hydroponic systems for fodder production can help overcome the challenges encountered in conventional production.

Previous research has shown that soaking barley seed before sowing increases the rate of germination, softens the seed coat, and breaks seed dormancy, though the number of hours recommended for soaking barley seed ranged from 3 to 28 h [15–18]. Similarly, the importance of using clean seeds for cultivation and seed sterilization during the soaking procedure cannot be overstated [9, 19]. Soaking seeds in a solution of 20% (bleach) for 30 min was recommended to prevent the formation of any fungal contamination [20, 21]. However, [22] using Mercuric chloride to prevent the proliferation of fungal contaminants while [23] tested both the effects of sodium hypochlorite (NaOCl) and Mercuric chloride (HgCl₂) on a range of pathogens and proposed that surface sterilization of the seed is important to remove unwanted fungal growth.

Although earlier studies showed a wide range of water types, including both mist and flood irrigation [14, 24, 25], there is a scarcity of data on the amount and frequency of water/irrigation used in a hydroponic chamber to germinate barley seed. This was necessary to address the challenges of nutrient imbalance in hydroponic systems, soil quality and productivity, and soil-based ecosystem services [26–28] affecting barley biomass production. In this study, an 8-day harvest cycle was used in conjunction with post-germination irrigation frequency to determine the most effective method to break seed dormancy, cause germination, and grow into a seedling mat and see the impacts of these treatments on the nitrogen value, protein content, fresh weight, and dry weight of the seedling forage mat post-harvest compared to the original untreated seeds. Because soil-based farming is facing serious challenges

in South Africa and other developing countries, fodder crop producers can use the study's findings to obtain critical information that will aid in the optimization of inputs and the efficient utilization of resources in hydroponic fodder production.

2. Materials and methods

2.1 Source of seeds

Viable seeds of *H. vulgare* sv13 were obtained from Kaap Agri Bedryf Ltd. located in Malmesbury, Western Cape. The seeds used originated from the Swartland District of the Western Cape. The seeds were first weighed on a balance that measures in 100 g increments.

2.2 Experimental design and hydroponic setup

The experiment was carried out in the plant tissue culture laboratory at the Cape Peninsula University of Technology's Bellville Campus. A 230 cm × 450 cm growing room was used to control light and temperature and determine the best growing conditions. Shelving units measuring 200 cm in height, 127 cm in length, and 40 cm in depth were installed in the growing room. The shelving unit had six shelves that were 37 cm apart and measured 120 cm × 40 cm. Two fluorescent light bulbs were installed on each shelf. For drainage, a corrugated fiberglass sheet was cut to the size of the shelf below and positioned at a 55-degree angle. The front, bottom end was fitted with a D-shaped gutter. This was used to collect the runoff from the fiberglass sheets. The run-off was then directed back to a sump via the gutter, resulting in an ebb and flow closed watering system. After cleaning and soaking the seeds, they were placed in perforated aluminum containers measuring 10 cm × 20 cm. The perforations were evenly spaced across the tray's bottom surface, with approximately 2 cm between each perforation. There was no need for a medium because the seeds germinated and formed a root mat that held the seedlings in place. The seed trays were then placed on the fiberglass sheeting, and each tray was fitted with an irrigation tube. Irrigation water was delivered to the seeds in their respective trays using a pump (HJ 1542 submersible), which delivered 622.5 mL/min to each tray for 2 min, for a total of 1245 mL. The pump was linked to a timer (MajorTech model MTD7), which controlled the amount of water delivered to each tray [29, 30]. Before the treated seeds were placed in the growing system, the entire setup, including the sump, Perspex shelves, and seed containers, was thoroughly cleaned and disinfected. The sump was filled with deionized water containing a 20% sodium hypochlorite solution, and the system was flushed to disinfect all surfaces [13].

