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ORIGINAL ARTICLE

Effect of Different Cultivation Beds, Species, and Types of Seed Culture on Benching Crown Formation in *Pistacia vera* L.

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K E Y W O R D S

Germination media;
Pistachio species;
Planting media;
Screw crown

A B S T R A C T

This study investigated the effects of soil texture, tree cultivars, and seed-sowing techniques on pistachio cultivars crowns' growth. The main research question addressed the effect of these factors on various morphological traits of pistachio seedlings, including leaf number, leaf area, stem length, and the occurrence of spiral crowns. A factorial experiment with randomized design and 5 replications. Seeds were sown in the orientation of seeds: up tip, down tip, and horizontal. Four ratios of culture media treatments using sand, peat moss, perlite, and farm soil were used. The findings indicated that the morphology of pistachio seedlings was significantly influenced by the selection of the culture medium. Besides, different types of seeds, including *P. vera* cv. Badami, *P. integerrima* and *P. mutica* affected the growth parameters. The highest leaf area was due to medium 3 with *P. vera* cv. Badami seeds were grown with their up tips (84.9 cm^2) and medium 1 with *P. integerrima* seeds were grown with their horizontal (67.5 cm^2). The highest number of leaves was found in medium 2 containing *P. vera* cv. Badami seeds are grown with down tips (28.2). The highest seedling stem diameter and stem length were associated with media 2 and 3 with *P. vera* cv. Badami cultivation in the form of up and horizontal tips averaged 7.6 mm, 35.4 cm, and 38.8 cm respectively. For spiral crown, media 1 and 2 had the highest percentage. In these media, all seeds showed a high percentage of spiral crowns.

Introduction

Pistacia vera L., commonly known as the pistachio tree, is a single species of subtropical plant belonging to Anacardiaceae family (Hossein *et al*., 2022; Nazoori *et al*., 2022). Among crops, it occupies a unique position as a strategic product. This product is the most significant export commodity, accounting for 14% of all non-oil exports (Fani *et al*., 2018). *P. vera* L. cv. Owhadi, *P. vera* L. cv. Akbari, *P. vera* L. cv. Ahmad-Aghaei, *P. vera* L. cv. Koleghoochi, *P. vera* L. cv. Badami Zarand, and *P. vera* L. Rezaei are among the most commonly

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cultivated plants in Iran (Rahemi and Pakkish, 2009; Taghizadeh *et al*., 2018). Iran is one of the major centers of diversity, cultivation, and the largest producer of pistachio in the world (Shamshiri and Hasani, 2015; Norozi *et al*., 2019; Sharifkhah *et al*., 2020). Selecting the appropriate seedling rootstock is one of the most critical decisions to make when establishing a pistachio orchard (Vahdati *et al.*, 2021). In Iran, *P. mutica* is chosen as a pistachio rootstock and a priceless plant that has gained popularity for its use in food, medicine, and industry (Bagheri *et al*., 2021). Some studies suggest that *P. mutica* leaves contain antioxidants (Altundag and Ozturk, 2011). Therefore, this plant possesses strong antiviral and antibacterial qualities (Adams *et al*., 2009).

Furthermore, to provide support, a suitable culture medium acts as a reservoir for nutrients, water, and oxygen, facilitating efficient gas exchange between roots and the environment (Abad *et al*., 2002). Instead of using soil directly, nurseries often create a seed culture mix combining peat moss and vermiculite for optimal results. However, concerns about the sustainability of peat moss led to the growing adoption of alternative ingredients such as vermiculite in professional potting mixes. These "waterless" mixes or artificial soils achieve properties similar to those of traditional peat-based options but with a lower environmental impact. Vermiculite is an indispensable component of horticulture due to its distinctive capacity to facilitate drainage and aeration while concurrently retaining substantial quantities of water, which are progressively released as plants require them (Meena *et al*., 2017).

