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# Enhancing *Asparagus officinalis* Seed Germination and Plantlet Development through *In vitro* Culture

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## Abstract

Asparagus (*Asparagus officinalis* L.) is a medicinal plant with high therapeutic value. To date, protocols for improving the *in vitro* germination of *A. officinalis* are extremely scarce. The aim of this study was to compare the germination, rooting and growth of asparagus under different growth conditions and to investigate the influence of the growth media, light and activated carbon. Asparagus seeds were sown in a greenhouse, in Petri dishes and *in vitro* culture. For *in vitro* culture, seeds were placed in Murashige and Skoog (1962) and ½ MS media; in the liquid or semisolid state, activated carbon was added to MS media. The results showed that *in vitro* culture produced the highest germination rate (80.55%) compared with greenhouse (25%) and Petri dish (44.44%) culture. Effective germination rates were obtained with ½ MS media (69.44%). These factors also had greater effects on plant development and growth. The average shoot and root lengths were 10.70 and 7.63 cm, respectively. Plant germination rates did not significantly differ between the liquid and semisolid media but did positively affect shoot length (9.72 cm) in the liquid media. When the seeds were incubated in a dark environment, the photoperiod (0/24 h light/dark) affected germination latency (7 days in the dark environment and 11 days in the light environment) but had no significant effect on germination. The highest germination rate was achieved with ½ MS semisolid media in the dark, ensuring a greater germination rate (80.55%). The germination of *A. officinalis* on activated carbon was effective, reaching 77.77%.

**Keywords:** Activated carbon; *Asparagus officinalis* L.; *In vitro* culture; Light; Physical state of medium; Seedling development

## Introduction

Asparagus (*Asparagus officinalis* L.), which belongs to the family Asparagaceae, is an economically important plant that can be harvested for years (Kaluzewicz *et al*. 2010; Iqbal *et al*. 2017). Originally from the Mediterranean region, it is widely cultivated in many countries (Ito *et al.* 2011). Due to its biological, pharmaceutical and industrial properties, it is considered a medicinal and nutrient-rich plant that contains large quantities of vitamin A, C, potassium, iron and calcium (Vora *et al.* 2017; Guo *et al.* 2020). This high nutrient content has an important economic effect, as it is used in the food industry (Nguyen *et al*. 2019) and in pharmaceuticals and has antifungal and anticancer activities (Li *et al.* 2016; Vora *et al.* 2017). In addition, the roots and seeds of *A. officinalis* have been used to treat uterine pain, elephantiasis, lithotriptic, pulmonary pain, bronchial catarrh and pulmonary tuberculosis (Akbar 2020).

To maintain the economic and therapeutic value of asparagus, improving its production and regeneration potential is essential. Germination is a critical stage in plant development. However, several species present obstacles that can lead to germination inhibition problems (Mezghenni *et al.* 2014). To overcome these difficulties, *in vitro* culture plays a vital role in the conservation and large-scale propagation of medicinal plants, as it provides a basis for studying the potential production of secondary metabolites that have therapeutic properties (Verma 2019). Similarly, light has a significant impact on the morphogenetic response of plants cultivated *in vitro*. Indeed, it positively affects the ability of explants to regenerate and produce roots (Kulus and Wozny 2020). In parallel, the physical state of the medium, either in the liquid or semisolid state, affects plant growth and development (Doğan 2022). In general, the basal media used for *in vitro* culture provide essential nutrients for plant growth and development (Brhadda *et al*. 2003). Activated charcoal is one of the elements studied *in vitro* culture and is frequently used to improve germination and seedling conservation, as well as an antioxidant in culture media (An *et al*. 2021). The use of activated carbon, due to its ability to absorb toxic substances produced and released by explants in

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culture media and to provide dark media, improves the rooting of plants *in vitro* (Thomas 2008; Sharma *et al*. 2012).