The temperature of the room was kept at 23°C, as it was found that a temperature range of 20–30°C did not have a significant impact on growth [25, 31]. Two Samsung Smart Inverter™ air conditioners were used to regulate the temperature. Fresh air was brought into the growing chamber through heap filters from outside the building. Lighting was provided with fluorescent tubes [32, 33]. The fluorescent bulbs used were Osram (L36/640) cool white fluorescent tubes with a light output of 5.96 kilo lux. The ExTech—Heavy Duty Digital Light Meter, model number HD 400, was used to measure the intensity of the light. A Panasonic TB178K timer control unit was used to set the lighting system to provide a photoperiod of 16 h day/8 h night [34, 35].

Treatment code	Description	Treatment code	Description	Treatment code	Description	Treatment code	Description	Treatment code	Description
T1	1 h soak-2 h irrigation	T6	3 h soak-2 h irrigation	T11	8 h soak-2 h irrigation	T17	16 h soak-2 h irrigation	T21	24 h soak-2 h irrigation
T2	1 h soak-4 h irrigation	T7	3 h soak-4 h irrigation	T12	8 h soak-4 h irrigation	T18	16 h soak-4 h irrigation	T22	24 h soak-4 h irrigation
T3	1 h soak-8 h irrigation	T8	3 h soak-8 h irrigation	T13	8 h soak-8 h irrigation	T19	16 h soak-8 h irrigation	T23	24 h soak-8 h irrigation
T4	1 h soak-10 h irrigation	T9	3 h soak-10 h irrigation	T14	8 h soak-10 h irrigation	T19	16 h soak-10 h irrigation	T24	24 h soak-10 h irrigation
T5	1 h soak-12 h irrigation	T10	3 h soak-12 h irrigation	T15	8 h soak-12 h irrigation	T20	16 h soak-12 h irrigation	T25	24 h soak-12 h irrigation

Table 1. Treatments of *H. vulgare* seeds soaked in 500 mL distilled water diluted with a 20% solution of sodium hypochlorite under different irrigation frequencies

2.3 Treatment preparation

There were 25 treatments, each with 10 repetitions. Each treatment included a pre-soaking period followed by a post-soaking irrigation period (**Table 1**). Each repetition began with 100 g of viable seeds placed in a sterile plastic container containing 500 mL of distilled water containing a 20% solution of sodium hypochlorite (bleach) at room temperature [8, 14]. It was decided to test a range of seed soaking times, namely: 1, 3, 8, 16 and 24 h, which was compared against the control of 16 h. After the allotted soaking time, the seeds were washed in running, deionized water and placed in their respective growing trays without being exposed to darkness. Each tray was 10 cm × 20 cm in size. This ensured that the washed seeds had a depth of 1 cm. After that, the containers were placed in the hydroponic system to germinate (**Figures 1 and 2**). The seeds were allowed to germinate and grow into a forage mat for 8 days at 23°C under a photoperiod of 16-h day/8-h darkness.

Drip irrigation tubes were used to flood each seed tray with 1245 mL of water, with the excess running off through drainage holes in the seed container. The runoff was collected and channeled back into the sump of the hydroponic system for reuse. When necessary, the sump was refilled with distilled water mixed with a 20% bleach solution to ensure disinfection. The five previously mentioned treatments were subjected to five different irrigation intervals, each with 10 repetitions. Flood irrigation was used to fill each seed tray with water every 2; 4; 8; 10; and 12 h, with the control being a 2 hourly water interval [24, 36].

2.4 Data collection

Before removing the seedlings from their trays at the end of the 8-day growing cycle, a grid of 2 cm × 2 cm blocks was placed over the surface of the container, dividing the space into 50 blocks. This was used to determine the average leaf height per block by measuring the height of each leaf in the respective 2 cm × 2 cm block. The average leaf height of the sample plants for each block was then measured to determine the container's overall average leaf height. The longest leaf in each tray was



Figure 1.
Photograph showing the hydroponic setup and irrigation supplied to each tray.



Figure 2.
Photograph of barley seedlings at harvest (photo by R.A. Smith).

also measured and recorded. Thereafter the seedling mat was removed from its tray and the depth of the root mat was recorded to determine whether the initial 1 cm of soaked seed had expanded over the 8-day growing period.