The spiral crown phenomenon is a recurring cause of significant reductions in pistachio crop yields. A physical constriction resembling a screw thread at the base of the stem can impede the flow of raw sap and nutrients to other parts of the plant. The pistachio rootstock is vulnerable to damaging elements in terms of screw crown. Another type of complexity associated with citrus exists where the roots are twisted as opposed to pistachio, which have twisted crowns. The newly widespread issue of root complications has a specific position among physiological disorders. Dry seed coat resistance to the formation of immature roots plays a major role in hypocotyl flexion. Citrus seeds frequently generate embryonic sacs in this configuration, and the terminal root may rotate at varying angles around the micropyle of the seed, where the root emerges during germination, in addition to conducting independent research (Konstantinou *et al*., 2023). This odd positioning of the baby may result in various degrees of curvature or even a full rotation of the root as it emerges, which may ultimately cause root issues. Growers of seedlings and gardeners may suffer financial losses due to this issue. This issue arises if care is not exercised while moving seedlings from the nursery to the mainland (Batchelor and Webber, 1948). Based on Taghdisi *et al*. (Taghdisi *et al*., 2011), heavier soils composed of river mud and sand significantly increased root complexity compared with lighter soils, across various tested citrus cultivars. This complexity, measured as "twist", was highest in citrange and lowest in citrumelo cultivars. Moreover, planting seeds horizontally in heavier soils was identified as an effective strategy to combat root rotation and promote the development of thicker, stronger seedlings.

In the screw crown of the pistachio, several factors are effective such as soil texture, type of seed, and how to plant pistachio seeds. To date, no research was conducted on the complexity of the crown in pistachio. To prove this topic, experiments were carried out on *P. vera* cv. Badami, *P. integerrima*, and *P. mutica*.

Materials and Methods

This study was conducted in the research greenhouse of Yazd University and nursery located in Mehriz City for three years. A plot of land was selected and marked between the planting rows with dimensions of 3*1 m. *P. vera* cv. Badami was purchased from Zare Nursery, and *P. integerrima* and *P. mutica* seeds were purchased from

Rafsanjan Pistachio Research Center. *P. vera* and *P. integerrima* seeds were soaked in water for 48 h, and their water was changed three times a day. *P. mutica* seeds were placed in plastic and preserved for 72 h at -18 °C to be ready for planting. After the time required for preparation, the seeds were soaked in 1 g l^{-1} of benomyl and then washed in 1 L of distilled water to prepare for planting. The culture media were prepared in different proportions and planted in potted plastic bags with a diameter of 10-15 cm and a height of 25–30 cm. The reason for utilizing these seeds was that the Badami is primarily used as a rootstock in these regions. Additionally, the research aimed to investigate the response of other pistachio species, including *P. integerrima* and *P. mutica*, to the benching crown.

-One unit of peat moss culture medium, one unit of perlite culture medium, and one unit of sand

-Two units of sand, one unit of perlite, and one unit of peat moss

-Two peatmoss units, one sand unit, and one perlite unit

-Control (sandy-loam soil)

Treatments for orientation of seed cultivation included up, down, and horizontal tips. The pots were then watered twice a day (morning and evening) for 15 days and then every other day.

Eight months after seed culture, pistachio seedlings from nursery and greenhouse were harvested. To assess the presence of "screw crown" formation, 1 cm of topsoil was removed. This allowed researchers to separate seedlings showing the screw crown phenotype from those without it. Subsequently, several morphological characteristics were measured for each seedling, including leaf count, stem length and diameter (using calipers), and leaf surface area (using a WIN AREA-UT-11 model).

The experiment used a replicated design. Each treatment was repeated three times to account for random variation. Data analysis was conducted using SPSS-20 software. In order to display the data and maybe spot patterns or correlations between the treatments and measured variables, graphs were made using Microsoft Excel 2018 software. Duncan's test was performed to compare the means of the various treatment groups.

Results

The results of the analysis of the variance of data showed that the effect of culture medium, seeds, and seed culture on some morphological characteristics of pistachios was significant (which level) (Table 1). Based on the results of comparing average data, the leaf area increased by 66% compared with the control, in a medium of *P. vera* cv. Badami, and in the third media, *P. vera* cv. Badami and up tip state compared to the control, increased by 74%, i.e., 84.9 cm^2 area (Fig. 1).

