

## Effects of Root Cutting and IBA Treatment on Root Quality and Seedling Growth of *Ammopiptanthus mongolicus* Postprint

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### Abstract

To address the forestry technical challenges of *Ammopiptanthus mongolicus*, characterized by long taproots, few fibrous roots, difficult seedling rooting, and low transplant survival rates, this study investigated different root pruning intensities combined with varying concentrations of hormone (IBA) treatments on seedlings at different developmental stages. After promoting root development, the effects of different treatment combinations on root morphology, root quality, and seedling growth of *A. mongolicus* were measured and analyzed to explore key measures for promoting root establishment and survival, providing a fundamental basis for seedling cultivation and afforestation techniques of *A. mongolicus*. The results showed: (1) Root pruning promoted the development of multiple taproots, as well as increases in lateral roots, total root mass, above- and below-ground biomass, root-shoot ratio, root activity, and root cation exchange capacity (CEC). Specifically, cutting 0.5 cm of the root tip when seedling root length reached 1.5–2.0 cm (F2) significantly promoted increases in lateral root number, root tip number, branch number, seedling quality index, and root activity, with root activity reaching the peak value among all root pruning treatments (significantly increased by 137.70% compared to the control). Root CEC was optimal under the treatment of cutting half the root length when root length reached 4.5–6.0 cm (T3). (2) The root-promoting and seedling growth effects were further enhanced by hormone application after root pruning. Specifically, after cutting 0.5 cm of the root tip at 1.5–2.0 cm root length, treatment with  $0.075 \text{ mg} \cdot \text{L}^{-1}$  IBA or  $0.125 \text{ mg} \cdot \text{L}^{-1}$  IBA (i.e., P3, P4) resulted in substantial and significant increases in root morphological indicators (except average root length) and root CEC. P4 treatment yielded the highest seedling fresh weight, root dry weight, root-shoot ratio, seedling quality index, and root activity. Under P3 treatment, soluble sugar and NSC contents in roots increased significantly by 26.04% and 29.07% compared to the control, respectively, while soluble protein content in roots peaked under P4 treatment

( $13.27 \text{ mg} \cdot \text{g}^{-1}$ ). Appropriate root pruning combined with IBA application can promote root absorption and nutrient storage in *A. mongolicus* by altering root morphology and enhancing root activity, thereby improving root quality and seedling growth potential.

## Full Text

### Abstract

To solve the technical forestry problems associated with *Ammopiptanthus mongolicus*—namely, its long main root, scarcity of capillary roots, difficult rooting, and low transplant survival rate—this study examined the effects of different root-cutting intensities and hormone treatments on seedlings at various developmental stages. After promoting root development through different treatment combinations, we measured and analyzed their impacts on root morphology, root quality, and seedling growth to identify key measures for enhancing root survival and provide a theoretical basis for seedling cultivation and afforestation techniques. The results showed that: (1) Root cutting promoted the development of multiple main roots in *A. mongolicus*, as well as increases in lateral roots, total root mass, aboveground and belowground biomass, root-shoot ratio, root activity, and root cation exchange capacity (CEC). Among the treatments, cutting 0.5 cm of the root tip when seedling root length reached 1.5–2.0 cm (F2) significantly promoted increases in lateral root number, root tip number, branch number, seedling quality index, and root activity, with root activity reaching a peak value that was 137.70% higher than the control. Root CEC was maximal when root length reached 4.5–6.0 cm. (2) The hormone treatment further enhanced root and seedling growth after root cutting. When 1.5–2.0 cm long roots were treated with 0.5 cm cutting +  $0.075 \text{ mg} \cdot \text{L}^{-1}$  IBA/ $0.125 \text{ mg} \cdot \text{L}^{-1}$  IBA (P3 and P4 treatments), root morphological indexes (except average root length) and root CEC increased significantly. Seedling fresh weight, root dry weight, root-shoot ratio, seedling quality index, and root activity were highest under the P4 treatment. Compared with the control, the content of soluble sugar and NSC in roots under P3 treatment increased significantly by 26.04% and 29.07%, respectively. Additionally, the content of soluble protein in roots reached its peak under P4 treatment ( $13.27 \text{ mg} \cdot \text{g}^{-1}$ ). Proper root cutting and IBA addition can promote root absorption and nutrient storage in *A. mongolicus* by altering root morphology and improving root activity, thereby enhancing root quality and seedling growth potential.

