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EFFECT OF IBA AND MEDIA ON THE ANATOMICAL STRUCTURE AND ROOT FORMATION IN *ZANTHOXYLUM BEECHEYANUM* PLANT

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ABSTRACT: *Zanthoxylum beecheyanum*, of ornamental plant (Chinese pepper) is a dense, evergreen shrub up to two meters tall. During the 2022/2023 and 2023/2024 growing seasons, a research study was carried out to determine the effects of indole-3-butyric acid (IBA) application (0, 1000, 2000 and 3000 ppm) on adventitious root formation (cut rooting) in *Z. beecheyanum* with peat moss + sand (1:1 v/v) and sand media at 60 and 75 days. The treatment of cuttings with 3000 ppm IBA and different media significantly enhanced rooting. At 60 days, the highest root number (10.0/plant) was obtained in sand medium, while at 75 days, it was 13.0/plant in peat moss + sand (1:1 v/v) medium. The longest roots (11.57 cm/plant) were obtained in peat moss + sand (1:1 v/v) medium, while sand medium produced roots with an average length of 12.43 cm. The highest fresh weight at 60 days was 0.579 g/plant in sand medium, 0.563 g/plant in peat moss + sand (1:1 v/v) medium, increasing at 75 days to 1.420 g/plant in peat moss + sand (1:1 v/v) medium, it was sand medium recorded 0.756 g/plant. Similarly, the highest dry weight at 60 days was 0.060 g/plant in peat moss + sand (1:1 v/v) medium and 0.054 g/plant with sand medium, improving further at 75 days. Overall, 3000 ppm IBA significantly enhanced rooting characteristics. Anatomical studies of the cutting bases revealed no structural barriers to rooting. It was found that treating the plant with IBA and media increased the rooting rate and reduced the rooting period.

Keywords: *Zanthoxylum beecheyanum*, rooting media, IBA, root formation

INTRODUCTION

Chinese pepper is a common name for various *Zanthoxylum*. *Z. beecheyanum* is a dense, evergreen shrub that can grow up to two meters high. The bark is brown with small spines and the leaves are compound: each leaf consists of approximately fifteen leaflets that are green, shiny, entire, and ovate, measuring 4-10 mm in length, with glands. The inflorescences are fasciculi of (1-2 mm) yellow to green flowers. *Z.beecheyanum* is usually propagated vegetatively; however, seeds are viable. This plant is indigenous to southern Japan and it is appropriate for bonsai fans (Weaver and Anderson, 2008).

Synthetic indole-3-butyric acid IBA and naturally occurring plant growth regulators share a lot of similarities in terms of both function and structure (Wiesman *et al.,* 1988; Pacurar *et al.,* 2014). IBA is used in a variety of ornamental, plants, to facilitate the rooting of cuttings (Akram *et al.,*2017; Gilani *et al.,* 2019).

The choice of medium plays an important role in providing essential nutrients, aeration, drainage, and care for the emerging root system (Vijaya and Syariful, 2018). The best medium for stem cuttings must have appropriate water retention and drainage to maintain proper adequate hydration without waterlogging (Tripathi *et al.*, 2014).

Treatments with exogenous auxin, such as IBA, have a significant impact on overcoming difficult root cuttings by encouraging the development of adventitious roots and inducing high rooting capacity (Hartmann *et al.,* 2007). It was found that narrow phloem tissue, cambial zone and vascular rays, in addition to the poor and slow vascular connection between the new roots and the vascular tissue of the cuttings, appeared to be the main causes of difficult to root lemon verbena cuttings. Lodama *et al.* (2016) found that treatment of *Lobostemon fruticosus* cuttings with IBA enhanced root initiation, and observed that callus tissue developed from the vascular cambium near the cut end of the cuttings. Parenchyma gaps in the phloem fiber ring at the cutting ends were also found.

The main objective of this study is to evaluate the effect of different levels of IBA and types of growing media on root formation, quality, and anatomical characteristics of the *Z. beecheyanum* plant.

MATERIALS AND METHODS

This investigation was conducted at a commercial nursery in Kafr Hakim, Giza, Egypt, during the two successive seasons (2022/2023 and 2023/2024), to develop an effective commercial propagation protocol for *Z. beecheyanum* through vegetative propagation studies.

Plant materials:

The *Z. beecheyanum* cuttings obtained from a private farm in Kafr Hakim, Giza, Egypt were used as a source of cuttings. The relative humidity and temperature in the plastic tunnel were regulated to maintain ideal and constant conditions for the growth of cuttings. The cuttings were monitored for 60 and 75 days until full rooting was achieved.