Table 1. Results of analysis of variance of seed effect, seed cultivation method, and culture medium on some morphological characteristics of pistachio seedlings

Treatments	df	Mean of square				
		Leaves area (cm^2)	Spiral crown %	Shoot length (cm)	Shoot diameter (mm)	Number of leaves
Seed (s)	2	19634.2**	437.7**	818.2**	32.5**	4065.6**
Seed culturing (C)	2	339.8**	125.9**	226.4**	0.3**	180.0**
Culture medium (M)	3	589.7**	606.8**	315.4**	16.1**	244.7**
M*S	6	713.2**	1250.4**	89.4**	17.0**	252.1**
M*C	6	1048.6**	5161.4**	162.9**	3.9**	109.5**
S*C	4	330.9**	2718.3**	139.5**	16.7**	98.3**
M*S*C	12	1060.5**	717.8**	181.8**	6.3**	66.6**

** Significance at the level of 1%

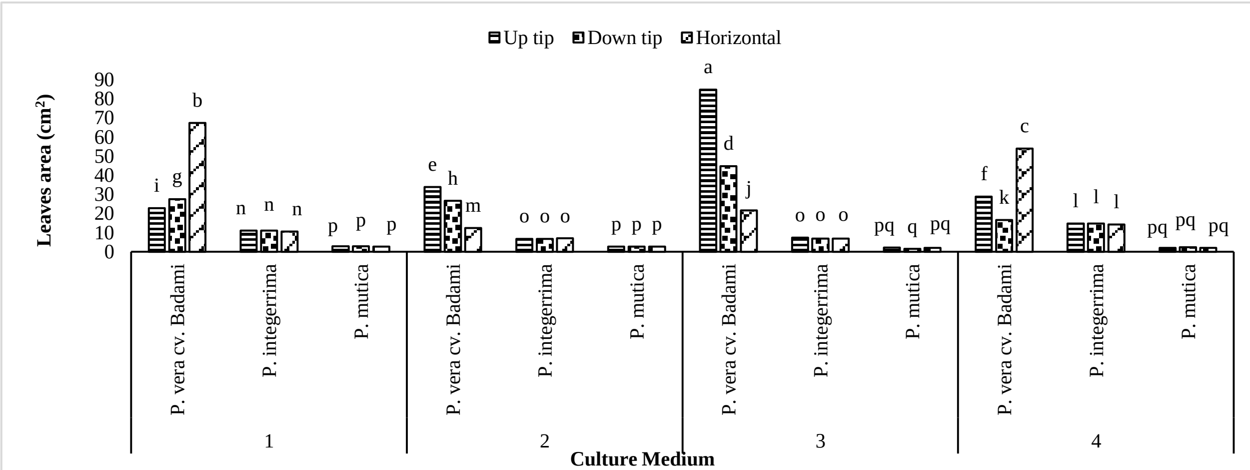

Fig. 1. The effect of seed type, seed planting method, and culture medium on the leaf area (1: One unit of peatmoss culture medium, one unit of perlite culture medium, and one unit of sand; 2: Two units of sand, one unit of perlite, and one unit of peatmoss; 3: Two peatmoss units, one sand unit, and one perlite unit, and 4: Control (sandy-loam soil)).

The results of comparing the mean interaction of culture medium, seed type, and seed sowing on the number of leaves of pistachio seedlings showed that using the second medium and *P. vera* cv. Badami seeds in up and down tip conditions, the number of leaves in pistachio seedlings increased compared to the control that amount of this is 28.2 (Fig. 2).

