Effect of treating alfalfa seeds with gibberellin and nano-fertilizer on productive characteristics and protein content in hydroponic cultures

Abstract. This study was carried out in an experiment in an incubator designed for hydroponic seed germination with tight thermal insulation in the laboratories of the Department of Plant Production Technologies at the Technical College of Agriculture in Mosul, which is affiliated with the Northern Technical University in Iraq, where the seeds of the Dutch variety alfalfa (*Medicago sativa*. L) were germinated commercially and four times between 2022 and 2024. The experiment was carried out in a completely randomized design (CRD) with two factors and three replicates in perforated dishes with dimensions of 28 cm in length and 19 cm in width (area 532 cm2) at a seed rate of kg / m2, resulting in an average seed weight of 53 g in one dish. The first experimental factor was the germination periods, while the second factor was soaking the seeds in solutions of varying amounts made from gibberellin and nano-compound fertilizer with the comparison treatment, which is soaking in water alone, as follows:

-Comparison. Water only and given the symbol w.

-Gibberellin 100 ppm and given the symbol GA 100ppm.

-Gibberellin 200 ppm and given the symbol 200ppm GA

-Nano fertilizer 20:20:20 NPK at a concentration of 2000 ppm and given - the symbol NPK2000ppm.

-Nano fertilizer 20:20:20 NPK at a concentration of 4000 ppm and given the symbol NPK4000ppm.

This study showed that the Gibberellin 200ppm GA treatment was significantly superior to the rest of the other soaking treatments in the characteristics of the height of the incubator (3.66 cm), as well as the wet weight of both seedlings and roots and their dry weight, which were (497.88, 56.97g/dish^{-1}), respectively, The fresh and dried root weights were considerably greater in the same treatment, at (400.86 and 43.86 g/dish^{-1}), respectively. while the comparison treatment was superior. Morally, it was significantly superior to the rest of the other soaking treatments in the characteristic of protein in the medium was (18.29%).

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As for the times of hydroponic seed germination, there were no significant differences between the times in all the studied characteristics, which enhances the high degree of incubator isolation, the accuracy of implementing the experiment, and the accuracy of taking the results.

Keywords. Hydroponics, alfalfa, seed soaking, gibberellin, nano fertilizer

1. Introduction Alfalfa (*Medicago sativa* L.) is a warm-season legume crop grown on around 30 million hectares globally. Alfalfa output is predicted to be 454 million tons per year worldwide (El-Shal et al., 2022). Alfalfa may be cultivated in salty circumstances, and as a suitable resource for grazing crops, it can adapt to both saline and alkaline environments (Lu et al., 2021). Alfalfa is relatively sensitive to salts (Emam et al., 2009), and exposure to salt stress of 0.05-0.2 ml lowers growth and production (Wang et al., 2017). Salt stress reduces dry matter production, which has been found at high salinity due to salt buildup and consequent harmful consequences (Bhattarai et al., 2020).

Seed soaking, which refers to the procedure of soaking seeds for a certain amount of time prior to planting (with growth regulators, fertilizers, amino acids, vitamins, salts, and so on), is one of the methods utilized that has proven to be beneficial and successful. Its advantages include tying the stimulation process to its influence on germination by enhancing emergence and making it regular and happening at the same time, as well as producing robust, homogenous, and stable seedlings with good and early field stability. The seed germination process using this technique may trigger a pre-adaptation reaction when various challenges arise, such as water scarcity, and subsequently lessen the harm caused by this stress in the plant's development and yield (Al-Ubaidi, 2015).

Nano-fertilizers are products containing extremely small particles that are totally soluble in water. They are one of the effective alternatives to traditional fertilizers because of the ease of nano-particles entering the cells and being absorbed by them due to their small size, association with protein carriers, and penetration of the cell wall, which enables the transfer of these nano-particles through the cells and their spread among them, which improves their functions and increases the efficiency of their effect (Grover et al., 2012).