For nutrient analysis, the trays were removed from the experiment and all excess remaining surface water was allowed to drain away after the allotted growth period of 8 days. The seedlings in their respective trays (**Figures 1** and **2**) were then weighed using a Kern KB 360-3 N scale that measures up to 0.01 g to determine their fresh weight. The weight of the seedling mat was calculated by subtracting the weight of the container from this measurement. Once the fresh weight of the plant material was determined, the seedling mat was removed from its tray and placed in brown paper bags before being dried in an oven (Labtech LDO-150F) at 60–70°C for 36–48 h. The plant material was weighed again after it had completely dried to determine its dry weight. Using a Culatti Typ MFC CZ13 mill, the dried plant material was ground and sieved after being weighed. Following that, samples of dried plant material were sent to the Agrifood Technology Station for protein and nitrogen analysis [37].

2.5 Statistical analysis

Data collected were analyzed using a two-way analysis of variance (ANOVA). The analysis was performed using Minitab 19.2.0/October 2, 2019, a stable release developed at the Pennsylvania State University, USA by Minitab LLC. Where F-value was found to be significant ($P \leq 0.05$), Tukey honest significant difference (HSD) was used to compare the interaction between soaking time and irrigation interval at $P \leq 0.05$ level of significance [38].

3. Results

3.1 Average leaf length and root mat expansion

When comparing all soak treatments in conjunction with all irrigation intervals on average leaf length, Treatment 20 with 16-h soaking time and 12-h irrigation duration

produced the longest leaf of 14.33 0.9 cm, while Treatment 9 produced the shortest leaf (6.23 0.40 cm) when compared to the control treatment. Both the independent and combined effects of soaking time and irrigation interval on leaf length were significant at $P \leq 0.05$ (**Figure 3**). Additionally, the root mat expansion was most

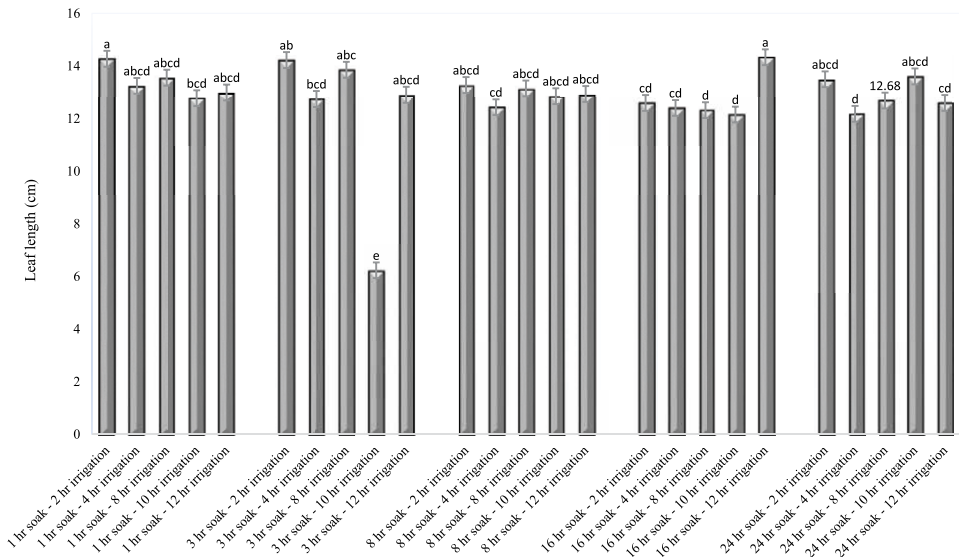


Figure 3.
Effect of soaking time and irrigation interval on average leaf length of *H. vulgare*. Means that do not share the same letters are significantly different.