Treatments:

IBA solutions with concentrations of 0 (control immersed in water), 1000, 2000 and 3000 ppm were prepared by dissolving 0, 1.00, 2.00 and 3.00 g of IBA, respectively, in distilled water, and the final volume was adjusted to one liter with water using a 1000 ml volumetric flask. The basal ends (approximately 2 cm) of the cuttings were dipped in IBA solutions (0, 1000, 2000 and 3000 ppm) for 10 seconds. The rooting medium surrounding the base of the cuttings was gently pressed to eliminate air pockets and ensure proper contact between the cutting base and the moist media.

Rooting media:

Two rooting media were used: peat moss + sand (1:1 v/v) and sand alone. The chemical analysis of irrigation water and media was conducted at the National Research Centre in Dokki, Cairo, Egypt, and the results are presented in Tables (1 and 2). The climatic conditions in the experimental area were recorded during the two seasons as shown in Table (3).

Cultivation:

The two rooting media (peat moss + sand (1:1 v/v) and sand alone) were filled into plastic propagation bags with drainage holes at the bottom. The cuttings were planted on the same day they were collected. Data on root number, root length, and root weight were recorded at 60 and 75 days after planting. The experiment was conducted during two successive seasons (2022/2023 and 2023/2024), with treatments applied in October of each season. The relative humidity and temperature in the plastic tunnel were maintained at specific values to ensure optimal growth conditions for the cuttings.

Measurements:

1. Roots number per plant.
2. Roots length cm.
3. Fresh weight of roots (g/plant).
4. Dry weight of roots (g/plant).

Table 1. Chemical analysis of the irrigation water utilized in this experiment.

Chemical examination of irrigation water								
K	**Na**	**Mg**	**Ca**	**SO_4**	**Cl**	**HCO_3**	**pH**	**EC**
0.04	1.03	4.13	7.29	3.35	4.13	5.04	7.55	1.27

Table 2. Chemical parameters of the experimental soil media employed in this study.

Ext. 1:1			Soluble cations (meq/100 g)				Soluble anions (meq/100 g)				O.C%	O.M%	Available macro-nutrients (mg/kg)		
Sample	**pH**	**EC**	**Ca^{++}**	**Mg^{++}**	**Na^{+}**	**K^{+}**	**$CO_3^{=}$**	**HCO_3^{-}**	**$SO_4^{=}$**	**Cl^{-}**			**N**	**P**	**K**
peat moss + sand	6.82	1.13	3.22	1.69	5.02	1.08	0.0	0.90	4.98	5.12	2.54	4.38	184.8	8.22	156
Sand	7.94	0.85	1.37	0.82	4.15	0.32	0.0	1.90	2.91	3.19	0.12	0.21	92.4	4.28	60

Source: Desert Research Center, The Egyptian Ministry of Agriculture and Land Reclamation, El-Matareya, Cairo, Egypt

Table 3. The climatic condition in the location of Kafr Hakim, Giza Governorate, Egypt where the experiments were conducted on *Z. beecheyanum* plant during 2022/2023 and 2023/2024 seasons.

The months from planting to the season end	The mean value of air temperature (°C)		
	2022	**2023**	**2024**
January	10.28	13.31	13.22
October	23.80	25.10	-
November	18.76	19.92	-
December	16.20	16.18	-
Mean	18.63	17.26	13.22

Anatomical studies:

Anatomical data were collected from cross-sections of *Z. beecheyanum* stems, focusing on specific characteristics, e.g., vascular bundle arrangement, cortex thickness, were prepared according to Sass (1958).

Statistical analysis:

The data set of characters studied was collected and analyzed using univariate statistics. The analysis of variance (ANOVA) for both seasons was carried out according to Gomez and Gomez (1984).

RESULTS

Effect of different IBA concentrations with peat moss + sand (1:1 v/v) and sand media on the root growth:

1. Roots number per plant:

As shown in Table (4) data in both seasons, at 60 days incubation period, 3000 ppm IBA recorded the best value (6.33 and 10.00/plant) with peat moss + sand (1:1 v/v) and the same concentration at 75 days in addition to the sand medium the best value was 13.00 and 10.00/plant, there were no significant differences between the concentrations of 1000, 2000 and 3000 ppm IBA in the peat moss + sand (1:1 v/v) medium and 3000 ppm IBA in the sand medium during the 1st season at 60 days, the best significance was in the 2nd season for the concentration of 3000 ppm IBA in the sand medium, while in both seasons at 75 days there were no differences between the concentrations of 2000 and 3000 ppm IBA and 1000 ppm IBA with two media in 2nd season and sand medium in the 1st season.

