De novo Propagation of Alfalfa Under Hydroponics

Liu Yang
Qindao University Normal College
Zhang Wenyu
Qindao University Normal College
Li Shuai
Qindao Agricultural University
Wu Yao
Qindao Agricultural University
Sun Xiaohui
Qindao Agricultural University
Li Siyu
Qindao Agricultural University
Wang Zongyu
Qindao Agricultural University
Ma Lichao
Qindao Agricultural University
Sun Juan
Qindao Agricultural University
Lili Cong (congli1985610@qau.edu.cn)
Chinese Academy of Agricultural Sciences https://orcid.org/0000-0002-6902-1737
Yang Guofeng
Qindao Agricultural University

Research

Keywords: Alfalfa, Hydroponic, Cutting propagation, Rooting rate

Posted Date: October 1st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-83257/v1

Abstract

Background: The legume plant alfalfa (*Medicago sativa* L.) is a widely cultivated perennial forage due to its high protein levels, palatability, and strong adaptability to diverse soil types and agro-ecological zones. This forage plant is a self-incompatible, cross-pollinated autotetraploid with tetrasomic inheritance. Therefore, maintaining excellent traits through seed reproduction is challenging in alfalfa. However, the cutting propagation technology could enable consistent multiplication of quality plants that are genetically identical to the parent plant, for use in breeding and other research applications. Most of previous alfalfa omics researches used varieties as material on omics and gene mining experiment due to poor growth consistency of cuttings by existing cutting methods, which generate genetically un-identical cuttings and thus compromise on the reliability of the results. Therefore, this study aimed to develop a simple, cost-effective, reproducible, and efficient hydroponic cutting method for the preservation of alfalfa plants and molecular research applications such as genomic, transcriptomic, and proteomic analyses.

Results: Alfalfa cultivar 'Wudi' grown under hydroponics for 30 days was used as source material for cuttings. The top, middle and bottom sections of its stem were used as cuttings. The rooting rate, root length, and stem height of the different stem sections were compared to determine the best segment for alfalfa propagation in four nutrient solutions (H^M, H^M+1/500H, H^M+1/1000H and d H^M+1/2000H). After 21 days of culture, the rooting rates of all the three stem types under four cutting nutrient solutions were above 78%, The rooting rate of the middle and bottom parts in H^M +1/1000 H and H^M +1/2000 H nutrient solutions reached more than 93% with higher health survey score (>4.70). Besides, root length and stem height in these two sections was exemplary.

Conclusions: This study developed a *de novo* cutting propagation method that can be used to conserve and propagate germplasm in breeding programs and research. This article is the first report on the cutting propagation of alfalfa by hydroponics, which could supplement the existing cutting propagation methods.

Background

Alfalfa (*Medicago sativa* L.), known as the "king of forage grass", is the most important leguminous grass worldwide, with the advantages of high nutritional value, palatability, high-stress resistance, and nitrogen-fixing ability [1, 2]. Rapid increases in livestock production have also increased demands for alfalfa forage in China in the last 50 years [3]. Besides, its rich genetic diversity enables cultivation under various environmental conditions. Therefore, alfalfa is not only a high-quality feedstock but also an important ecological protection crop [4].

The recent transformation and upgrading of animal husbandry in China have increased the social and economic value of alfalfa in the development of modern grass husbandry. Thus, the demand for alfalfa cultivars has witnessed an unprecedented increase. However, cultivated alfalfa is a self-incompatible

cross-pollinated autotetraploid (2n = 4 × 8 = 32) with tetrasomic inheritance, in which bivalent pairing is not preferential [5]. Therefore, the maintenance of excellent traits through seed reproduction is challenging. However, the cutting propagation could enable consistent multiplication of quality plants that are genetically identical to the parent plant for breeding and research applications. Compared to propagation from seeds, propagation from stem cuttings is simple, cheap and plant growth is usually fast. Besides, propagation from cuttings also saves the time required for seeds to germinate; thus, cuttings grow more quickly than seedlings [6].

With the rapid development of omics research and advancement in genome editing technology, the molecular breeding of herbage has become more precise and in-depth to be used in the breeding of new cultivar. Therefore, consistent quality plant materials are required to avoid background interference of experimental results. Cross-pollination in alfalfa results in the same variety producing seeds with different genotypes. Previous studies on alfalfa omics and gene mining experiments used variety as source material, which typically generate plant with diverse genetic backgrounds [7–10]. Experimental material with such a mixed-type genotype would result in significant background interference, thus impairing an accurate bioinformatics analysis and precise identification of target genes. However, the primary benefit of cuttings is that all offspring are clones with the same genetic material and characteristics as the mother plant. Therefore, recent researches in alfalfa omics try to use clonal lines of an individual plant as the source material. The reported cutting methods, such as field cutting, soil culture conducted under greenhouse, and sterile cutting, offer specific advantages in clonal preservation. However, these methods also have significant shortcomings, especially the inability to quickly and growth consistently provide clones with excellent genetic uniformity.

Hydroponic cutting propagation a form of plant propagation in which cuttings are multiplied in water or nutrient solution as a medium instead of soil. This method is advantageous because of the short cycle, rapid screening and year-round production. Plants grown under hydroponics systems typically produce higher yields, require less space, and conserve soil and water. This system is ideal when outdoor gardening space is limited [11]. Moreover, the systems enable easier control of key factors in the growth environment, including temperature, light intensity, and moisture, which is conducive to maintain consistent quality of plant materials for research [12]. Meanwhile, it also has the advantage of conveniently observing the entire process of root growth [13].

However, the hydroponic cutting system of alfalfa has not been reported. Therefore, this study established a hydroponic cutting propagation system in alfalfa, which is simple, time-saving, and has a high propagation coefficient for clone preservation. The optimized method can provide genetically identical alfalfa clones for use in scientific research, conventional alfalfa breeding, and molecular studies. These study results particularly unblock the bottleneck experienced in existing cutting methods, which typically generate genetically un-identical lines from the same source material.