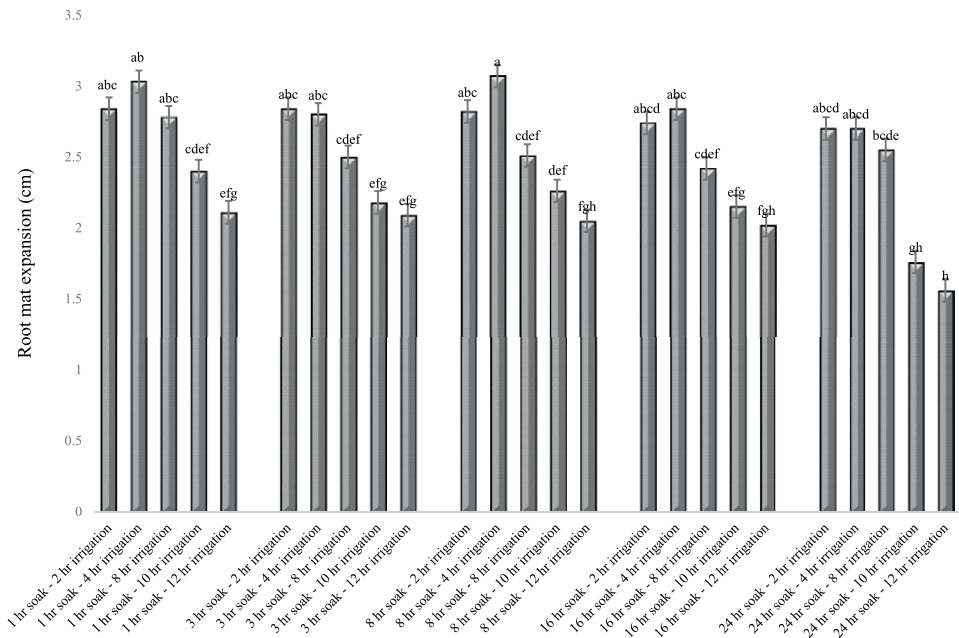


Figure 4.
Effect of soaking time and irrigation interval on root mat expansion of *H. vulgare*. Means that do not share the same letters are significantly different.

significant in Treatment 12 (8 h soak–4 h irrigation) and less significant in Treatment 25 (24 h soak–12 h irrigation) at a 95% confidence limit (**Figure 4**).

3.2 Nitrogen, protein, fresh weight and dry weight analyses

The highest nitrogen yield was obtained from seeds that had been soaked for 1 h and irrigated for 12 h (Treatment 5), while the lowest yield was obtained from Treatment 17 (**Figure 5**). Similarly, the protein content of the samples follows the same pattern, with the highest and lowest nitrogen yields recorded in Treatments 5 and 17, respectively (**Figure 6**). Furthermore, the freshly harvested *H. vulgare* sample

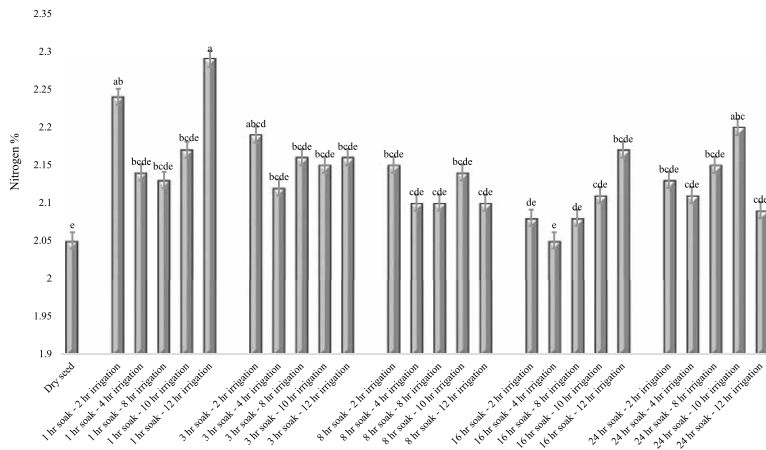


Figure 5. Effect of soaking time and irrigation interval on the nitrogen content of *H. vulgare*. Tukey pairwise comparisons was used to compare means of combined effects of soaking time and irrigation interval at $P \leq 0.05$. Means that do not share the same letters are significantly different.