2. Roots length (cm):

According to the information in Table (4) in both seasons, at 60 days incubation period, 3000 ppm IBA recorded the best value (7.83

Table 4. Effect of different IBA concentrations with rooting media on roots number, roots length, fresh and dry weight of roots at 60 and 75 days from planting in both seasons (2022/2023 and 2023/2024) in *Z. beecheyanum* plant.

Treatments	IBA concentrations															
	0.0 ppm	1000 ppm	2000 ppm	3000 ppm	0.0 ppm	1000 ppm	2000 ppm	3000 ppm	0.0 ppm	1000 ppm	2000 ppm	3000 ppm	0.0 ppm	1000 ppm	2000 ppm	3000 ppm
	1st season								2nd season							
	60 days from planting				75 days from planting				60 days from planting				75 days from planting			
Roots number																
Peat moss + sand	2.00 d	5.00 a-d	5.00 a-d	5.67 a-d	3.00 ef	6.33 b-f	7.00 a-f	13.00 a	3.00 cd	4.67 cd	4.67 cd	5.67 cd	3.33 b	5.67 ab	5.67 ab	10.00 ab
Sand	1.67 d	2.33 cd	3.00 cd	6.33 a-d	2.67 ef	8.00 a-f	8.33 a-f	10.00 a-d	1.33 d	5.00 cd	6.00 bc	10.00 a	4.00 b	6.33 ab	10.00 ab	13.00 a
Roots length																
Peat moss + sand	2.97 cd	7.37 ab	7.73 a	7.83 a	5.93 c-f	8.17 a-d	9.93 a-c	11.07 ab	5.63 a-d	7.83 a-d	8.00 a-d	9.83 ab	8.70 a-e	10.27 a-c	10.63 a-c	11.57 ab
Sand	1.37 d	1.40 d	1.57 d	3.53 cd	1.67 f	2.33 f	4.67 d-f	6.83 b-e	2.47 e	8.27 a-d	8.57 a-d	8.60 a-d	8.60 a-e	10.73 a-c	11.90 ab	12.43 a
Fresh weight of roots																
Peat moss + sand	0.007 g	0.355 c-g	0.434 c-f	0.563 a-e	0.282 de	0.882 a-e	0.888 a-e	1.420 a	0.016 f	0.136 d-f	0.220 c-f	0.341 a-d	0.192 a-d	0.336 a-d	0.450 a-d	0.738 ab
Sand	0.012 g	0.056 g	0.078 g	0.143 fg	0.084 e	0.365 c-e	0.371 c-e	0.462 b-e	0.011 f	0.223 c-f	0.287 b-f	0.579 a	0.160 b-d	0.400 a-d	0.427 a-d	0.756 a
Dry weight of roots																
Peat moss + sand	0.001 g	0.045 b-e	0.046 b-e	0.060 a-c	0.036 de	0.086 a-e	0.094 a-e	0.151 a-c	0.004 de	0.017 c-e	0.030 b-e	0.041 a-d	0.023 c	0.034 c	0.057 a-c	0.092 ab
Sand	0.002 g	0.006 fg	0.008 fg	0.016 e-g	0.005 e	0.036 de	0.039 de	0.046 c-e	0.001 e	0.027 b-e	0.028 b-e	0.054 a-c	0.017 c	0.043 bc	0.045 a-c	0.063 a-c

and 9.83 cm/plant) with peat moss + sand (1:1 v/v) and the same concentration at 75 days in addition to the sand medium, with 2nd season the best value was 11.07 and 12.43 cm/plant, there were no significant differences between 1000, 2000, and 3000 ppm IBA in the peat moss + sand medium of 1000, 2000 and 3000 IBA in peat moss + sand (1:1 v/v) medium during the 1st season at 60 days, while there is no notable variations were detected in 2nd season at concentrations of 1000, 2000 and 3000 IBA in the media, similar results were observed between peat moss + sand (1:1 v/v) and the control in 1st season. In 2nd season, there were no significant differences between all concentrations other than 0.0 ppm IBA in sand medium.