**Keywords:** *Ammopiptanthus mongolicus*; root cutting; hormone treatment; root morphology; root activity; seedling growth

### Introduction

The root system serves as the dynamic interface between soil and plants, representing a crucial organ for material and energy exchange with the external environment and for stabilizing the plant. Root quality (encompassing root growth

status and vitality) directly influences plant nutrition and biomass composition, playing a vital role in plant growth, development, and biomass accumulation. When characterizing plant root quality, important metrics include root architecture, root morphology, root activity, and physiological-ecological characteristics related to nutrient absorption and storage, which reflect the spatial arrangement of roots in soil and represent plant strategies for soil nutrient utilization.

Fine roots (diameter < 2 mm) exhibit plasticity and represent the most sensitive and active portion of the plant in responding to environmental changes. Plants can alter root branching patterns to adapt to habitat variations, forming extensive root systems to improve effective utilization of soil nutrients and maintain normal growth, development, and resistance to adverse conditions. Currently, auxin is recognized as a crucial factor affecting root development, particularly for the continued development and growth of lateral roots. When new lateral roots penetrate the root epidermis, the meristem is believed to be activated, and lateral root meristems possess the capacity to produce auxin, though the stage at which primordia become independent of auxin supply from the primary root remains unclear.

*Ammopiptanthus mongolicus*, belonging to the genus *Ammopiptanthus* in the Fabaceae family, is a precious evergreen broadleaf shrub in China's desert regions and a Tertiary relict plant with high ecological, economic, and research value. Its broad application prospects and unique, excellent utilization characteristics have attracted considerable attention from researchers both domestically and internationally. *A. mongolicus* primarily reproduces through seed propagation, with slow natural regeneration and scarce germplasm resources. As a taproot plant with long, deeply penetrating main roots and few lateral roots, *A. mongolicus* exhibits extremely low root branching, making vegetative propagation difficult and transplant survival extremely challenging. This has become a bottleneck issue urgently needing resolution for the breeding and development of *A. mongolicus*.

Based on root structural plasticity, certain cultivation measures can improve root structure, promote lateral root formation, and enhance seedling water and nutrient absorption efficiency, thereby addressing the technical challenge of low transplant survival. Therefore, this study employed different root-cutting lengths combined with different IBA concentrations to investigate the effects of root cutting and IBA treatment on root quality and seedling growth in *A. mongolicus*, providing a theoretical foundation for seedling cultivation and afforestation techniques.

## Materials and Methods

### 1.1 Experimental Materials

The experimental material consisted of *A. mongolicus* seeds collected from Min-qin, Gansu Province, after reaching maturity in the current year.

## 1.2 Seed Germination

Plump, intact, and uniformly sized seeds were selected and soaked in 0.1% potassium permanganate for 10 minutes, then rinsed with distilled water three times. After the seeds cracked and showed white radicles, they were placed in a 60°C water bath for 5 minutes and then arranged neatly in seedling trays for germination.

## 1.3 Root Cutting and IBA Hormone Treatment

On the 5th day after seed germination, when radicle lengths reached 0.5 cm, 1.5-2.0 cm, 3.0-4.0 cm, and 4.5-6.0 cm, root tip cutting treatments of 0.5 cm were performed. Non-cut seedlings at each developmental stage served as controls (F1-F4). Based on preliminary experiments showing better root development after cutting, the treatment with root length of 1.5-2.0 cm was selected for combined root cutting + IBA treatments at concentrations of  $0.075 \text{ mg} \cdot \text{L}^{-1}$  and  $0.125 \text{ mg} \cdot \text{L}^{-1}$ , with non-IBA treatment as control (S1-S3). A total of 15 treatments were established (Table 1), with three replicates per treatment and 30 seedlings per replicate.