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Gibberellins are a stimulating plant hormone. Gibberellins were identified in infected rice fields in Japan, where they were seen to grow in strikingly elongated plants, which were linked to the fungus Gibberellafujikuro. It was discovered that treating healthy seedlings with fungal extract caused the plant to elongate and exhibit the same symptoms of illness. Gibberellin is an active chemical isolated from the fungus. Later research led to the discovery of its occurrence in plants, which are terpenoid molecules with 19 or 20 carbon atoms. Gibberellins travel freely inside the plant, up and down without obstruction. Gibberellins promote stem elongation by driving cell elongation and expansion, and they also modulate cell membrane permeability. (Syed Mohammed, 1982).

Hydroponics: the process of germination of plant seeds for the purpose of producing green fodder is considered one of the most important reasons for the success of ideal livestock development, which has a high nutritional value, is rich in carbohydrates and protein, and has good palatability, because this technology provides green fodder throughout the year and does not require large areas for fodder production compared to traditional cultivation of fodder crops (Hayawi & Taha, 2021).

2. Materials and Methods

The laboratory study was conducted in the laboratories of the Technical Agricultural College in Mosul, which is affiliated with the Northern Technical University in Iraq, in an air-conditioned incubator with a very high insulation ratio, ensuring that the temperature is maintained at 18-22 degrees Celsius. The incubator has shelves supplied with artificial illumination with LED bulbs that are lighted at regular intervals for 12 hours a day, On the crop of *Medicago sativa* L. Dutch variety, with four meals, so that the germination process was carried out in each season of the year at a rate of one meal during the year 2022-2023, and the meals were implemented, which is the first factor for laboratory experiments, on the following dates:

1 – 2 / 4 / 2022
2 – 28 / 7 / 2022
3 – 1 / 10 / 2022
4 – 15 / 1 / 2023

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The second factor of the laboratory experiment is the process of soaking the seeds for 24 hours in aqueous solutions represented by the following materials and levels and their interactions, after which the grains were left to air dry at room temperature (25°C) for three days until the time of germination, and the solutions were as follows:

1-Comparison. Water only and give the symbol w.
2-Gibberellin 100 ppm and give the symbol GA 100ppm.
3-Gibberellin 200 ppm and give the symbol GA 200ppm.
4-Nano fertilizer 20:20:20 NPK at a concentration of 2000 ppm and give the symbol NPK 2000ppm.
5- Nano fertilizer 20:20:20 NPK at a concentration of 4000 ppm and give the symbol NPK 4000ppm. (Al-Obaidi and AlRijabo, 2021)

The experiment was carried out in a completely randomized design (CRD) with two factors and three replicates in perforated dishes with dimensions of 28 cm length, 19 cm width (area 532 cm2) at a seed rate of kg/m2, resulting in an average seed weight of 53 g in one dish. Each meal lasted 26 days, beginning with the soaking procedure and concluding with the commencement of data collecting, as described below:

1- 1First stage: One day for soaking (24 hours).
2- 2Second stage: Three days for drying the soaked seeds.
3- 3Fourth stage: Twenty days for the seed germination process.
4- 4Fifth stage: Leave the seeds to dry a little before starting to take readings.

The dishes are sprayed every six hours with equal amounts of water.

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3.Results and Discussion

Table (1).The effect of soaking treatments, meals and their interaction on the height of the alfalfa culture (cm)

Soaking / batch	W	GA 100ppm	GA 200 ppm	NPK 2000 ppm	NPK 4000 ppm	Average batchs
batch1	2.42 h	3.33 c - e	3.62 a – c	2.87 g	2.87 g	3.02 a
batch2	2.39 h	3.28 d - e	3.61 a - d	2.79 g	2.94 f - g	3.00 a
batch3	2.29 h	3.44 a - e	3.68 a - b	2.75 g	g	3.01 a
batch4	2.37 h	3.36 b - e	3.68 a - b	3.22 f - g	2.71 g	3.07 a
Average soaking coefficients	2.37 d	3.35 b	3.66 a	2.91 c	2.85 c	

Identical coefficients are not significantly different from each other at the 5% probability level.