3. Fresh weight of roots (g/plant):

As shown in Table (4), in both seasons, at the 60 days incubation period, 3000 ppm IBA recorded the best value (0.563 and 0.579 g/plant) with peat moss + sand (1:1 v/v) and sand media, with 2nd season the best value was 1.420 and 0.756 g/plant with peat moss + sand (1:1 v/v) and sand media. There were no significant changes between concentrations of 3000 ppm IBA in sand medium during the 2nd season at 60 days. In the 1st season at 75 days there were no significant differences between peat moss + sand (1:1 v/v) at all concentrations compared to the control and also in the 2nd season, in addition to the sand medium and the control in peat moss + sand (1:1 v/v) medium similar results were observed.

4. Dry weight of roots (g/plant):

As shown in Table (4) in both seasons, at the 60 day incubation period, 3000 ppm IBA recorded the best value (0.060 and 0.054 g/plant) with peat moss + sand (1:1 v/v) and sand media; the same concentration at 75 days resulted in better values (0.151 and 0.092 g/plant) with peat moss + sand (1:1 v/v) medium in both seasons. It was noted that there was no significance at 60 days in the 2nd season between the two media at 3000 ppm IBA. At 75 days, no differences were observed between the concentrations in both seasons.

Anatomical structure:

The anatomical studies on the stem cutting of *Z. beecheyanum* and microphotographs presented in Fig. (1) proved that there is no obstacle in the rooting process in this plant, likes the sclerenchyma cycle between the phloem and cortex in the pericycle region. Whereas the pericycle region in this plant consists of parenchyma cells alternating with sclerenchyma cells. It was observed that the rooting process started from the pericycle parenchyma. Based on the anatomical studies, root development at the base of stem cutting occurs in three phases; (a) the root initiation and the beginning of callus formation (Fig., 1 B), (b) the root primordium formation (Fig., 1 C), and (c) root growth and development (emergence and elongation of roots) (Fig., 1 D). It can be concluded that the rooting process in this plant is not difficult and the addition of IBA is not always necessary to induce root elongation. However, when used, it shortens the time needed to reach the top of rooting. Likewise, the time and the media are factors that determine and stimulate the rooting process in this plant and reduce its duration.

DISCUSSION

The findings verified the significance of exogenous IBA applications and selection of appropriate doses (Canli and Bozkurt, 2009). In Compared to the untreated groups, IBA had a significant effect on fresh weight, dry weight, root length and number of roots (per cutting). Which could attributed to the accumulation of metabolites at the application site for auxins, call expansion, accelerated glucose hydrolysis, cell division, and protein synthesis (Strydom and Hartmann, 1960). A suitable medium provides the plant with sufficient support, acts as a reservoir for water and nutrients, facilitates oxygen reach to the roots, and allows gas exchange between the roots and the atmosphere, according to the findings of (Agro, 1998). Sand is a commonly used natural substrate that greatly accelerates the process of root formation due to its unique physical and chemical components (Wei *et al.*, 2017). These results align with those