Results

Effect of different stem segments on the propagation of cuttings

The data presented in Fig. 1 indicates the cuttings were well rooted after 21 days of culture. The rooting rates of all the three stem types under four cutting nutrient solutions were above 78%, indicating that the new cutting rooting method developed in this experiment has an excellent rooting efficiency (Table 1).

Under the modified 1/2 Hoagland nutrient solution (H^M), there were no significant differences in the rooting rates and root lengths between the top, middle, and bottom segments. However, the bottom segments gave the highest percentage (91.67%) of the rooted cuttings compared with the top and middle sections. Meanwhile, the root length of the middle segment cuttings was the longest (13.37 cm), with an 86.67% rooting rate (Fig. 1a). The stem height of the bottom segment was highest (18.74 cm). Under H^M+1/500 HB-101 (H^M+1/500 H) nutrient solution, rooting rates of the top, middle, and bottom parts were not significantly different. However, the bottom segment demonstrated the best cutting propagation response, according to the rooting rate, root length, and stem height. Figure 1b also shows that the bottom part rooted well and generated more healthy new plants in this nutrient solution, relative to the top and middle segments. Under H^M+1/1000 HB-101 (H^M+1/1000 H) and H^M+1/2000 HB-101 (H^M+1/2000 H) nutrient solutions, the rooting rates of the middle and bottom parts could reach more than 93%, which was significantly higher than that of the top segment, and the health survey score of rooted plants were higher than others treatments (Table 1) ,the plants grows well with healthy root system and leaves(Fig. 1c, d). Under the H^M+1/2000 H nutrient solution, 100% of the middle segment rooted, and all cuttings grew into healthy plants with the health survey score reached 4.86. The rooting rate of the bottom section was 98.33% in the two nutrient solutions.

Table 1
Influence of different stem nodes on cutting propagation

Stem type for cutting	Nutrient solution	Rooting rate (%)	Root length (cm)	Stem height (cm)	Health survey score
Top	H^M	83.33 ± 1.67 a	9.99 ± 0.55a	9.51 ± 0.47b	3.44 ± 0.14b
Middle	H^M	86.67 ± 6.01 a	13.37 ± 2.13a	14.61 ± 3.29ab	3.74 ± 0.13b
Bottom	H^M	91.67 ± 1.67 a	9.33 ± 0.59a	18.74 ± 1.48a	4.43 ± 0.12a
Top	H^M +1/500 H	83.33 ± 1.67a	6.86 ± 0.50b	8.36 ± 0.57b	2.92 ± 0.14c
Middle	H^M +1/500 H	93.33 ± 1.67a	7.80 ± 0.17ab	15.87 ± 2.53a	3.36 ± 0.09b
Bottom	H^M +1/500 H	93.33 ± 4.41a	8.58 ± 0.20a	19.18 ± 1.0a	4.60 ± 0.13a
Top	H^M +1/1000 H	78.33 ± 6.01b	7.37 ± 0.25b	8.47 ± 0.68c	3.34 ± 0.11b
Middle	H^M +1/1000 H	93.33 ± 3.33a	8.93 ± 0.06b	15.09 ± 1.62b	4.72 ± 0.09a
Bottom	H^M +1/1000 H	98.33 ± 1.67a	12.72 ± 1.70a	19.45 ± 0.27a	4.88 ± 0.04a
Top	H^M +1/2000 H	90.00 ± 0.00b	7.85 ± 0.79b	10.09 ± 1.30b	3.10 ± 0.16b
Middle	H^M +1/2000 H	100.00 ± 0.00a	9.00 ± 1.14ab	16.70 ± 2.69ab	4.86 ± 0.05a
Bottom	H^M +1/2000 H	98.33 ± 1.67a	10.94 ± 0.50a	20.95 ± 2.39a	4.76 ± 0.09a

Influence of different nutrient solutions on rooting

After 21 days of culture, the highest rooting frequency for the upper stem segment was observed in H^M+1/2000H (90.00%), while there were no significant differences in the rooting rates of the other three nutrient solutions (83.33%, 83.33%, and 78.33% for H^M, H^M+1/500H, and H^M+1/1000H, respectively). However, H^M led to greater root length (9.99 cm), but no significant differences were found in the other three nutrient solutions (Fig. 2). In terms of the stem height of the cuttings, H^M+1/2000H gave the best

rooting rates (average of 10.09 cm), followed by H^M, H^M+1/500H, and H^M+1/1000H, respectively, but with no significant differences.

The rooting rates of the middle segment reached above 93% in H^M+1/500H, H^M+1/1000H, and H^M+1/2000H nutrient solutions (Fig. 3). Especially in the H^M+1/2000H nutrient solution, all the cuttings rooted well, and the rooting rate reached 100%. The lowest rooting rate (86.67%) and the longest root length (13.37 cm) were found in the H^M nutrient solution. There were no significant differences in the stem height among the four nutrient solutions, but the stem height could reach more than 14 cm.

Figure 4 shows that the rooting rate of the bottom stem segment showed no significant difference among the four cutting nutrient solutions, all of which were higher than 90.00%. The highest rooting frequency was observed in H^M +1/1000 H and H^M + 1/2000H (98.33%), followed by H^M +1/500H (93.33%), and H^M gave the lowest (91.67%). The root growth was best in H^M +1/1000 H, with the length of the root reaching 12.72 cm, followed by H^M + 1/2000H (10.94 cm) and H^M (9.33 cm). Root growth was lowest in H^M + 1/500H, in which the root length (8.58 cm) was 4.14 cm shorter than in H^M + 1/1000H.