3.1 **The impact of soaking treatments, meals, and their interactions on the height of the alfalfa culture (cm)**

The results of Table (1) revealed no significant changes in the average height of the incubator for the four meals. The average of the fourth meal (3.07 cm) had the highest value, while the average of the second meal was the lowest (3. cm). In terms of soaking treatments, all treatments outperformed the reference treatment, which was soaking in water and measured (2.37) cm. The GA200ppm soaking procedure produced the greatest average of 3.66 cm. This is consistent with (Elangbam et al., 2017) , (Al-Amri, 2018).The reason for this is the effect of the gibberellin solution on the soaked seeds, which stimulated the plant to elongate by stimulating cell elongation and increasing their size, resulting in a greater increase in plant height than other soaking treatments, which caused rapid germination, and thus a significant increase in plant height was generated by gibberellin or nano-fertilizer. The interaction between the two components revealed that GA200ppm had a superior interaction with both meals. The fourth was (3.68 cm), and the lowest value was (2.29 cm) because of the interaction between the comparative treatment and the third meal.

Table (2): Effect of soaking treatments, meals and their interaction on the total wet weight of the alfalfaculture (g/dish^{-1})

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Soaking / batch	W	GA 100ppm	GA 200 ppm	NPK 2000 ppm	NPK 4000 ppm	Average batchs
batch1	438.61 g	470.83 b - e	497.90 a	459.88 f	475.29 b - c	468.50 a
batch2	437.51 g	478.34 b	497.63 a	464.59 e - f	473.98 b - d	470.41 a
batch3	435.45 g	475.86 b - c	497.26 a	464.73 e - f	470.89 b - e	468.84 a
batch4	432.15 g	474.85 b - d	498.73 a	470.06 d - e	467.33 d - e	468.62 a
Average soaking coefficients	435.93 d	474.97 b	497.88 a	464.81 c	471.87 b	

Identical coefficients are not significantly different from each other at the 5% probability level.

3. 2 The effect of soaking treatments, meals and their interaction on the total wet weight of the alfalfa culture (g/dish^{-1})

Table (2) revealed that there were no significant variations in the averages of the total wet weight of the culture over the four meals. The second lunch had the greatest average (470.41) g/dish^{-1}, whereas the first meal had the lowest average (468.50) g/dish^{-1}. In terms of soaking treatments, all treatments greatly surpassed the comparator treatment, which was soaking in water (435.93 g/dish^{-1}).

The GA200ppm soaking treatment produced the highest average (497.88) g/dish^{-1}, greatly surpassing the other treatments. This finding is consistent with those reported by(Zhang et al, 2020) and Darkatanian and Saeed (2020). This is owing to the speed with which the seeds treated with the highest concentration of gibberellin were processed, giving precedence to its seeds and later its components in the production of materials. The stored meal had a good effect on the weight. The interaction between the two components revealed that the interaction of GA200ppm with all meals was superior, with no significant differences between them. The greatest value (498.73) g/dish^{-1}was associated with the fourth meal, whereas the lowest value (435.93) g/dish^{-1}was associated with the comparison treatment and the same meal.

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Table (3): Effect of soaking treatments, meals and their interaction on the total dry weight of the alfalfa culture (g/dish^{-1})

Soaking / batch	W	GA 100ppm	GA 200 ppm	NPK 2000 ppm	NPK 4000 ppm	Average batchs
batch1	46.87 f	52.97 c - d	57.00 a	50.86 c - e	48.59 e - f	51.25 a
batch2	47.86 e - f	53.37 b - c	57.67 a	48.70 e - f	48.43 e - f	51.21 a
batch3	47.73 e - f	52.62 c - d	55.92 a - b	50.17 d - e	48.38 e - f	50.96 a
batch4	48.32 e - f	52.64 c - d	57.31 أ	48.35 e - f	48.55 e - f	51.03 a
Average soaking coefficients	47.70 d	52.90 b	56.97 a - b	49.52 c	48.49 c - d	

Identical coefficients are not significantly different from each other at the 5% probability level.