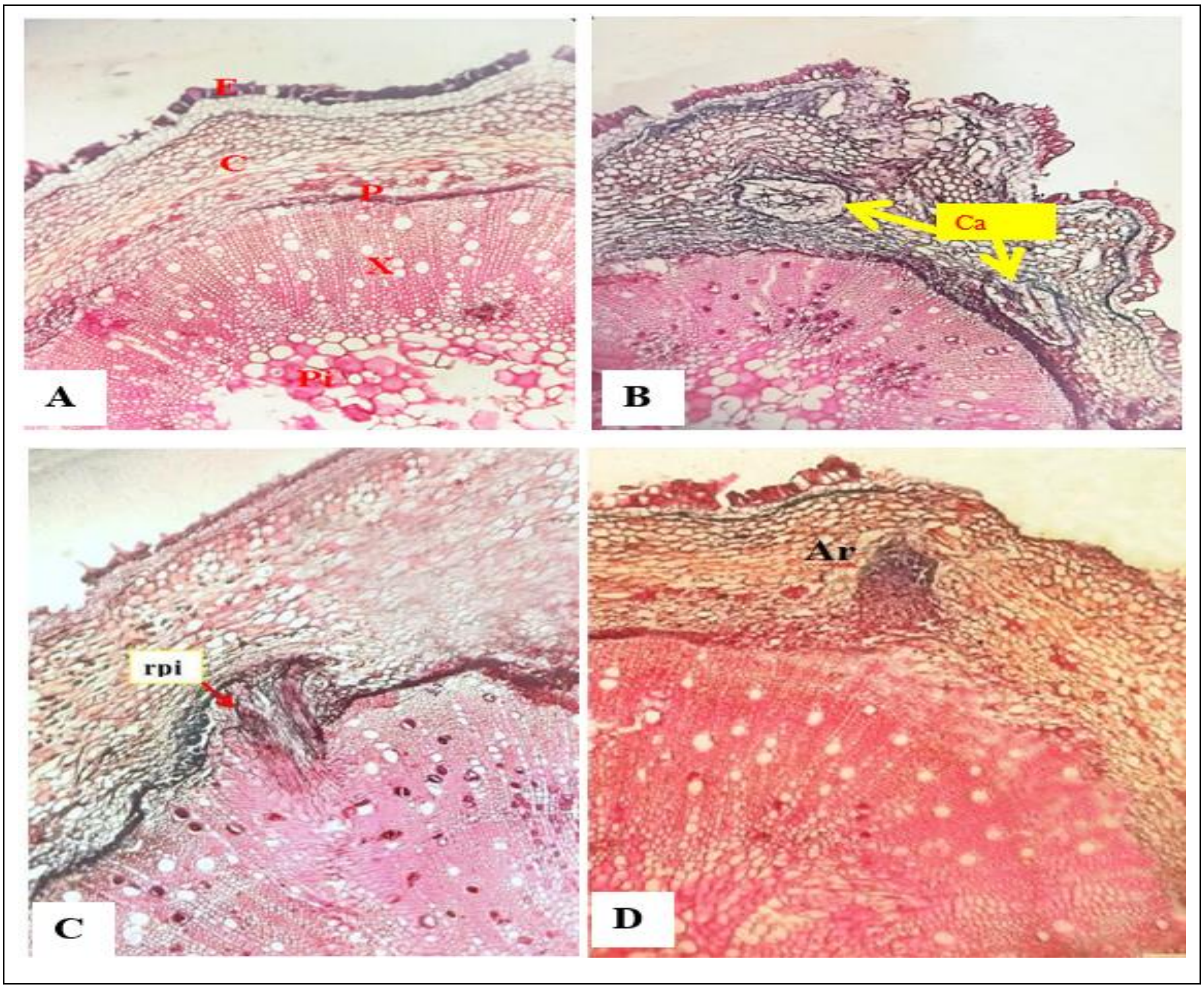

Fig. 1. Transverse parts of rooting zone of *Z. beecheyanum* plant cuttings reaction to the application of IBA at 1000, 2000 and 3000 ppm. A: untreated plants as control (E: epidermis, P: pericycle, C: cortex, X: xylem, Pi: pith), B: the root initiation and the beginning of callus formation (Ca: callus), C: the root primordium formation and root growth (rpi: root primodium initials), D: development as root elongation and emergence (Ar: adventitious root). for A and B X= 100 while for C and D X= 200.

reported by El-Naggar and Esmaiel (2022) on Dracaena cuttings. Additionally, Abdulraahman and Ayoub (2023) found that rooting quality (number of roots/plant), (root length) and (dry weight of roots) were significantly impacted by the propagation medium. Through anatomical study, we found that the pericycle layer in this plant consists of parenchyma cells, alternated with other sclerenchyma cells and there is no connected sclerenchyma ring between the phloem the cortex, which hinders the rooting process. Also, we found that the effect of indole3-butyric acid (IBA) dipped at concentrations of 1000, 2000 and 3000 ppm, the media, and the time are factors that determine and stimulate the rooting process in this plant and reduce its duration. Such results are in line with Mohamed *et al.* (2017) and Rahdari *et al.* (2014) who stated that the anatomical study of the rules of the Dracaena cutting showed that the difficulty of rooting in them is due to the presence of ring sclerenchyma cells between the bark and cortex, which constitutes a mechanical obstacle to the emergence of roots. The anatomical study of Cordyline showed the presence of an abundance of root primordia in its tissues due to the ease of rooting. Furthermore, Macedo *et al.* (2013) studied in detail the formation of adventitious roots in explants of the *Galega vulgar* L. cultivar with four to five nodes and found that the first signs of differentiation for root formation appeared 96 hours after IBA treatment and that the first cell divisions occurred approximately 144 hours after IBA treatment in outer phloem cells. El-Nashar (2008) reported that treating *Jasminum sambac* L. and *Pittosporum tobira* L. cuttings with IBA (100 or 200 ppm) encouraged the adventitious roots to start growing and affected the sclerenchymatous tissue, making it loose, so that the root initial can easily emerge. Hartmann *et al.* (2007) found that adventitious roots can grow naturally on the base of stem cuttings. During adventitious root production, some anatomical changes occur. Adventitious roots on stem cuttings of herbaceous plants often form between and outside the vascular bundles, whereas in woody plants, they originate from secondary phloem, live parenchymatous cells, cambium, phloem, vascular rays, and callus tissues. Furthermore, adventitious roots develop from callus tissue generated at the cutting base and are associated with difficult to root species.