## 1.4 Root Promotion Cultivation

All treated seedlings were subjected to hydroponics, with the nutrient solution (N:P:K = 1/500) replaced every 3 days. The hydroponic liquid level was maintained at the boundary between the root system and aboveground portion. The hydroponic materials were placed in a light incubator at 25°C with 22000 Lux illumination for a 16-hour light/8-hour dark cycle. After 30 days, when seedling height reached 2.5-5.6 cm with 2-3 leaves, and cut-root seedlings showed obvious multiple main roots and fine root growth with root tip numbers  $>10$  and differential performance among treatments, sampling and root quality analysis were conducted.

## 1.5 Measurement Indicators

**1.5.1 Root Morphology** Root systems were scanned using an Epson Perfection V800 Photo scanner, and WinRHIZO 2019 root analysis software was used to obtain data on root surface area, root length, total root projection area, average root diameter, root tip number, total root volume, crossing number, and branch number.

**1.5.2 Seedling Growth and Physiological Indicators** After root promotion cultivation, seedling height and ground diameter were measured with a digital vernier caliper (at the hydroponic liquid boundary). Aboveground and belowground portions were separated, root surface moisture was absorbed with filter paper, and fresh weights of aboveground and root parts were measured with an electronic balance. Fresh samples were collected to determine soluble protein content by UV absorption method and root activity by TTC method.

Remaining portions were placed in paper bags and oven-dried at 105°C for 15 minutes, then at 80°C to constant weight to determine dry weights of each part. Dried root, stem, and leaf samples were ground and passed through a 0.25 mm sieve. Soluble sugar and starch contents were determined by sulfuric acid-anthrone method, and root cation exchange capacity (CEC) was determined by leaching method. For analysis, non-structural carbohydrates (NSC) represented the sum of soluble sugar and starch, seedling quality index = seedling total dry weight (g) / [seedling height (cm) / ground diameter (mm) + aboveground dry weight (g) / root dry weight (g)], and root biomass (g) = root dry weight (g).

## 1.6 Data Processing

Data analysis was performed using SPSS 22.0 statistical software. One-way ANOVA was used for significance testing, with Duncan's method for multiple comparisons ( $\alpha = 0.05$ ). Pearson correlation analysis was conducted among root morphological and physiological indicators.

# Results

## 2.1 Effects of Root Cutting and IBA Treatment on Root Morphology of *A. mongolicus* Seedlings

**2.1.1 Root Architecture Analysis** Figure 1 shows that root cutting significantly altered primary and lateral root growth and overall root architecture. Non-cut seedlings (P1-P4) exhibited long, single main roots with few capillary roots. Root cutting treatments (S2-S3) resulted in multiple main root systems with lateral roots distributed on main roots. Under the 1.5-2.0 cm root length + 0.5 cm cutting treatment (T2-T4), lateral root number, root tip number, and branch number increased significantly. Meanwhile, root length at each developmental stage decreased compared with controls. Different intensities of root cutting showed varying effects. After cutting, root average diameter, surface area, total projection area, and total volume showed no consistent patterns. However, the 1.5-2.0 cm root length + 0.5 cm cutting treatment increased lateral root number, root tip number, branch number, root average diameter, surface area, total projection area, and total root volume compared with controls, while decreasing root length. Comparing the gradient treatments, the 1.5-2.0 cm root length + 0.5 cm cutting + 0.125 mg·L<sup>-1</sup> IBA treatment was most effective for promoting seedling quality.