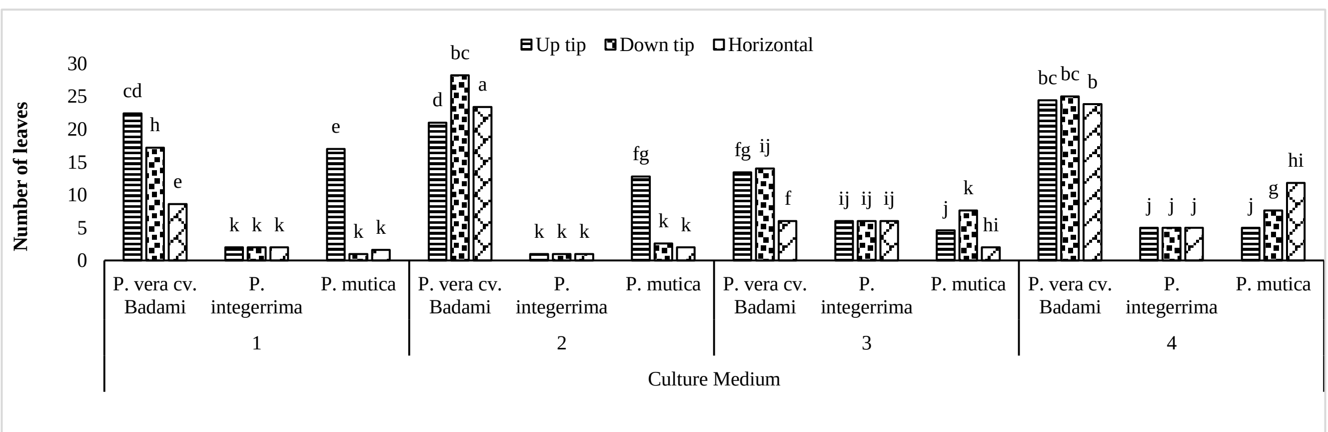

Fig. 2. The effect of seed type, seed planting method, and culture medium on the number of leaves (1: One unit of peatmoss culture medium, one unit of perlite culture medium, and one unit of sand; 2: Two units of sand, one unit of perlite, and one unit of peatmoss; 3: Two peatmoss units, one sand unit, and one perlite unit, and 4: Control (sandy-loam soil))

The mean interaction of culture medium, seed type, and seed dispersal method on the stem diameter of pistachio seedlings was compared, and the results indicated that *P. vera* cv. Badami, and up tip conditions, seedling stem diameter increased compared to the control (7.6 mm), and in the second media, stem diameter increased with *P. integerrima* seed treatment and low and horizontal planting conditions (Fig. 3).

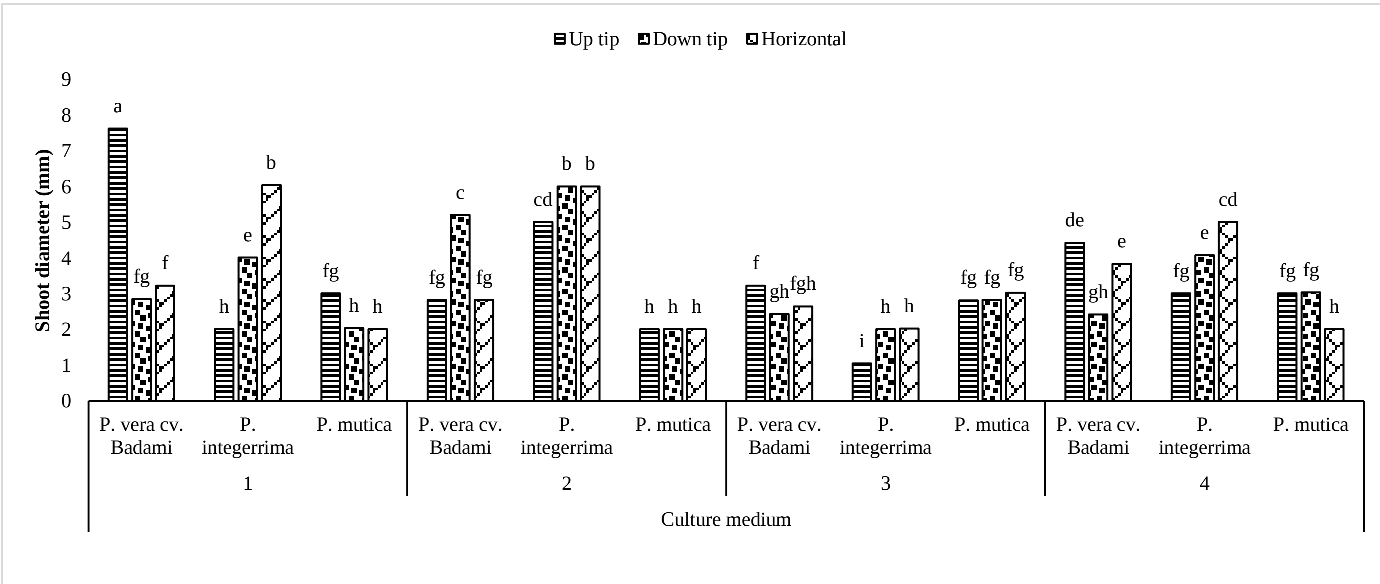

Fig. 3. The effect of seed, seed sowing method, and culture medium on stem diameter (1: One unit of peatmoss culture medium, one unit of perlite culture medium, and one unit of sand; 2: Two units of sand, one unit of perlite, and one unit of peatmoss; 3: Two peatmoss units, one sand unit, and one perlite unit, and 4: Control (sandy-loam soil))