3.3 The effect of soaking treatments, meals and their interaction on the total dry weight of the alfalfa culture (g/dish^{-1})

Table (3) showed no significant changes in the average dried root weights from the culture for the four meals. The first dinner had the greatest average value (51.25) g/dish^{-1}, whereas the third meal had the lowest average (50.96) g/dish^{-1}. In terms of soaking treatments, all treatments greatly beat the reference treatment, which was soaking in water and weighed (47.70 g/dish^{-1}.

The GA200ppm soaking treatment produced the highest average (56.79) g/dish^{-1}, greatly surpassing the other treatments. This is similar with the findings of (Guo et al., 2022) and (Nedunchezhiyan et al., 2023), as the superiority of the plant's moist weight was mirrored in its dry weight. The interaction between the two parameters revealed that the GA200ppm interaction was superior with all meals, which did not differ substantially from one another. The maximum value was (57.67) g/dish^{-1}, which overlapped with the second meal, and the lowest value was (46.87) g/dish^{-1}, caused by the interaction of the first meal with the comparison treatment.

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Table (4): The effect of soaking treatments, meals and their interaction on the root weight trait in the -*- culture (g/dish^{-1})

Soaking / batch	W	GA 100ppm	GA 200 ppm	NPK 2000 ppm	NPK 4000 ppm	Average batchs
batch1	351.35 f	380.51 b - d	400.53 a	370.30 e	385.36 b - c	377.61 a
batch2	351.30 f	387.17 b	400.23 a	375.36 d - e	383.75 b - c	379.56 a
batch3	348.52 f	384.50 b - c	401.06 a	376.16 d - e	381.76 b - d	378.40 a
batch4	344.73 f	383.92 b - c	401.65 a	379.87 c - d	376.46 d - e	377.32 a
Average soaking coefficients	348.98 d	384.02 b	400.86 a	375.42 c	381.83 b	

Identical coefficients are not significantly different from each other at the 5% probability level.

3.4 Effect of soaking treatments, meals and their interaction on root weight in the alfalfa culture (g/dish^{-1})

Table (4) showed no significant changes in average root weights from the culture for the four meals. The second meal had the greatest average (379.56) g/dish^{-1}, whereas the fourth meal had the lowest average (377.32) g/dish^{-1}. In terms of soaking treatments, all treatments greatly beat the comparator treatment, which was soaking in water, with a weight of (348.98) g/dish^{-1}. The GA200ppm soaking treatment produced the highest average (400.86) g/dish^{-1}, greatly surpassing the other treatments.

The increased germination rate is linked to metabolic repair during imbibition and osmotic adaptation, as indicated by (Darkatanian and Saeed, 2020). The interaction between the two components revealed that the interaction of GA200ppm with all meals was superior, with no significant differences between them. The greatest value was (401.65 g/dish^{-1}, which was interacted with the fourth meal, and the lowest value was (344.73 g/dish^{-1}, which did not vary significantly from all interactions between the comparator treatment and the four meals.

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Table (5): Effect of soaking treatments, meals and their interaction on the weight of dried roots from the alfalfa culture(g/dish^{-1})

Soaking / batch	W	GA 100ppm	GA 200 ppm	NPK 2000 ppm	NPK 4000 ppm	Average batchs
batch1	37.68 d	40.85 b - c	43.66 a	39.96 c - d	39.01 b - d	40.23 a
batch2	38.33 b - d	40.67 b - c	44.38 a	38.91 b - d	39.25 b - d	40.31 a
batch3	38.19 b - d	40.25 b - d	42.58 a - b	40.43 b - c	39.18 b - d	40.13 a
batch4	38.78 b - d	40.22 b - d	44.12 a	39.37 b - d	39.12 c - d	40.32 a
Average soaking coefficients	38.25 d	40.50 b	43.68 a	39.67 b - c	39.14 c - d	

Identical coefficients are not significantly different from each other at the 5% probability level.