In addition, there is increasing awareness of the need for *in vitro* germination to ensure plant survival under changing environmental conditions and there is a risk of losing some commercially viable plant species that will go extinct due to unfavorable climates and unfavorable conditions characterized by high temperatures and salt-stressed conditions (Gao *et al*. 2021; Ortega-García *et al*. 2021). To develop conservation strategies, seed germination studies of this medicinal plant have been useful. This highly promising approach will enable us to construct a bank of materials that can be used for other biotechnology applications, notably somatic embryogenesis and micropropagation. Further molecular biology studies will allow us to genetically improve an important medicinal plant, such as *A. officinalis*. Moreover, *in vitro* culture techniques offer promising prospects for mass production, as well as plant sanitation and genetic improvement; such studies have been very limited on *A. officinalis,* even though some interesting results have been obtained on micropropagation (Khunachak 1978; Carmona-Martín 2014; Rasad *et al.* 2019), somatic embryogenesis (Pontaroli and Camadro 2005; Abbasi *et al*. 2020) and synthetic seed preservation (Sallam *et al.* 2023). However, there are no reports on *in vitro* seed germination, elongation or rooting capacity. In addition, germinated seedling banks are difficult to maintain due to low seed viability (Encina and Regalado 2022). This study investigated the germination capacity of *A. officinalis* under different conditions. For the first time, an *in vitro* study of the effects of culture medium, light, the physical state of the media and activated carbon on seed germination, rooting and growth of *A. officinalis* seedlings and their acclimatization was carried out and to determine if asparagus seeds are subjected to *in vitro* culture with activated carbon, culture media and controlled light conditions, their germination efficiency and rate will be significantly greater than those of seeds grown under other conditions. A deeper understanding of the conditions that enhance germination would contribute to optimizing the production and genetic quality of this important plant.

## Materials and Methods

### Plant material

Seeds of *A. officinalis* variety d'Argenteuil were collected from the Agro Sarnese-Nocerino region, Italy. Under a laminar flow hood, the seeds were disinfected by soaking for one minute in 70% ethanol, followed by immersion for 5 min in 60% sodium hypochlorite. The plants were then rinsed 3-4 times with sterile distilled water and dried on sterile filter paper before sowing in various culture media. Observations of *A. officinalis* seed germination were made daily and continued until germination percentages stabilized at approximately 40 days. The emergence of the radicle is considered the first indicator of germination.

### Effect of growing conditions on germination

**Greenhouse:** Seeds were germinated in plastic pots containing 50/50 v/v peat and sand at an average of 2-3 seeds per pot, sown to a depth of 1 cm and placed in a greenhouse under natural light at 25 ± 2°C and 60% relative humidity. To ensure optimum germination, the pots were covered with plastic paper for 1-2 days and the seeds were watered with 100 mL of Hoagland solution once or twice a week (Hoagland and Arnon 1950).

**Petri dishes:** Seeds were placed in 100-mm-diameter Petri dishes lined with sterile filter paper and moistened with sterile distilled water at an average of 12 seeds per dish. To ensure germination without contamination, the Petri dishes were sealed with parafilm and incubated under a 16 h photoperiod at 25 ± 2°C.

***In vitro* culture:** The basic medium used for *in vitro* culture is that of Murashige and Skoog (1962), which the micro- and macro-elements required for *in vitro* plant development supplemented with 30 g/L sucrose and 8 g/L agar. Before adding the agar, the pH of the medium was adjusted to 5.7 ± 0.1 using a solution of HCl or NaOH. The medium was sterilized by autoclaving at 121°C under a pressure of 1 bar for 20 min. Seeds were grown in test tubes and placed in a culture chamber at a temperature of 25 ± 2°C under a photoperiod of 16 h with a light intensity of 45 $\mu$moL $m^{-2}$ $s^{-1}$.

### Effect of various factors on germination *in vitro*

**Culture medium:** MS media as well as ½ MS media obtained by diluting macro elements by half were used. Seeds were grown in test tubes or flasks filled with medium and placed in a culture chamber at a temperature of 25 ± 2°C and a photoperiod of 16 h of light and 8 h of darkness. The number of germinated seeds was recorded every day for a period of 40 days.

**Light:** To determine the effect of light on the initiation of seed germination and the development of new *A. officinalis* seedlings on MS and ½ MS media (liquid and semisolid), the seeds were incubated under a photoperiod of 24 h dark or 16 h light until germination.