CONCLUSION

The application of IBA with peat moss + sand (1:1 v/v) and sand media had a positive effect on *Z. beecheyanum* plant, during the observation period in October. In general, the concentration of 3000 ppm had the desired effect on roots number, roots length, fresh and dry root weight at 75 days compared to the control with different media. Both media (peat moss + sand and sand) positively influenced root number and fresh weight, while root length was favored in the sand medium, and dry weight was highest in the peat moss + sand medium. In the first season, the peat moss + sand medium yielded the highest dry weight. In the second season, the sand medium was preferable at 60 days, but by 75 days, the peat moss + sand medium showed the best results. No significant differences were observed between the two media in root number, length, or weight during the second season. These findings demonstrate that the combination of IBA at 3000 ppm with peat moss + sand (1:1 v/v) and sand media significantly enhances root development in *Z. beecheyanum*. The peat moss + sand medium, in particular, appears to be highly effective for promoting root growth and dry weight, making it a promising option for propagation and cultivation practices. To further optimize root development in *Z. beecheyanum*, future studies could explore the effects of other plant growth regulators (e.g., NAA) in combination with IBA on root formation.

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تأثير IBA والأوساط الزراعية على التركيب التشريحي وتكوين الجذور في نبات الكزاليزم

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نبات الكزاليزم الإسم الشائع (الفلفل الصيني) وهو شجيرة كثيفة دائمة الخضرة يصل ارتفاعها إلى مترين. كانت الدراسة خلال موسمي ٢٠٢٣/٢٠٢٢ و٢٠٢٤/٢٠٢٣ لتقييم تأثير تركيزات مختلفة من حمض أندول-٣-البيوتريك بتركيزات مختلفة (٠,٠ و١٠٠٠ و٢٠٠٠ و٣٠٠٠ جزء في المليون) مع أوساط البيتموس + الرمل (١:١ حجم/حجم) والرمل على تكوين الجذور العرضية لنبات الكزاليزم وتم اخذ القراءات عند ٦٠ و٧٥ يوم. أدت معاملة العقل بتركيز ٣٠٠٠ جزء في المليون من حمض أندول -٣-البيوتريك مع الأوساط المختلفة إلى تحسين التجذير. عند ٦٠ يوما، كان أعلى عدد جذور (١٠,٠ جذر/نبات) في وسط الرمل، بينما عند ٧٥ يوما، بلغ ١٣,٠ جذر/نبات في وسط البيتموس + الرمل (١:١ حجم/حجم). تم تحقيق أطول الجذور (١١,٥٧ سم/نبات) في وسط البيتموس + الرمل (١:١ حجم/حجم)، بينما كان أفضل طول (١٢,٤٣ سم/نبات) في وسط الرمل. بلغ أعلى وزن طازج للجذور عند ٦٠ يوما ٠,٥٧٩ جم/نبات فى وسط الرمل و ٠,٥٦٣ جم/نبات فى وسط البيتموس + الرمل (١:١ حجم/حجم)، وارتفع عند ٧٥ يوما إلى ١,٤٢٠ جم/نبات فى وسط البيتموس + الرمل (١:١ حجم/حجم) و ٠,٧٥٦ جم/نبات فى وسط الرمل. كما بلغ أعلى وزن جاف عند ٦٠ يوما ٠,٠٦٠ جم/نبات فى وسط البيتموس + الرمل (١:١ حجم/حجم) و ٠,٠٥٤ جم/نبات فى وسط الرمل عند ٧٥ يوماً. بشكل عام، أدى استخدام ٣٠٠٠ جزء في المليون من حمض أندول -٣-البيوتريك إلى تحسين خصائص التجذير بشكل ملحوظ. أظهرت الدراسة التشريحية لقواعد العقل في نبات الكزاليزم عدم وجود صعوبة في عملية التجذير وذلك لعدم وجود عائق في التركيب الداخلي، وقد وجد أن معاملة النبات بالأندول-٣-البيوتريك أدى إلى زيادة معدل التجذير وخفض فترة التجذير مع أوساط الزراعة.