**2.1.2 Root Morphological Index Analysis** Table 2 shows that root cutting at different developmental stages (three different root lengths) increased lateral root number, root tip number, and branch number, with significant differences among intensities at each stage. The 0.5 cm root tip cutting treatment (F2) when root length reached 1.5-2.0 cm significantly promoted increases in lateral root number, root tip number, branch number, seedling quality index, and root activity, with root activity reaching the peak for root cutting treat-

ments (137.70% higher than control,  $P < 0.05$ ). Root CEC was maximal when root length reached 4.5–6.0 cm. The hormone treatment further enhanced root and seedling growth after cutting. Among these, the 1.5–2.0 cm root length + 0.5 cm cutting + 0.075 mg · L<sup>-1</sup> IBA/0.125 mg · L<sup>-1</sup> IBA treatments (P3 and P4) showed the best results. Root morphological indexes (except average root length) and root CEC increased significantly after 1.5–2.0 cm root length + 0.5 cm cutting + 0.075 mg · L<sup>-1</sup> IBA treatment. Seedling fresh weight, root dry weight, root-shoot ratio, seedling quality index, and root activity were highest under the P4 treatment.

## 2.2 Effects of Root Cutting and IBA Treatment on Seedling Growth of *A. mongolicus*

Table 3 and Figure 2 show that root cutting treatments (F1-F2, S1-S2, T1-T2) increased ground diameter, aboveground fresh weight, root fresh weight, root-shoot ratio, and root dry weight compared with controls, but aboveground dry weight showed no significant increase. Root cutting at different developmental stages (three root lengths) increased root activity compared with controls (F1-F2). Among different intensities, cutting 0.5 cm when root length reached 1.5–2.0 cm (F2) significantly increased root activity by 137.70% compared with control (F1) and was higher than other root development stages. After root cutting, IBA treatment further increased root activity, with the 1.5–2.0 cm root length + 0.5 cm cutting + 0.125 mg · L<sup>-1</sup> IBA treatment showing the best effect. Root cutting also promoted seedling growth, with root dry weight and seedling quality index significantly increased. After root cutting, hormone treatment further enhanced seedling growth, with the 1.5–2.0 cm root length + 0.5 cm cutting + 0.125 mg · L<sup>-1</sup> IBA treatment being most ideal, showing the highest seedling quality index.

## 2.4 Effects of Root Cutting and IBA Treatment on Nutrient Absorption and Storage in *A. mongolicus* Seedling Roots

### 2.4.1 Root Cation Exchange Capacity

Root CEC reflects the ability of roots to absorb and utilize mineral element nutrients effectively. Figure 3 shows that root cutting at different developmental stages (three root lengths) increased root CEC in continuously cultured seedlings (F1-F2, S1-S2, T1-T2). When root length reached 4.5–6.0 cm, the 0.5 cm cutting treatment (T2) produced maximal CEC, significantly increasing by 386.47% compared with control (T1). After root cutting, IBA treatment increased root CEC, with the 1.5–2.0 cm root length + 0.5 cm cutting + 0.075 mg · L<sup>-1</sup> IBA treatment (P3) showing the highest value, significantly higher than control (S2) and other treatments.

### 2.4.2 Root Nutrient Content

Figure 4 shows that root cutting at different developmental stages increased root soluble protein content, with significant increases at root lengths of 1.5–2.0 cm (62.39%) and 3.0–4.0 cm (95.29%). Adding IBA on the basis of root cutting further increased soluble protein content, which

was higher than control and peaked at  $13.27 \text{ mg} \cdot \text{g}^{-1}$  under the 1.5–2.0 cm root length + 0.5 cm cutting +  $0.125 \text{ mg} \cdot \text{L}^{-1}$  IBA treatment (P4). Root cutting also promoted increases in root soluble sugar and NSC content, with the 1.5–2.0 cm root length + 0.5 cm cutting +  $0.075 \text{ mg} \cdot \text{L}^{-1}$  IBA treatment (P3) significantly increasing these by 26.04% and 29.07% compared with control, respectively.