**Physical state of the medium:** Thus, MS and ½ MS media were used either in semisolid media containing 8 g/L agar or in liquid media. The flasks of liquid media were kept under continuous agitation during the incubation period. All tubes or flasks were adjusted to 15 mL of culture medium.

**Activated carbon:** Activated carbon is known for its ability to adsorb polyphenols that inhibit plant aerial and root growth and development. It was therefore used at concentrations of 0, 5 and 10 g/L to improve germination and rooting. To enhance the efficiency of the MS semisolid media, activated carbon was added to the media. All media were incubated under a photoperiod of 24 h dark or under a photoperiod of 16 h light.

**Acclimatization**

Seedlings obtained by *in vitro* germination and ready for acclimatization were transplanted into pots containing a mixture of 50% sand and 50% peat and watered once or twice a week with Hoagland solution.

**Evaluation of seedling growth and measured parameters**

Germination observations were carried out daily to monitor germination kinetics. The number of germinated seeds was recorded each day, while radicle emergence was observed as the first indicator of germination. Observations of germination and hypocotyl elongation were made regularly every two days for a period of 40 days. Germination curves were used to determine latency and germination time.

**Statistical analysis**

Three replicates were used in each experiment, and each replicate had 12 explants. Using SPSS (Statistical Package for the Social Sciences 21) software, two separate tests were used to compare means. First, the independent sample t test was used to assess the equality of the means. Then, for a more in-depth analysis of comparisons between means, we applied the Student–Newman–Keuls test (ANOVA) at a significance level of 5%.

## Results

**Effect of growing conditions on germination**

Statistical analysis revealed significant differences among the three growing conditions. Indeed, the highest germination rate of 80.55% was observed for seeds grown *in vitro,* whereas the percentage of seeds that germinated in petri dishes (44.44%) or in greenhouses (25%) was significantly lower (Fig. 1a-b).

The curves of variation in the germination rate as a function of days showed that the seeds followed different kinetics under all the conditions tested and the germination speed varied remarkably. Indeed, seeds that germinated *in vitro* or in petri dishes showed very short latency times, reaching 7 and 9 days, respectively. On the other hand, seeds that germinated in the greenhouses showed prolonged latency, extending for more than 25 days (Fig. 1c). However, we also observed a slower average germination speed for seeds germinated in the greenhouse. On the other hand, the average growth rate of the seeds germinated *in vitro* was greater than that under the other two conditions.

**Effect of medium**

There were significant differences in the effect of culture media on germination between MS and ½ MS media ($P \leq 0.05$). Notably, in the ½ MS media, the germination rate of the seeds was 69.44%, which was significantly greater than that in the MS media (47.22%). Comparison of the germination kinetics on the two media revealed a latency of 7 days for the ½ MS media and 14 days for the MS media. Similarly, for the ½ MS media, we obtained a maximum germination rate after 29 days compared with that of the MS media, which enabled us to obtain a maximum rate after 33 days.

**Effect of light**

Statistical analysis revealed no significant difference between the germination rates of seeds under light (51.38%) and dark (65.27%) conditions. However, it should be noted that light prolonged the latency. Dark-incubated seeds germinated 4 days earlier than light-incubated seeds at 7 and 11 days, respectively.

**Effect of the physical state of the culture medium**

Statistical analysis revealed that the germination rates of *in vitro-grown* seeds in liquid and semisolid growth media were similar. However, the germination rate varied according to the day. For the liquid medium, we observed a longer latency time of 11 days compared with that of the semisolid medium, where this phase was reduced to only 7 days.

**Culture medium-light interactions**

The results of the medium-light interaction experiments are shown in Fig. 2a and indicate that the germination of seeds is influenced by the incubation conditions, including light and culture media. First, the seeds exhibited greater germination under dark conditions, whether in MS culture media or ½ MS media, with germination rates of 58.33 and 72.22%, respectively. Significant germination was also observed in ½ MS media under light conditions, with a rate of 66.66%. However, the lowest germination rates were recorded under light conditions, notably in the MS media, at only 36.11%.