Discussion

At present, the commonly used cutting methods in alfalfa include field cuttings and indoor seedling cuttings, both of which are soil-based. Although the survival rate of some germplasm by these methods can reach 85%, the growth consistency of plants generated by the above methods is low, and thus unsuitable for molecular biology research, as they cause background results interference. Therefore, establishing hydroponic systems for cutting propagation could solve these challenges. Hydroponic plants have become more popular because they save on space, are dirt-free, and generate genetically consistent cutting plants within a short time [14]. We demonstrated that the rooting rate of alfalfa cuttings is influenced by many factors, including the position of the cutting in the stem and the cutting nutrient solution. Although all the stem segments in this experiment gave higher rooting rates than other methods reported previously [15], it was evident that the rooting ability of the bottom stem segment is the optimal choice for cutting propagation under the hydroponics system, followed by the middle part. Meanwhile, the rooting rate of the top stem section was lower than that of the bottom and mid segments, and its clonal growth was weak. Moreover, flower buds were formed in the later stage of cutting development, which affected the vegetative growth and was not conducive to the growth and utilization of propagated clones from the top stem segment. It is possible that the middle and bottom stem segments are fully developed and can provide the primary nutrients for rooting and initial growth of cuttings. In contrast, the developmental stage of the top stems is relatively young, and the inadequate storage of nutrients is not conducive for rooting [16–18]. Similarly, a reported study about alfalfa cutting in soil also demonstrated that middle and bottom stem segments have better rooting capacity, but the middle segment obtain the highest rooting rate (85 .4%). The possible reason for this result is the different growth period of the cuttings.

In this preliminary experiment, we found that the composition of the nutrient solution was very important for stimulating the rooting rate under the hydroponics system and that the rooting rates of the three stem segment types reached a maximum when cultured in H^M+1/2000H. Supplementing the cutting nutrient solution with HB-101, a natural plant activity solution improved the total rooting rate. This result suggests that HB-101 supplementation at concentrations of 1/500, 1/1000, and 1/2000 could stimulate the growth of adventitious roots. Root growth was, however, limited in H^M+1/500 H, resulting in the shortest root lengths in all the three stem segments. This result could be because this concentration of HB-101 was too high, which probably affected root growth and development. Nutrient solutions H^M + 1/1000H and H^M +1/2000 H gave the best rooting rates, and cutting clonal plants demonstrated optimal growth. It is possible that a low concentration of HB-101 can promote rooting but could inhibit plant growth when used at higher concentrations. Therefore, H^M + 1/2000 H is an ideal nutrient solution for hydroponics culture of alfalfa cuttings.

Conclusions

Plant propagating from cuttings is one of the most used methods of clonal multiplication. The results obtained indicate the technique optimized in this study is feasible for cutting propagation under the hydroponics system. This paper is the first time to explore the hydroponic cutting technology for alfalfa. In nutrient solutions H^M+1/1000H and H^M+1/2000H, the rooting rate can be more than 93%, and plants developed a better root system. This *de novo* cutting method for alfalfa has the potential for application in conventional breeding and molecular studies to provide uniform propagation material. In particular, the technique can unlock the bottlenecks experienced in soil propagation of alfalfa cuttings, which it is difficult to provide large quantities of cloning materials with growth consistency for molecular research applications such as genomic, transcriptomic, and proteomic analyses. When using the hydroponics propagation system, stem cuttings for successful propagation can be obtained at almost any time during the active growth period of parent plants. The experiment developed a convenient and efficient method for producing clones with high genetic consistency by the cutting method. The optimized method can be applied for alfalfa molecular biology research, and also for conventional propagation and conservation of the alfalfa germplasm. In conclusion, this new method is recommended for the propagation of alfalfa cuttings.

Methods

Preparation of cutting materials

The ‘Wudi’ alfalfa cultivar was selected as the parent plant for stock material. Alfalfa seeds were sterilized in 70% ethanol for 1 min and rinsed in sterilized water five times. The seeds were placed in Petri dishes containing two sheets of sterile filter papers and maintained in a growth chamber under controlled conditions (25 ± 1 °C day/ 20 ± 1 °C night, 80% relative humidity, 16 h light/8 h dark). Seven days after germination, uniform seedlings were transferred into specially designed pots containing modified

Hoagland nutrient solution (Table 2) and incubated under the following conditions: 16 h light (25ºC)/ 8 h darkness (20ºC), the light intensity of 300 mol/m^2·s, and relative humidity of 75%. The nutrient solution was refreshed every seven days.

Table 2
Preparation of individual nutrient solutions for the culture of alfalfa cuttings

Base solution	Composition	Amount
A solution (200×)	$Ca(NO_3)_2•4H_2O$	189.00g
	KNO_3	121.4g
B solution (200×)	$NH_4H_2PO_4$	23.0g
	$MgSO_4•7H_2O$	98.6g
C solution (1000×)	H_3BO_3	2.86g
	$MnSO_4•4H_2O$	2.13g
	$ZnSO_4•7H_2O$	0.22g
	$CuSO_4•5H_2O$	0.08g
	$(NH4)_4Mo_7O_{24}•4H_2O$	0.02g
D solution (200×)	$FeSO_4·7H_2O$	5.561g
	EDTA-$Na_2·2H_2O$	7.485g
	ddH_2O	To 1L
Note: When preparing the nutrient solution, each solute should be dissolved separately and then mixed. The prepared base solution should be protected from light during storage. When preparing modified 1/2 Hoagland nutrient solution, separately add 2.5 mL base solution A, B, and D and 0.5 mL solution C into 1L distilled water.		