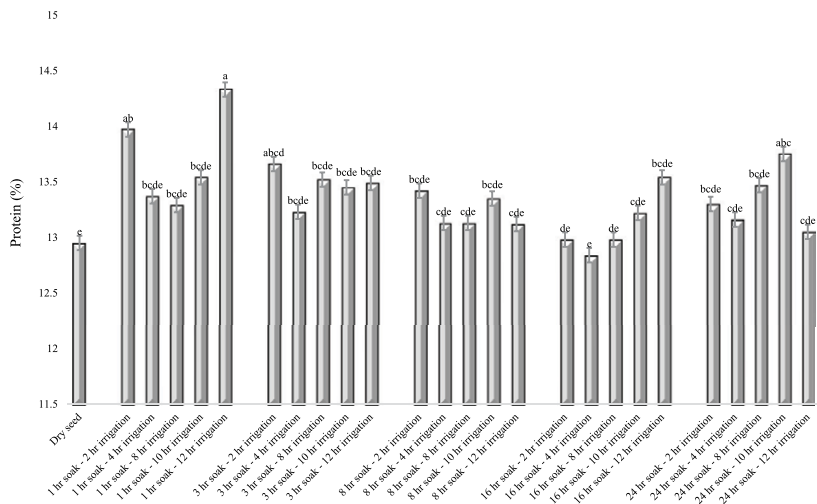


Figure 6. Effect of soaking time and irrigation interval on the protein content of *H. vulgare*. Means that do not share the same letters are significantly different.

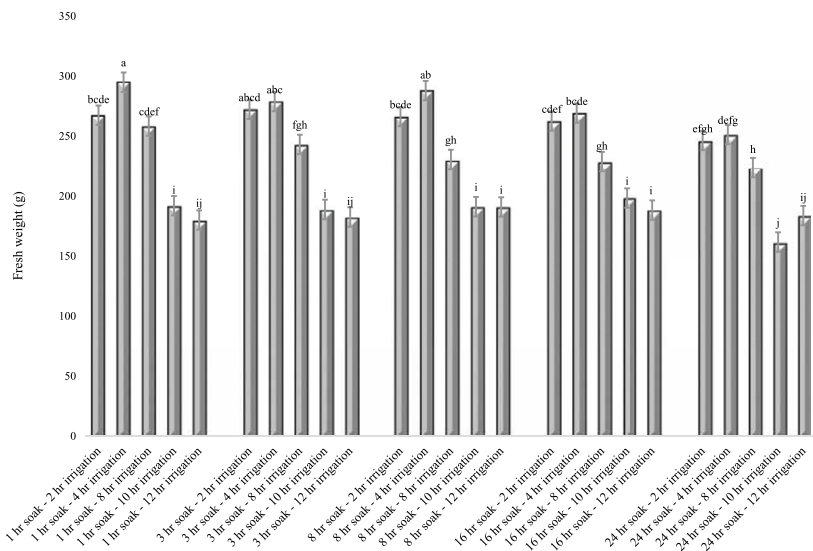


Figure 7.
 Effect of soaking time and irrigation interval on fresh weight of *H. vulgare*. Means that do not share the same letters are significantly different.