**Interactions between the physical state of the medium and light**

The interaction between the state of the medium and light revealed that *in vitro* germination rates were not significantly affected by incubation conditions such as light or the physical state of the culture medium. Both media (liquid and semisolid) under dark conditions showed similar germination rates, reaching 65.27%. On the other hand, when the seeds were exposed to light, both the liquid and semisolid media had statistically similar effects, with rates of 48.61 and 54.16%, respectively (Fig. 2b).

**Interactions between the culture medium and the physical state**

The results revealed significant differences in germination

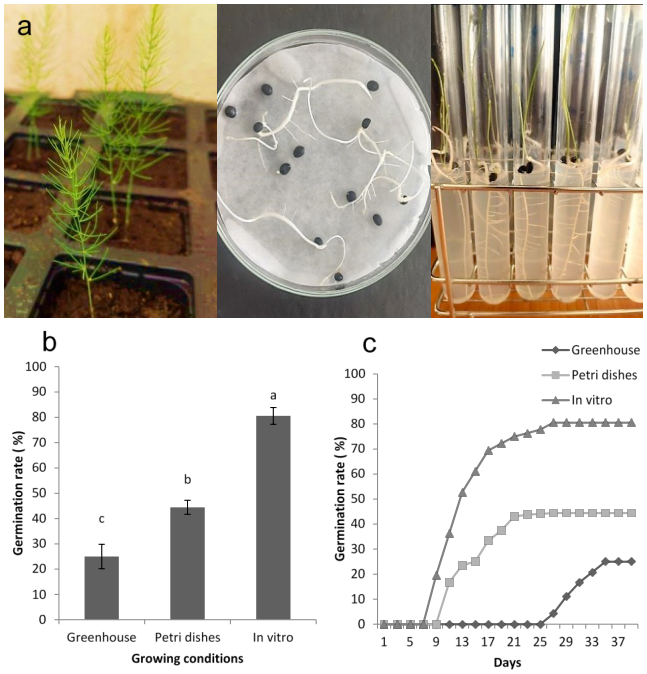


**Fig. 1:** Germination of *Asparagus officinalis* L. seeds under the three growing conditions tested (**a**, **b**) and germination kinetics of *A. officinalis* under different growing conditions (**c**)

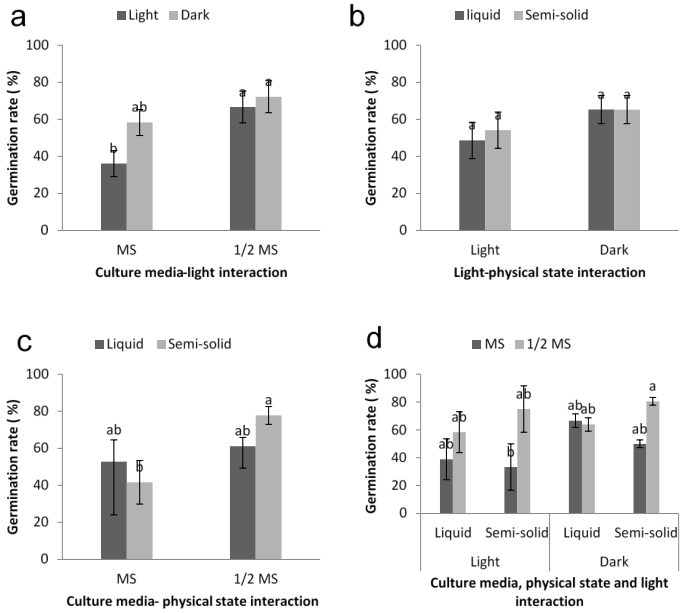


**Fig. 2:** Effect of the interaction of media and light on the *in vitro* germination of *Asparagus officinalis* L. seeds (**a**), effect of the interaction between the physical state of the media and light on the germination of seeds *in vitro* (**b**), effect of the interaction between the culture media and physical state of the media on the germination of seeds *in vitro* (**c**) and effect of the interaction between the culture media, physical state of the media and light on the germination of seeds *in vitro* (**d**)

in both the liquid and semisolid-states on MS and ½ MS media. The highest germination rate (77.77%) was observed for the ½ MS semisolid media. The lowest germination rates were recorded for the MS media on both the liquid and semisolid media, at 52.77 and 41.66%, respectively (Fig. 2c).