## 2.5 Correlation Analysis Between Root Morphological and Physiological Indexes

Correlation analysis between root morphological and physiological indexes of *A. mongolicus* seedlings (Table 4) showed that root activity was significantly positively correlated with lateral root number, root tip number, branch number, root surface area, total root projection area, and total root volume ( $P < 0.01$ ), and extremely significantly positively correlated with root CEC ( $P < 0.001$ ). Root soluble sugar and NSC were extremely significantly positively correlated with root soluble starch ( $P < 0.001$ ). Root soluble protein showed no significant correlation with other root indexes. Total root projection area was extremely significantly positively correlated with root surface area, total root volume, lateral root number, root tip number, branch number, and root activity ( $P < 0.001$ ). This indicates that root cutting can increase root morphological parameters, thereby promoting root activity and nutrient absorption capacity.

## Discussion

### 3.1 Effects of Root Cutting and IBA Treatment on Root Architecture and Morphology of *A. mongolicus* Seedlings

Root cutting disrupts the apical dominance and downward growth function of the main root, enhancing meristematic capacity and promoting lateral and fibrous root growth, thereby altering root architecture and morphology. In this experiment, root cutting transformed *A. mongolicus* seedling root systems from single main roots with few capillary roots to multiple main root branch systems, while increasing lateral root number, root tip number, and branch number. Concurrent hormone addition further increased these parameters. Root cutting activates root meristems, and under the supply of exogenous growth hormone IBA, root growth points rapidly form primary roots that generate new roots. These new roots penetrate the root epidermis, actively optimizing root architecture, increasing lateral root and root tip numbers, and enlarging root surface area.

### 3.2 Effects of Root Cutting and IBA Treatment on Seedling Growth

Changes in root architecture and morphology directly affect root growth and seedling development. In this study, as IBA concentration increased under root cutting conditions, *A. mongolicus* seedling root architecture became more complex. Under  $0.075\text{--}0.125 \text{ mg} \cdot \text{L}^{-1}$  IBA treatments, root morphological indexes

such as lateral root number, branch number, and total root mass increased significantly, corresponding to significant increases in root fresh weight, root-shoot ratio, root dry weight, and root biomass, with significantly improved seedling quality index. This indicates that root cutting combined with IBA treatment promoted root metabolism and seedling growth. The increased root tip number and surface area enlarged the contact area between roots and the environment, increasing absorption points and area, thereby improving root physiological activity and effectively promoting seedling growth.

However, seedling height, ground diameter, and aboveground growth showed no significant increase under root cutting and hormone treatment. This relates to the developmental stage of seedlings in this experiment, which were measured after 30 days of hydroponics following 3–5 days of cutting. Although root architecture and morphology changed significantly, seedlings remained in the root construction and development stage, leading to imbalance between nutrient absorption and consumption. Accumulated nutrients were prioritized for root system self-improvement, resulting in faster belowground growth than aboveground growth, which had not yet reached the rapid growth phase. Consequently, differences among treatments were not significant for aboveground parameters.

### 3.3 Effects of Root Cutting and IBA Treatment on Root Activity

Root cutting increased seedling root activity compared with non-cut controls, with hormone treatment after cutting showing more significant effects. The 1.5–2.0 cm root length + 0.5 cm cutting + 0.125 mg·L<sup>-1</sup> IBA treatment produced maximal root activity, significantly higher than other treatments. Studies show that root activity is closely related to root length density and root metabolism. The thick, dense growth tendency of *A. mongolicus* roots after treatment enhanced metabolic activity and improved root activity. Additionally, plants exhibit growth compensation effects after root cutting. In this experiment, all root cutting treatments showed higher root soluble protein content than controls, which can be interpreted as the growth compensation effect promoting protein storage in seedling roots. However, nutrient storage showed no regular pattern among different cutting treatments. Further research is needed on nutrient storage, distribution among organs, and related metabolic processes to better understand the physiological mechanisms of root and seedling growth.