Design of culture containers

As shown in Fig. 5, the culture containers consisted of white plastic pots (17.5 cm long, 11.5 cm wide, and 5 cm high). The outer surfaces of the culture containers were covered by a black tape to maintain a dark environment to promote root growth and prevent the growth of green algae in the nutrient solution. Further, the pots were covered with polyvinyl chloride (PVC) board, which was perforated with 15 evenly spaced holes (1.2 cm diameter) (Fig. 5a). The cut sponge strips (1 cm thick, 5–6 cm long and 1.8-2.0 cm wide) were washed 1–2 times with tap water and then washed once with distilled water. The culture pots,

PVC boards, and the cut sponges were washed with 75% alcohol and put in an ultra-clean platform for 4 h UV disinfection. PVC board can be reused many times and is easy to sterilize.

Preparation of nutrient solutions

As shown in Table 2 and Table 3, different concentrations of HB-101 natural plant vitality solution (v/v, 1/500, 1/1000, and 1/2000) were added to 1/2 modified Hoagland base solution, respectively, and stored at room temperature until use for cultivation of cuttings.

Table 3
Preparation of culture medium for alfalfa cuttings

Composition	Volume
A solution	2.5 mL
B solution	2.5 mL
C solution	0.5 mL
D solution	2.5 mL
HB-101	2 mL (1/500);1 mL (1/1000);0.5 mL (1/2000)
ddH_2O	To 1L
Note: The preparation protocols for solutions A, B, and C are shown in Table 2.	

Cutting method

After 30 days of hydroponics culture, branches with a stem base diameter of 1.6 ~ 1.8 mm and from healthy, disease-free plants were selected as source material for propagation cuttings. Each branch was divided into three segments: Top part (the top of the branch), middle part (the fifth stem segment), and the bottom part (the first stem segment). Stem sections of size 6–8 cm with 1–2 leaves and one bud were sliced at a 45-degree angle. While cutting the stems, it was ensured that the cuttings were not too large because big cuttings would not root well or, if rooted, the plants would be tall and lanky instead of being compact. Cuttings were transplanted into pots containing 750 mL of cutting nutrient solution and held in position with a 1 × 3 cm sponge strip (~ 1 cm thick). All jars and sponge strips were sterilized before use with 75% ethanol for 2 h and then placed under ultraviolet light in a laminar flow hood for another 2 h. Each treatment contained 20 cuttings and was done in 3 replications. Preparation of cuttings was done in the early morning between 7 to 8 am when the plant cells were turgid, as the stems were easier to cut and would have higher chances of maintaining turgidity after culture.

Management after cutting

In the first week after cutting, the pots were placed in a chamber under controlled conditions of temperature (25 ± 1 °C day/ 20 ± 1 °C night), humidity (80%), and photoperiod (16 h light (200 mol/m^2·s)/ 8 h dark). After one week, the culture conditions were changed to 300 mol/m^2·s light intensity, 16 h (25ºC) day/ 8 h night (20ºC), 75% relative humidity, and the nutrient solution was refreshed every seven days. The plant growth parameters of five cuttings per treatment were assessed 21 days after culture, including the percentage of rooted cuttings, root length, and stem height. 5 Rooted plants per treatment were used for health survey. The health survey were visually scored on a scale of 4, where: 1–2 scores = Plant was severely stunted, leaves discolored or the plant completely dead; 2–3 scores = Plant grow weakly, roots and leaves discolored, root length is short (< 5 cm); 3–4 scores = Plant grows well, root system developed; 4–5 scores = Plant has more lateral roots, plant grow luxuriant. Rooted alfalfa cuttings were subsequently cultivated for another seven days and could then be used for experiments or planted in pots. Of note, the young plants will need special care to ensure optimal growth.

Data Analysis

Analyses of variance (one-way ANOVA) were conducted using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA) for significant differences among treatments and to analyze the influence of different stem node parts and various nutrient solutions on the rooting rate, root length and stem height. Means were separated using Duncan's multiple range test at P = 0.05).

Abbreviations

H^M: Modified 1/2 Hoagland nutrient solution

H^M +1/500H: 1/500(v/v) HB-101 added to modified 1/2 Hoagland nutrient solution

H^M +1/1000H: 1/1000(v/v) HB-101 added to modified 1/2 Hoagland nutrient solution

H^M +1/2000H: 1/2000(v/v) HB-101 added to modified 1/2 Hoagland nutrient solution

Declarations

Acknowledgements

Not applicable.

Author contributions

LC supervised, conceived, and designed the experiments. YL, WZ, and SL performed the experiments and collected the data. WZ analyzed the data, LC and YL wrote the manuscript. WY and XS prepared nutrient

solutions for the experiments. SL and WZ assisted in some of the experiments. LM provided advice. JS and GY acquired funding and were involved in project administration and supervision. All authors read and approved the final manuscript.

Funding

This research was financially supported by the “National Natural Science Foundation of China” (No. 31802119), Qingdao Agricultural University high-level talent research fund (No.1120003), and “First Class Grassland Science Discipline programme in Shandong Province, China”.

Availability of data and materials

All the data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Shen C, Du HL, Chen Z, Lu HW, Zhu FG, Chen H, et al. The Chromosome-level genome sequence of the autotetraploid alfalfa and resequencing of core germplasms provide genomic resources for alfalfa research. Mol Plant. 2020; 139: 1250-1261.
2. Ma JT, Qiu DY, Gao HW, Wen HY, Wu YD, Pang YZ, et al. Over-expression of a γ-tocopherol methyltransferase gene in vitamin E pathway confers PEG-simulated drought tolerance in alfalfa. BMC Plant Biol. 2020; 20:226.
3. Bai ZH, Ma WQ, Ma L, Velthof GL, Wei ZB, Havlík P, et al. China’s livestock transition: driving forces, impacts, and consequences. Sci Adv. 2018; 4, eaar8534.
4. Radović J, Sokolović D, Marković J. Alfalfa-most important perennial forage legume in animal husbandry. Biotechnology in Animal Husbandry. 2009; 25: 465-475.
5. Dilkova M, Bingham E. Microsporogenesis of alfalfa cultivars and selected genotypes II. Medicago Genet. Rep. 2017; 17:1-16.
6. Howard BH. Propagation techniques. Scientific Horticulture. 1971; 23, 116-126.