## Interactions among the medium, its physical state and light

The interactions between the different culture media and the environmental conditions, particularly the physical state of

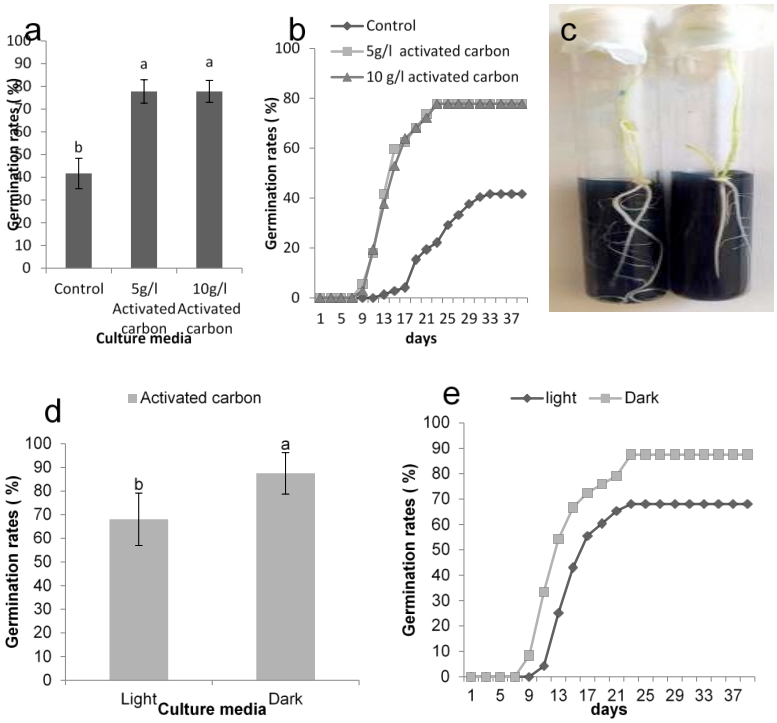


**Fig. 3:** Germination rate of *Asparagus officinalis* L. seeds under different activated carbon concentrations (**a**), effect of activated carbon on germination kinetics (**b**), effect of activated carbon on the germination of *A. officinalis* (**c**), effect of activated carbon-light interactions on the germination of seeds *in vitro* (**d**), effect of light and activated carbon on germination kinetics (**e**)

the medium as well as the incubation conditions in terms of exposure to light, contributed to the determination of the optimal parameters for the *in vitro* culture of *A. officinalis* seeds. The results showed that the best growth conditions were observed when the seeds were placed in ½ MS medium in the semisolid-state during dark incubation, with the maximum rate reaching 80.55%. On the other hand, the lowest rate was recorded when the seeds were grown in MS media, in the semisolid state, and incubated in the light, with a germination rate of only 33.33%. In the case of the liquid medium, both MS and ½MS showed similar and significant germination rates, 38.88 and 58.33%, respectively, in the dark treatment and 66.66 and 63.88%, respectively, in the light treatment (Fig. 2d).

**Activated carbon effect**

**Activated carbon concentration:** Analysis of variance revealed that the addition of activated carbon significantly increased the seed germination rate. Activated carbon concentrations of 5 and 10 g/L resulted in similarly high germination rates, reaching 77.77% compared with 41.66% for the control (Fig. 3a). By tracking germination rates as a function of time, we concluded that compared with activated carbon media, control media prolonged the latency time, which was 11 days and reduced the latency time to a mere 9 days. The concentrations of 5 and 10 g/L had similar effects on the germination kinetics (Fig. 3b-c).