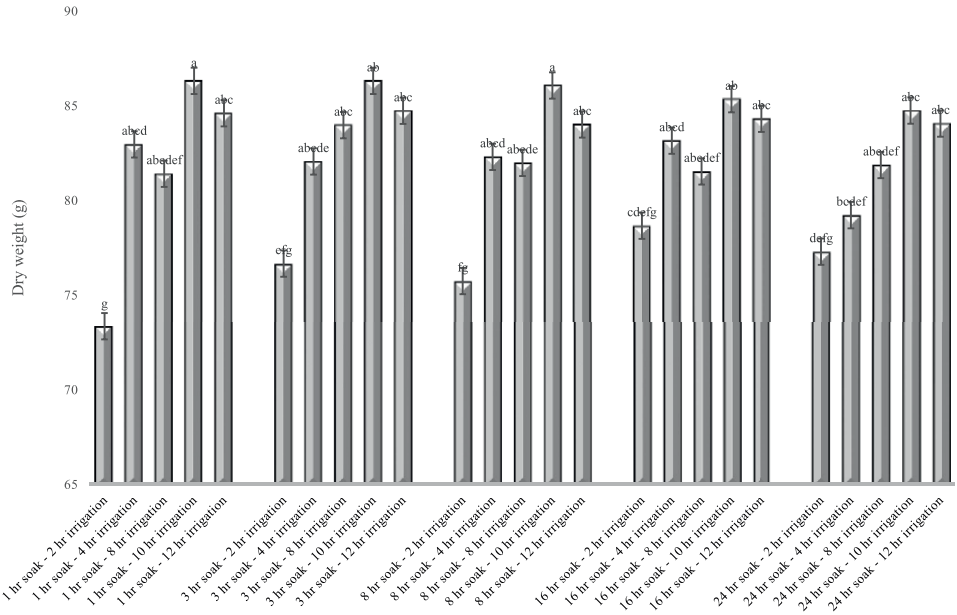


Figure 8.
 Effect of soaking time and irrigation interval on the dry weight of *H. vulgare*. Means that do not share the same letters are significantly different.

weighed the most in Treatment 2 (1 h soak–4 h irrigation) and the least in Treatment 24 (**Figure 7**). The dry weight of *H. vulgare*, on the other hand, was highest in Treatments 4 and 14, and lowest in Treatment 1 (**Figure 8**).

Furthermore, the two-way ANOVA revealed that soaking time and irrigation interval had no independent effect on nitrogen and protein yield of *H. vulgare*, as well

as the interaction of the two factors. Similarly, the fresh weight was not significantly affected by the two factors independently, but soaking time significantly affected the dry weight of the harvested plant samples. The interaction of the two factors had a significant effect on the dry weight of the sample, although the irrigation interval indicated otherwise.

4. Discussion

Findings from this study suggest that the seeds of *H. vulgare* responded more favorably to a shorter soaking time for a longer period of irrigation. The average leaf height increases as the irrigation period lengthens, with mean average heights ranging from 8 to 12 cm. For example, Treatment 6 with a 3-h soaking time and a 2-h irrigation interval had the greatest effect on average leaf height. Treatments 1 and 3 (**Figures 3–5**) were of slightly less significance because they both had a 1-h soaking treatment but were subjected to 2 hourly and 8 hourly irrigation intervals. These treatments differed greatly from the soaking control of 16 h as reported in [33, 39] but in agreement with [24] with an irrigation control of 2 h. Although Treatment 20 had the highest mean length value of 14.33 cm, it appeared that the seedlings and their corresponding lengths responded more to the increased irrigation frequency of 2 h, confirming the irrigation control used in this study (**Figure 4**). This was consistent with the soak control of 16 h, but it differed significantly from the irrigation control of 2 h [33, 40]. Also, the average height of barley recorded in this experiment differed significantly from previous studies where heights of 14.0 cm and 6.2 cm were respectively recorded by [20, 41].

Treatment 12 (8-h soak with 4 hourly irrigation) had the highest mean root expansion (3.07 cm) after the 8-day growing cycle, which was significantly different from the control. Treatment 2 followed with a mean of 3.03 cm (1-h soak with a 4-hourly irrigation interval; **Figure 5**), which was less significant than Treatment 12 due to a marginal difference of 0.04 cm, which is significant. Treatment 2 would thus allow the cultivator to reduce soaking time again to achieve similar results, with both treatments having the greatest effect with an irrigation frequency of 4 h, though other researchers have reported that transient exposure of *H. vulgare* roots to heavy metals may also have an adverse effect on root mat expansion [35, 42]. It would be interesting to see if changing the irrigation type, from drip to spray, or the mineral/trace metals composition of the irrigation water, would improve seedling root expansion, and if adding liquid fertilizer would do the same.