### 3.4 Effects of Root Cutting and IBA Treatment on Nutrient Absorption and Storage

Root CEC is an important indicator of nutrient absorption capacity, with biomass accumulation being the most direct manifestation. This study found that root cutting combined with IBA treatment significantly increased root biomass allocation while relatively decreasing aboveground biomass allocation, significantly improving seedling quality index. The 1.5–2.0 cm root length + 0.5 cm cutting + 0.125 mg·L<sup>-1</sup> IBA treatment showed significantly higher root

CEC than controls, indicating that appropriate root cutting and IBA addition can improve nutrient absorption and storage by altering root morphology and increasing root activity. However, root soluble protein, soluble sugar, and NSC contents showed no consistent patterns with cutting intensity or hormone concentration gradients, and correlations with root morphological indexes and root activity were not significant. Root cutting breaks the original developmental balance, causing nutrients to be preferentially allocated to roots for reconstruction while simultaneously disrupting original root absorption capacity. This leads to differences in nutrient balance and storage capacity during root development depending on cutting timing and intensity.

## Conclusions

1. Root cutting induced multiple main root development and increased lateral roots, total root mass, aboveground and belowground biomass, and root-shoot ratio in *A. mongolicus*. Cutting 0.5 cm of root tip when root length reached 1.5–2.0 cm significantly promoted increases in lateral root number, root tip number, branch number, and seedling quality index. Root activity reached a peak value 137.70% higher than the control.
2. Hormone treatment after root cutting further enhanced root and seedling growth. The 1.5–2.0 cm root length + 0.5 cm cutting + 0.125 mg · L<sup>-1</sup> IBA treatment was most effective, producing the highest seedling fresh weight, root dry weight, root-shoot ratio, seedling quality index, and root activity.
3. Root cutting increased root activity and CEC, with the 1.5–2.0 cm root length + 0.5 cm cutting treatment showing the best effect. IBA treatment after cutting further improved root activity, with the 1.5–2.0 cm root length + 0.5 cm cutting + 0.125 mg · L<sup>-1</sup> IBA treatment being optimal. Correlation analysis showed that appropriate root cutting and IBA addition can increase root complexity and morphological parameters, thereby enhancing root activity and absorption function.
4. The 1.5–2.0 cm root length + 0.5 cm cutting + 0.075 mg · L<sup>-1</sup> IBA treatment significantly increased root soluble sugar and NSC content by 26.04% and 29.07%, respectively, compared with the control. Root soluble protein content peaked at 13.27 mg · g<sup>-1</sup> under the 1.5–2.0 cm root length + 0.5 cm cutting + 0.125 mg · L<sup>-1</sup> IBA treatment. Appropriate root cutting and IBA addition can promote nutrient storage in *A. mongolicus* roots by altering root morphology and improving root activity, thereby enhancing root quality and seedling growth potential.

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## Figures

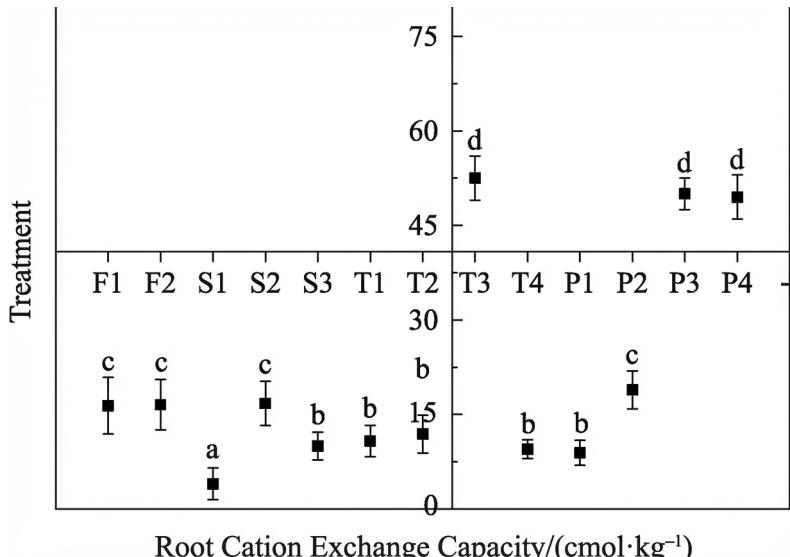


Figure 1: Figure 4

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