**Interaction between light and activated carbon:** The results obtained showed very different reactions of germinating seeds depending on the medium used with activated carbon and the incubation factor. In fact, germination was more progressive and faster in the presence of activated carbon in the obscurities, reaching the optimum germination rate (87.5%) on day 23, compared with 68.05% germination in the presence of light (Fig. 3d-e). The kinetics of germination showed that there was a general improvement in germination in the presence of activated carbon. The activated carbon media resulted in the shortest dark and light latency times of 7 and 9 days, respectively, with the highest germination speeds and rates. However, the latency time reached 17 days in the light control group and 11 days in the dark group (Fig. 3e).

**Development of aerial and root parts of seedlings obtained using different study factors**

**Effect of culture medium:** Statistical analyses revealed that ½ MS media promoted the development of the aerial and root systems of the plants, which reached lengths of 10.70 cm and 7.63 cm, respectively. In the MS media, however, they grew to barely 4.67 and 5.72 cm, respectively (Fig. 4).

**Effect of the physical state:** The development of the aerial and root systems of *A. officinalis* is clearly influenced by the physical state of the medium. Indeed, the results of the analysis of variance highlighted that the maximum shoot

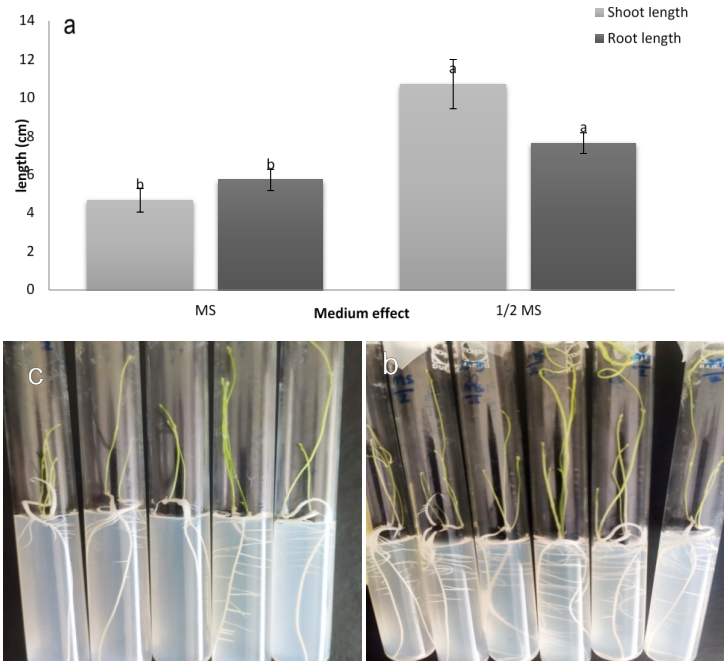


**Fig. 4:** Effect of media on the development of *Asparagus officinalis* L. aerial systems and root systems (**a**) on ½ MS media (**b**) and MS media (**c**)

length obtained in the liquid medium reached 9.72 cm, while in the semisolid medium, the length reached 5.65 cm. With regard to root length, the data revealed no significant difference between the two media, liquid or semisolid, with lengths of 6.16 and 7.19 cm, respectively, after 5 weeks of germination (Fig. 5a-dd).

**Interaction between the medium and the physical state:** The results revealed that the ½ MS medium in the liquid state was the most effective for growth of the aerial part, with a length of 15.44 cm, while the semisolid-state length was found to be only 5.97 cm. On the other hand, the MS media resulted in lower shoot growth, both in the liquid and semisolid state, with lengths of 4.01 and 5.33 cm, respectively, after 5 weeks of germination. In addition, a significant improvement in root length was observed in the ½ MS media, particularly in the semisolid state, with a length of 7.80 cm. The lowest values (4.86 cm) were recorded in the liquid MS media after 5 weeks of germination (Fig. 5b).

**Effect of activated carbon:** A statistical analysis demonstrated that activated carbon media favored the most development of the aerial and root systems of the plant, with lengths of 14.70 cm and 10.65 cm, respectively. On the other hand, the plants in the control medium exhibited the lowest growth (5.33 and 6.58 cm) (Fig. 5eg). In terms of acclimatization, after all the seedlings obtained from *A. officinalis* were transplanted into pot substrates under controlled environmental conditions, the survival rate was on the order of 93.33% and all the asparagus plants exhibited notable and normal development of their cladodes during the first few weeks of acclimatization (Fig. 6).