7. Cong LL, Sun Y, Long RC, Kang JM, Zhang TJ, Li MN, et al. Modulation of protein expression in alfalfa (*Medicago sativa*) root and leaf tissues by *Fusarium proliferatum*. J Integr Agr. 2017; 16: 2558-2572.
8. Cui WT, Yao P, Pan JC, Dai C, Cao H, Chen ZY, et al. Transcriptome analysis reveals insight into molecular hydrogen-induced cadmium tolerance in alfalfa: the prominent role of sulfur and (homo)glutathione metabolism. BMC Plant Biol. 2020; 20:58.
9. Kang WJ, Jiang ZH, Chen YG, Wu F, Liu C, Wang HF, et al. Plant transcriptome analysis reveals specific molecular interactions between alfalfa and its rhizobial symbionts below the species level. BMC Plant Biol. 2020; 20:293.
10. Xiong X, Wei YQ, Chen JH, Liu N, Zhang YJ. Transcriptome analysis of genes and pathways associated with salt tolerance in alfalfa under non-uniform salt stress. Plant Physiol Bioch. 2020; 151:323-333.
11. Wang LP, Sheng QQ, Zhang YL, Xua JY, Zhang HH, Zhua ZL. Tolerance of fifteen hydroponic ornamental plant species to formaldehyde stress. Environ Pollut. 2020; 265: 115003.
12. Lee JY, Yang H, Lee TK, Lee CH, Seo JW, Kim J, et al. A short-term, hydroponic-culture of ginseng results in a significant increase in the anti-oxidative activity and bioactive components. Food Sci Biotechnol.2020; 29: 1007-1012.
13. Delden SH, Nazarideljou MJ, Marcelis LFM. Nutrient solutions for *Arabidopsis thaliana*: a study on nutrient solution composition in hydroponics systems. Plant Methods.2020; 16:72.
14. Wang LP, Sheng QQ, Zhang YL, Xua JY, Zhang HH, Zhu Z Tolerance of fifteen hydroponic ornamental plant species to formaldehyde stress. Environ Pollut. 2020; 265(null), 115003.
15. Niu S, Han Q F, Jia Z K. Effects of different treatments on rooting capacity of *Medicago Sativa*. Acta Agriculturae Boreali-occidentalis Sinica.2007;16:149-152.
16. Hackett WP. Effects of phase change on cutting rooting. In Davis TD, Sankbla N (eds). adventitious root formation in cutting. Portland Oregan: Dioscorids Press, 1988, 11.
17. Haissing BE, Riemenschneider DE. Study on rooting habit of cutting. Physid Plant.1992;86:1.
18. Paton DM. On rooting mechanism of *Eucalyptus robusta* Ann Bot.1981; 47:836.