3.5 Effect of soaking treatments, meals and their interaction on the weight of dried roots from the alfalfaculture (g/dish^{-1})

Table (5) showed no significant changes in the average weight of dried roots from the culture for the four meals. The fourth meal had the greatest average (40.32) g/dish^{-1}, whereas the first meal had the lowest average (40.13) g/dish^{-1}. In terms of soaking treatments, all treatments performed much better than the comparator treatment, which was soaking in water at (38.25) g/dish^{-1}. The GA200ppm soaking treatment produced the highest average (43.68) g/dish^{-1}, greatly surpassing the other treatments. This is congruent with what was mentioned by(Zhang et al. , 2020).

In the interaction between the two factors, it became clear that the GA200ppm interaction was superior to all meals, which did not differ significantly among them. The highest value was (44.38) g/dish^{-1}, which was overlapped with the second meal. The lowest value was for the interaction of the comparison treatment with the first meal (37.68) g/dish^{-1}.

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Table (6): The effect of soaking treatments, meals and their interaction on the protein percentage in the alfalfa culture%.

Soaking / batch	W	GA 100ppm	GA 200 ppm	NPK 2000 ppm	NPK 4000 ppm	Average batchs
batch1	18.59 a	17.14 c	16.80 c	16.81 c	17.01 c	17.27 a
batch2	18.17 a - b	17.15 c	16.74 c	16.78 c	16.86 c	17.14 a
batch3	18.02 a - b	16.91 c	17.48 b – c	16.85 c	17.05 c	17.26 a
batch4	18.39 c	16.93 c	16.79 c	17.06 c	17.04 c	17.24 a
Average soaking coefficients	18.29 a	17.03 b	16.95 b	16.87 b	16.99 b	

Identical coefficients are not significantly different from each other at the 5% probability level.

3.6 The effect of soaking treatments, meals and their interaction on the protein percentage in the alfalfa culture %

The results of Table (6) showed that there were no significant variations in the average protein percentages in the culture medium between the four meals. The greatest value was the average of the first meal (17.27%), while the lowest was the average of the second meal (17.14%). In terms of soaking treatments, the comparison treatment fared much better than the other treatments, with a value of (18.29%). The remaining soaking treatments showed no significant differences. The lowest result was obtained by soaking NPK 2000ppm (16.87%). The cause is the activity of the developing plant from treated seeds.

Whether they were gibberellin solutions or nano-fertilizer, the plant grew quicker than a seed soaked in water alone, which is consistent with the findings of (Al-Sal, 2019). The interaction between the two parameters revealed that the comparative treatment with all meals performed much better than the other interactions. The greatest result was for the interaction of the first meal with it (18.59%), while the lowest was for the interaction of the soaking treatment GA 200 ppm with the second meal (16.74%).

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Conclusions

Soaking seeds before planting or germinating them in gibberellin growth regulator solution had a prominent and clear effect, especially at higher concentrations, on increasing fodder yield, whether from the legume crop (alfalfa) or the grass crop (bonicam), primarily through its effect on plant height, which contributed to increasing plant weight, whether wet or dry, and thus increasing fodder production. Regarding the impact of soaking in the compound nano-fertilizer solution , Its effect was less than that of the gibberellin growth regulator solution, and this effect, whether from gibberellin or the compound nano-fertilizer, stemmed from the plant's need for both substances at the start of its unfolding and formation of its first parts, which contributed to the expansion and elongation of the cells of the plant parts after the stage of water absorption and the decomposition of the stored materials in the seed (endosperm).

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