## Discussion

The aim of the present research was to improve the germination, rooting and growth conditions of *A. officinalis* our results revealed a strong correlation between growth conditions and the impact of growth conditions on the germination and development of the plants. Indeed, statistical analysis revealed that the most favorable germination rate was observed for *in vitro* culture. Compared with greenhouse and Petri dish culture*, in vitro* culture improved the germination rate and reduced the latency time. These findings are similar to those of Mezghenni *et al.* (2014), who proved that argan trees show high germination rates *in vitro* and that the germination kinetics obtained using *in vitro* techniques are faster than those obtained *via* greenhouse cultivation. Additionally, the percentage of seeds that germinated increased when *in vitro* culture or priming treatment was used, especially for seeds with low physiological quality. These findings are consistent with those of Evans and Pill (1989) and Bittencourt *et al*. (2004), who discovered that priming treatments promote asparagus seed germination and seedling emergence more successfully than do greenhouse cultivation or simply soaking the seeds in water.

As noted by Sidik *et al.* (2024), the basal medium plays a critical role in plant tissue growth and productivity, comprising macronutrients, micronutrients, vitamins, carbon sources, and solidifying agents. Varying proportions of these components in culture media can significantly impact plant responses and their development. The response of the seeds to MS and ½ MS media indicated a significant

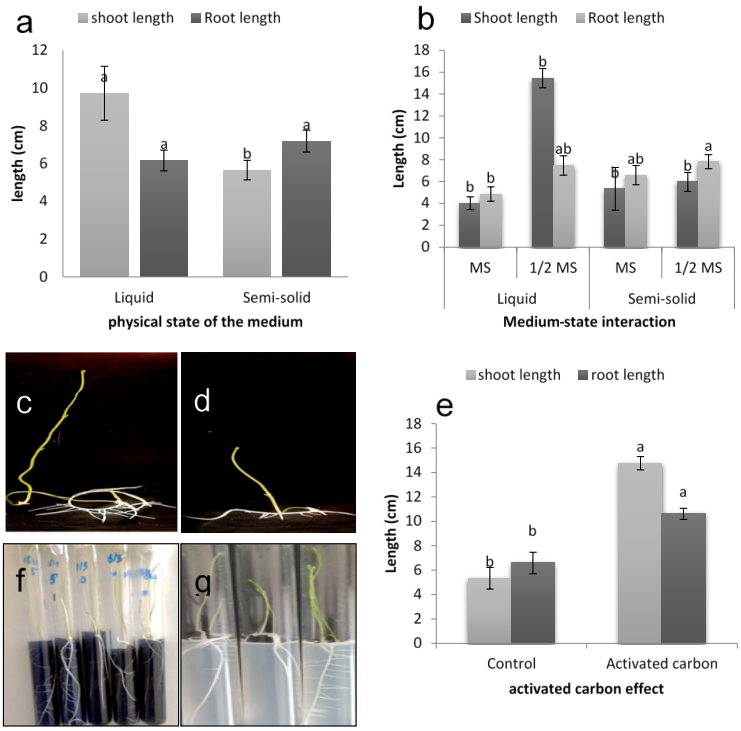


**Fig. 5:** Effect of culture media on plantlet development (**a**), effect of culture media and physical state interaction on plantlet development (**b**), effect of physical state on plantlet development liquid media (**c**) and semisolid media (**d**), effect of activated carbon on the development of *A. officinalis* (**e**), effect of activated carbon on root development in activated carbon media (**f**) and control media (**g**)

**Fig. 6:** Acclimatization of *Asparagus officinalis* L. seedlings

difference in the germination rate *in vitro*. Similarly, variation in the rhizogenesis of *in vitro*-grown plants between these two media was detected. Therefore, ½ MS medium with macroelements diluted by half was more advantageous for germination and root induction. Our findings are in line with those of Fadel (2010), who studied *Mentha spicata*, as well as Brhadda *et al.* (2003), who studied the olive tree *Moroccan picholine* and revealed that a less concentrated mineral medium stimulates plant elongation and rooting. Moreover, Li *et al.* (2024) demonstrated that the concentration of medium salts significantly impacted the induction of rooting. On the other hand, Dönmez *et al.* (2022) reported that the mineral salt concentration does not influence root number in Spathiphyllum plants. Similarly, in *Pecteilis radiata,* Kim *et al*. (2019) reported that the most favorable germination was observed on MS media compared with ½ MS media.