Figures

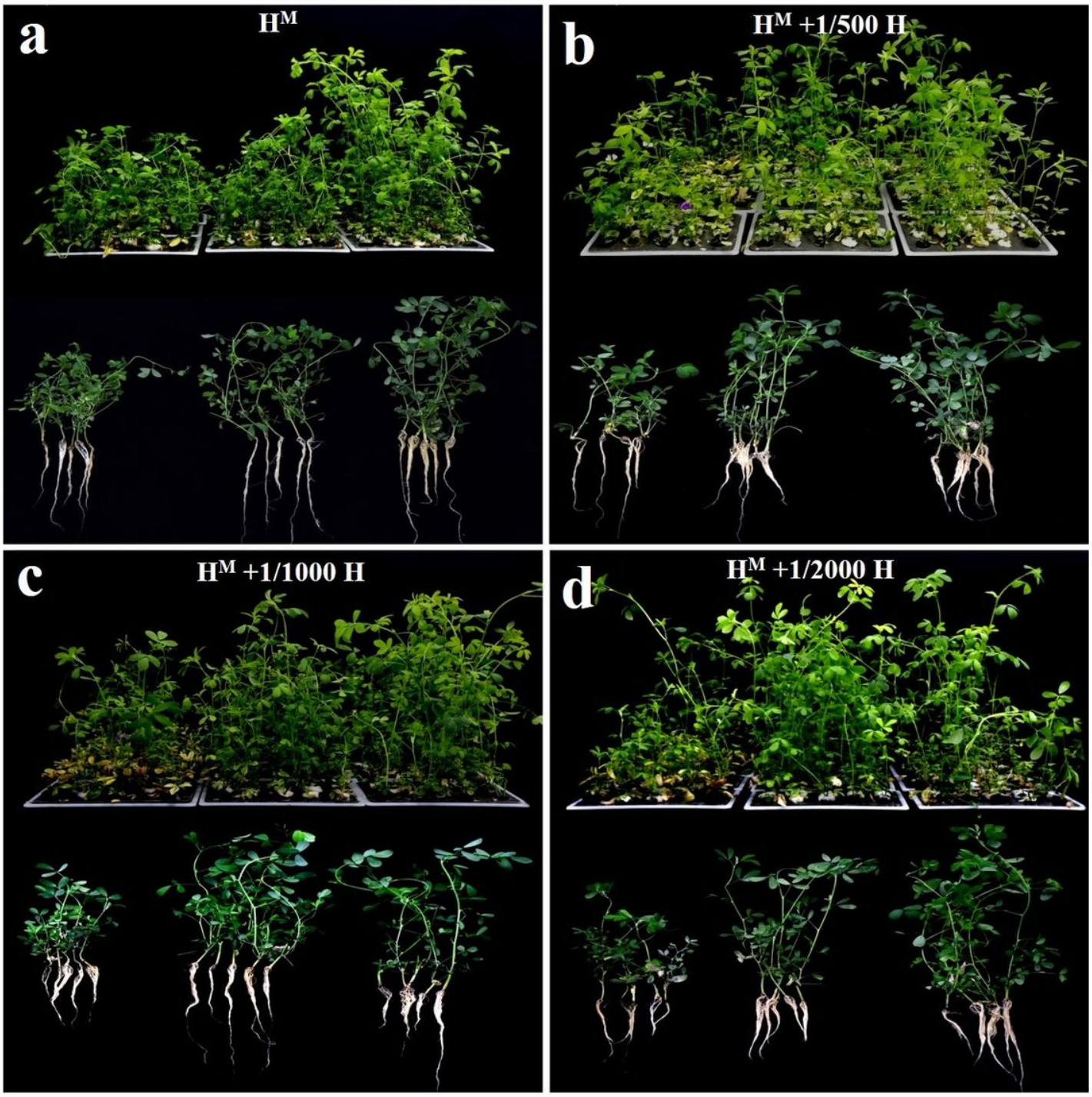

Figure 1

Influence of different stem parts on the propagation of cuttings under four nutrient solutions: a HM nutrient solution. b HM+1/500H nutrient solution. c HM+1/1000H nutrient solution. d HM+1/2000H nutrient solution.

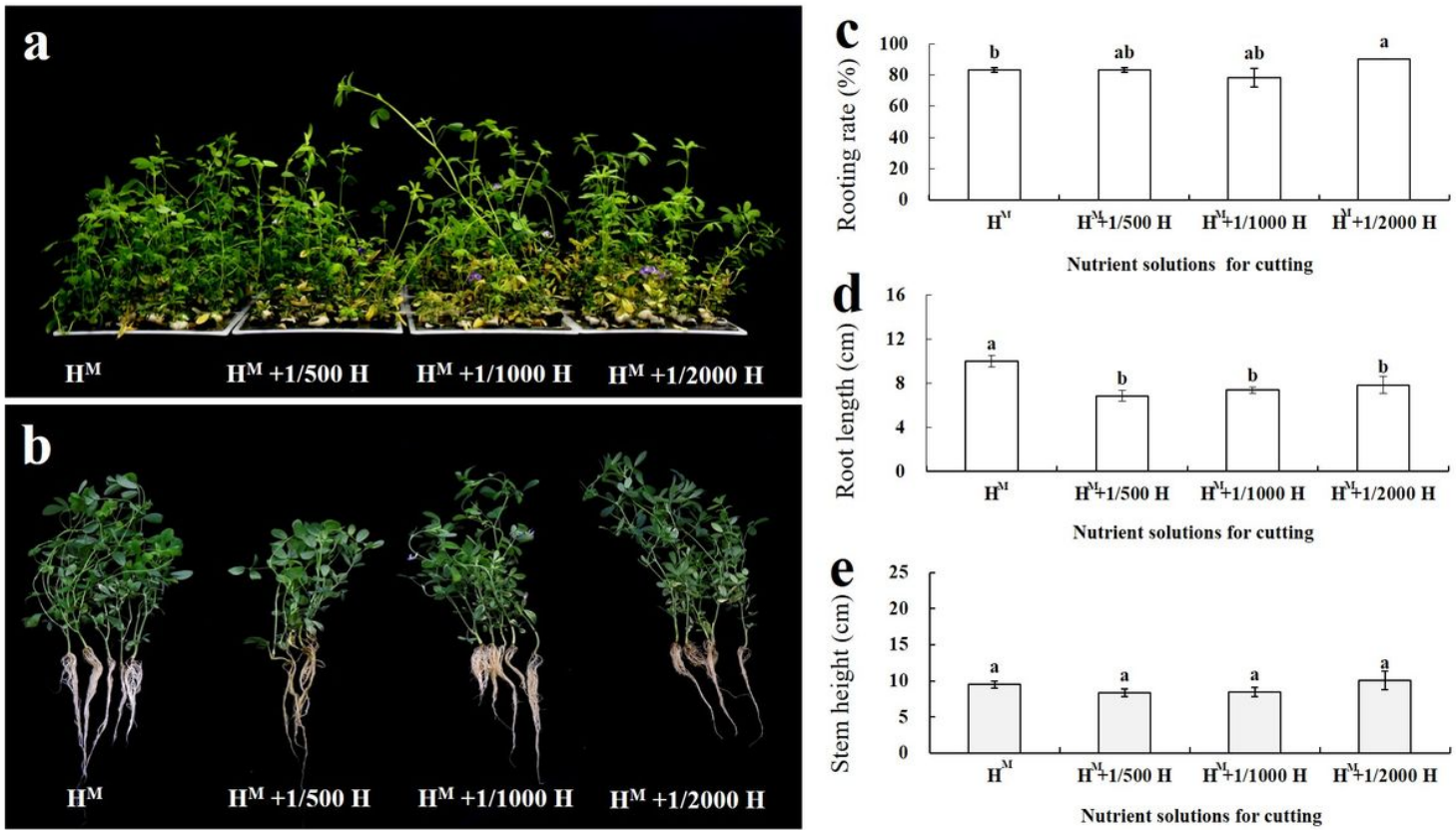

Figure 2

Rooting rates, root lengths, and stem heights of the top stem segment obtained in the four nutrient solutions: a Cuttings grown in the four nutrient solutions. b Rooted plants in the four nutrient solutions. c Rooting rate. d Root length. e Stem height.