Furthermore, the highest nitrogen and protein concentrations were obtained in Treatment 5 with a 1-h soaking treatment and a 12-h irrigation interval (**Figures 6 and 7**). These results did not agree with the controls of 16 h of soaking and 2 hourly irrigation intervals. This indicated that the seed requires a shorter soaking treatment (1 h) as well as a longer (12 h) irrigation interval to achieve the highest level of nutrients in the seedling at harvest time. However, as discovered during the growth experiment, a 12-h irrigation interval is not beneficial to seedling growth. It was interesting to note that the next highest statistical mean belonged to treatment 1 (1-h soak with a 2-h irrigation interval), which was only marginally less than the highest mean achieved in treatment 5, which also had a 1-h soak time but a 12-h irrigation interval. This was consistent with the control and resulted in seedlings that were stronger and healthier. The shorter soaking time benefits the grower by reducing the time spent pre-soaking the seed, but it does not help with water conservation because it is

irrigated every 2 h. However, as the salinity of the hydroponic medium changes, this may change. However, this might change as the salinity of the hydroponic medium changes [43, 44]. Only crude nitrogen and protein were tested in this experiment, and further research into trace element levels would be required to determine the seedlings' full nutrient spectrum post-harvest. Other studies found that shortening the growing period from 8 to 4 days resulted in higher nutrient levels, which could be investigated further.

Furthermore, all of the highest dry weight means were obtained using a 10-h irrigation interval. The most significant results were obtained with soaking treatments lasting 1 and 3 h, as shown in Treatments 4 and 9, respectively. Although less significant, Treatment 14 with an 8-h soaking time was only 0.24 g lighter than treatments 4 and 9. This indicated that the seed pre-soaking time before germination could be reduced to 1 h. Under a 10 hourly irrigation interval, all three treatments (Treatments 4, 9, and 13) produced the highest dry weight. The highest fresh weight recorded came from that of Treatment 12, with a soaking time of 8 h and an irrigation interval of 4 h. It can be deduced that the seedlings benefitted from longer pre-soaking treatment. They still required moderate watering as the irrigation interval was every 4 h. Neither of the treatments agreed with the controls for soaking nor irrigation. The second highest mean value was achieved with Treatment 2 with a 1-h soak and 4 hourly irrigations. This proved that the pre-soaking time could be reduced without it affecting the total weight of the seedling post-harvest, however, the water consumption required to enable growth remained relatively high. Therefore, the disparity recorded in the weight of fresh samples compared to dry samples agrees with the results of the previous study as reported by Emam [20]. An investigation into the use of a nutrient solution to the irrigation water would be required to establish if this would improve overall fresh weight, post-harvest and if the introduction of a nutrient solution would allow the irrigation interval to be decreased thereby saving water.

5. Conclusion

This study reveals that a 1-h pre-soaked treatment, under 4 or 12 hourly irrigation intervals (T2 and T5) was the best treatment for cultivating barley hydroponically to achieve better yield for optimal fresh fodder production. For dry fodder weight, the highest yields were obtained with irrigation intervals of 10–12 h. Even though other growth parameters investigated such as length and root map expansion deviated from this trend, shorter soaking time at increased irrigation frequencies, proved to be beneficial to the farmer in terms of weight, nutrient yield, and height of *H. vulgare*. In the future, it would be helpful to ascertain if a change in irrigation type, from drip to spray, or increasing the mineral/trace metals composition of irrigation water would improve the root expansion of the seedling or bring about a marked decrease in irrigation water, thereby saving water.

Authors' contributions

Ryan Anthony Smith: Designed and performed the experiments; collated and analyzed data; wrote the original paper.

Muhali Olaide Jimoh: Analyzed and interpreted collated data; revised the manuscript with technical inputs.

Charles Petrus Laubscher: Conceived and designed the experiments; sourced for funding; supervised the experiments; edited the manuscript.

Funding

The authors wish to acknowledge the financial assistance received from the Cape Peninsula University of Technology through the University Research Fund.

Competing interests

Authors declare no conflict of interest.

Data availability statement

All data associated with this research are available on reasonable request from the corresponding author.

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