Furthermore, our results disagree with those of Rezali *et al.* (2017) on *Typhonium flagelliforme* and Pandey *et al.* (2010) on *Rauwolfia serpentina* L. These authors showed that decreasing the macroelement concentration of the MS medium led to a decrease in shoot and root length.

These results indicate that genetic factors are crucial in the choice of growing medium applied, the nutritional requirements and the adaptation of the mineral composition of the medium to the genotype to be regenerated. However, elevated concentrations of macroelements can lead to increased osmotic stress, making it difficult for seeds to absorb.

Germination rates in liquid and semisolid media were similar, and our results concur with those of Tsai and Chu (2008), who reported that seed germination rates in *Doritaenopsis* are unaffected by the physical state of the medium. However, compared with that in semisolid media, the growth of asparagus seedlings in liquid media was significantly greater. Our result is similar to that obtained by Pateli *et al*. (2003) for *E. radicans*. Clearly, the liquid media promoted better nutrient uptake, leading to better plant growth. Biswas *et al*. (2024) demonstrated that the physical state of the growth medium influences the growth, development, and maturation of the target plant species.

Compared with light conditions, dark conditions allowed a significant number of seedlings to be produced in a relatively short time. Our results are similar to the findings reported by Karunarathna *et al.* (2023) for *Madhuca longifolia* and Yadukrishnan *et al.* (2020) for *Arabidopsis thaliana*. These authors have shown that the average germination speed in the light is slower than that in the dark. This can be explained by the fact that seed incubation under dark conditions promotes an increase in phytohormones, which increases protein accumulation and promotes germination. Therefore, the requirement for light is likely a genetic characteristic and is mediated by phytohormones that stimulate the synthesis of substances that promote growth and germination. Furthermore, Routhier (2006) findings on the germination of *Eugenia repanda* and *Hexaclamys edulis* indicated that exposure of the seeds to light favors their germination. Moreover, Abohatem *et al*. (2024) demonstrated that dark incubation significantly improves germination rates and facilitates the conversion of date palm somatic embryos into plants. These results led to the conclusion that different plant species react differently to light. Light-induced decomposition of carbonic acid gas leads to carbon fixation and seed structure reinforcement, hindering germination. Additionally, light promotes oxygen release, which is vital for germination. Conversely, in darkness, carbonic acid gas remains intact and oxygen levels remain conducive to germination.

The present research focused on the positive influence of activated carbon on seed germination and rooting. Similar results were obtained by Sorgato *et al.* (2020) for *Dendrobium nobile* and Poniewozik *et al.* (2022) for *Paphiopedilum insigne*. This improvement can be attributed to the fact that activated carbon provides a dark environment and absorbs phenolic substances that encourage root growth. Moreover, Thomas (2008), Selvaskanthan and Eeswara (2024) explained the role of activated carbon in terms of the ability to absorb noxious substances produced and released from the explant or associated with minerals in the culture medium.

## Conclusion

Composition of the growing medium plays a crucial role in seed germination. *In vitro* culture of *A. officinalis* on ½ MS media in the semisolid-state and in the dark produced the highest germination rates. This technology opens up new avenues for the mass production of selected materials. Moreover, *in vitro* plants were found to be more vigorous and more resistant to disease, offering very promising prospects for the production of medicinal plants, as well as for the genetic improvement of plants.

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## Author Contributions

All authors have read and agreed to the published version of this manuscript.

## Conflicts of Interest

All authors declare no conflict of interest.

## Data Availability

All data generated or analyzed during this study are included in this manuscript and are available from the corresponding author upon reasonable request.

## Ethics Approval

Not applicable to this paper.

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