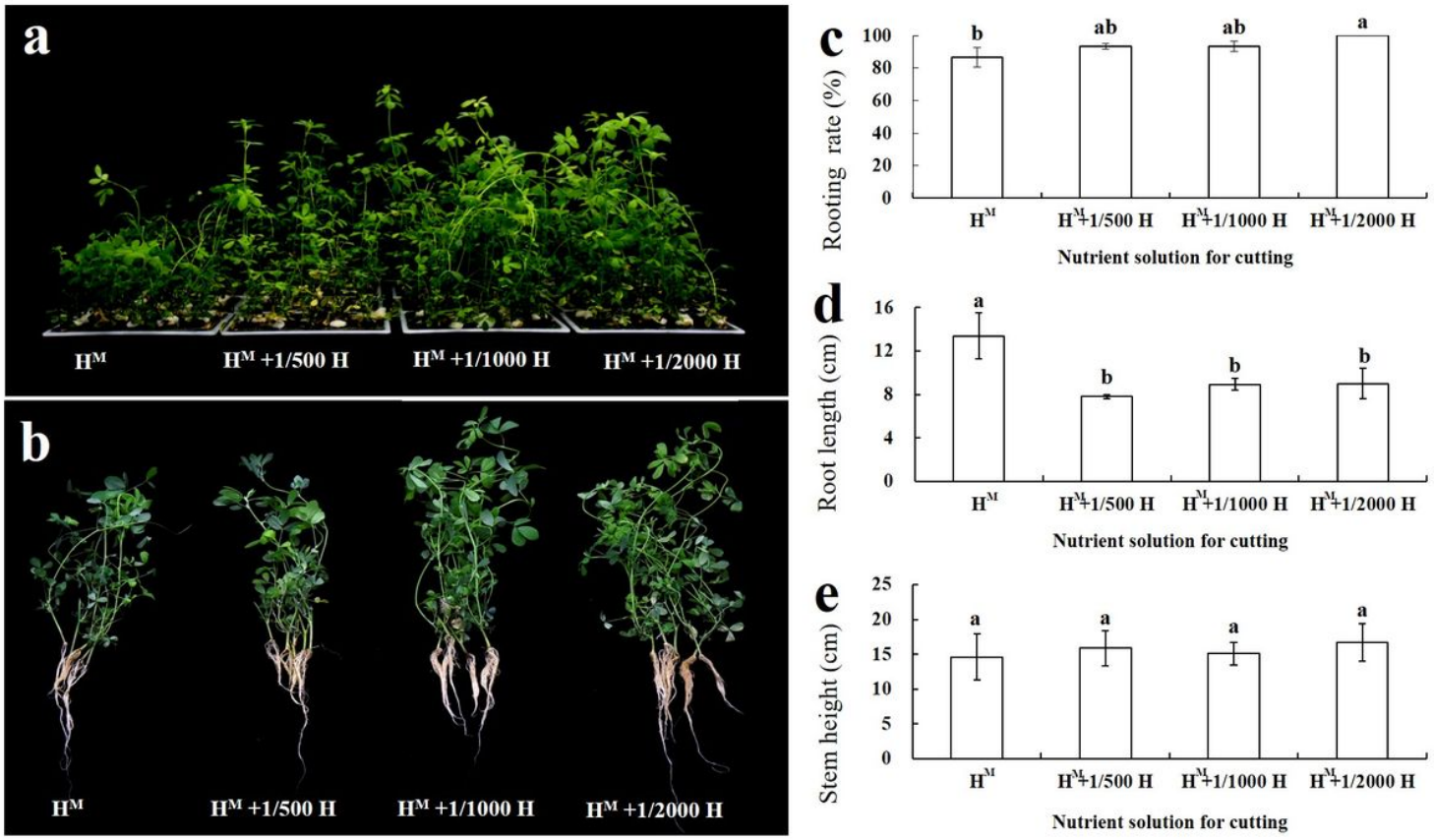

Figure 3

Rooting rates, root lengths, and stem heights for the middle stem segment under the four nutrient solutions: a Cuttings grown in the four nutrient solutions. B Rooted plants in the four nutrient solutions. c Rooting rate. d Root length. e Stem height.

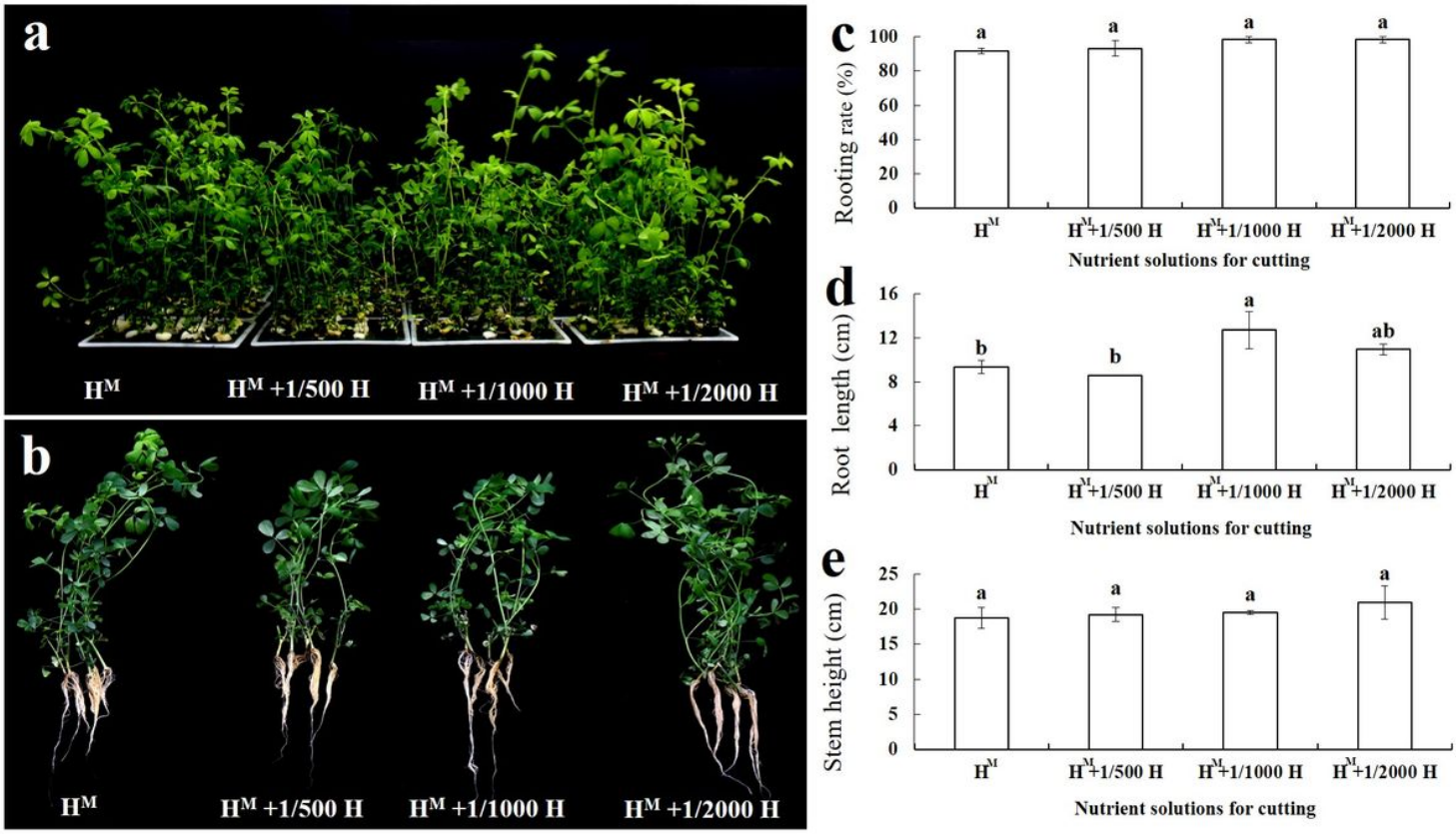

Figure 4

Rooting rates, root lengths, and stem heights for the lower stem segment grown under the four cutting nutrient solutions: a Cuttings grown in four nutrient solutions. b Rooted plants in the four nutrient solutions. c Rooting rate. d Root length. e Stem height.

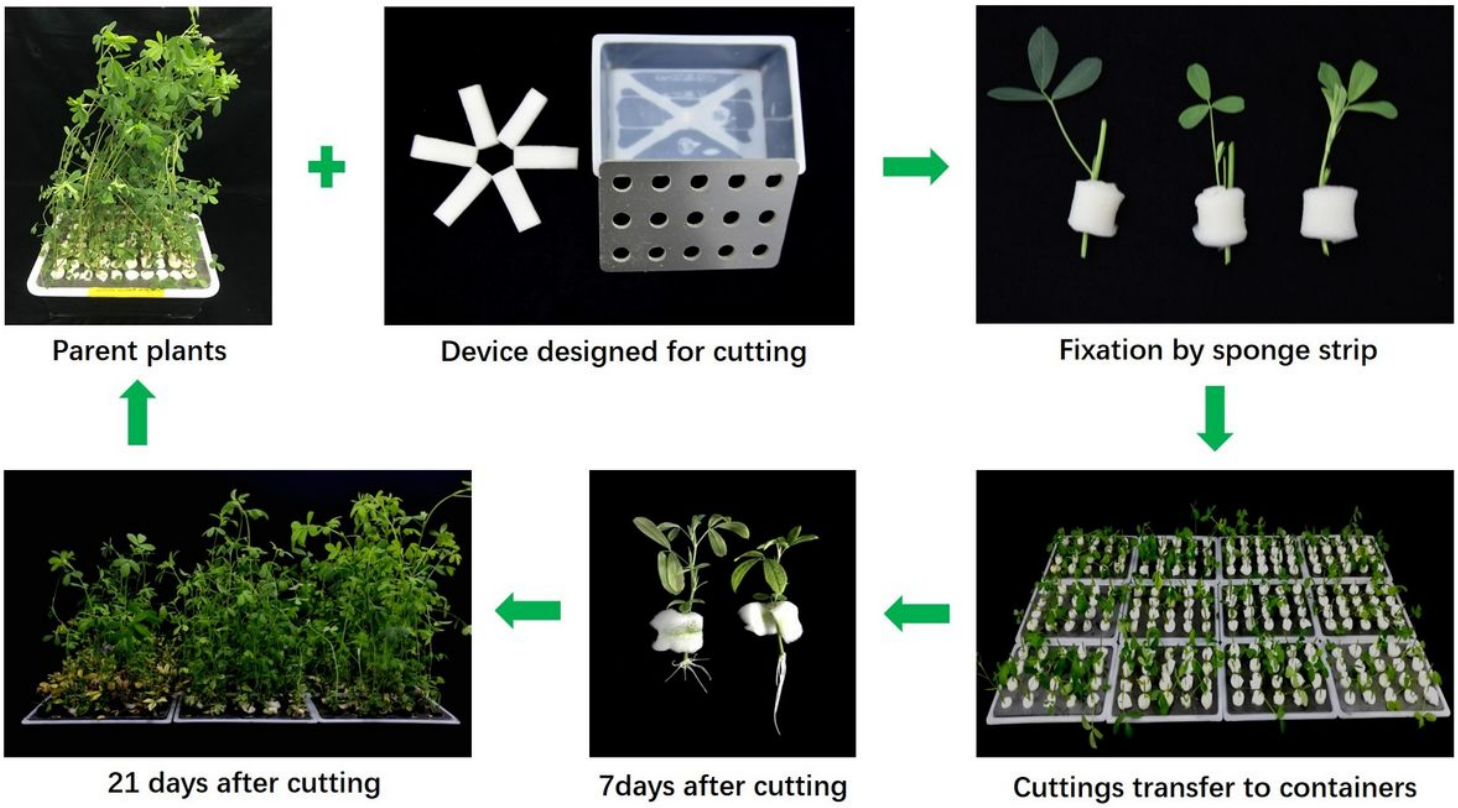

Figure 5

Schematic overview of alfalfa cutting under the hydroponic system