The results of comparing the mean interaction of culture medium, seed type, and seed sowing method on pistachio seedling stem length showed that the second media, *P. vera* cv. Badami, and up tip planting mode caused an increase compared with the control with the amount of 35.4 cm. In the third media, *P. vera* cv. Badami seeds with up tips increased with the amount of 38.8 cm and in the same media, *P. mutica* seeds with horizontal tips increased compared to the control with amount of 30.5 cm (Fig. 4).

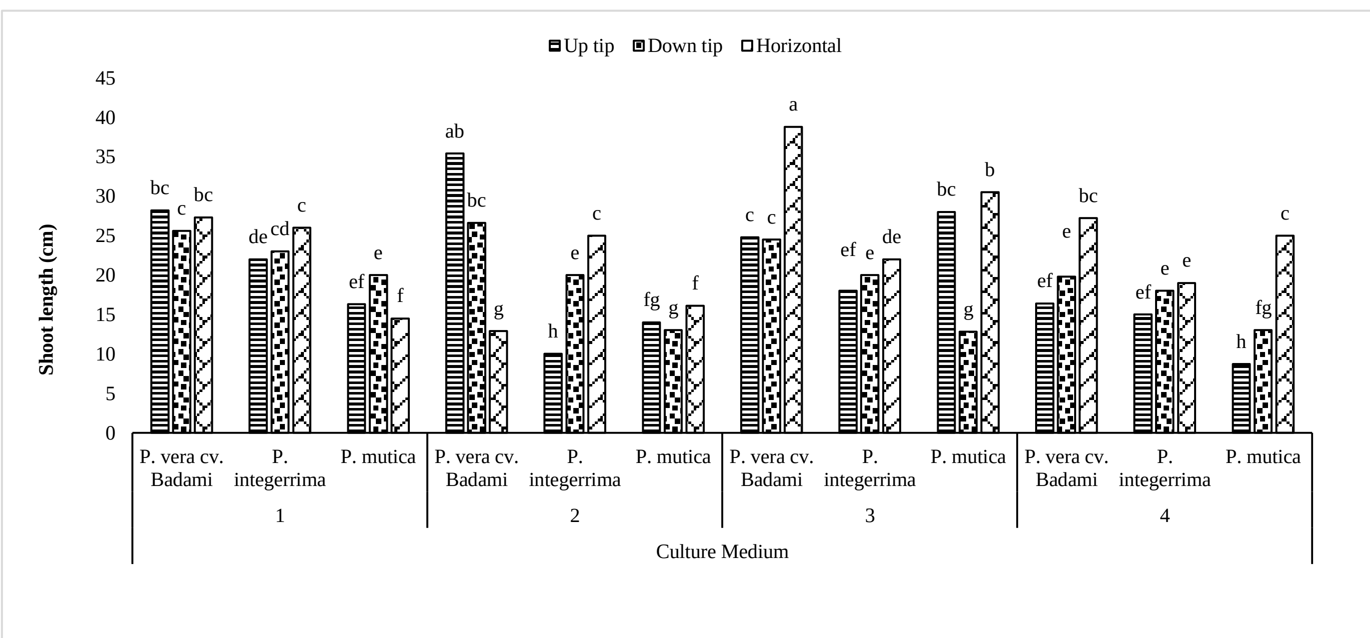

Fig. 4. The effect of seed, seed sowing, and culture medium on stem length (1: One unit of peatmoss culture medium one unit of perlite culture medium, and one unit of sand; 2: Two units of sand, one unit of perlite, and one unit of peatmoss; 3: Two peatmoss units, one sand unit, and one perlite unit, and 4: Control (sandy-loam soil)).

The results of comparing the mean interaction of culture medium, seed type, and seed sowing method on the crown of pistachio seedlings showed that using the first medium increased the spiral crown with horizontal tip when compared to the control. In this medium, up and horizontal tip caused increasing in spiral crown. In the second media, *P. integerrima* with a horizontal tip showed a spiral crown with 81.6% (Fig. 5). Pictures of the number of seedlings with a crown are shown (Fig. 6).

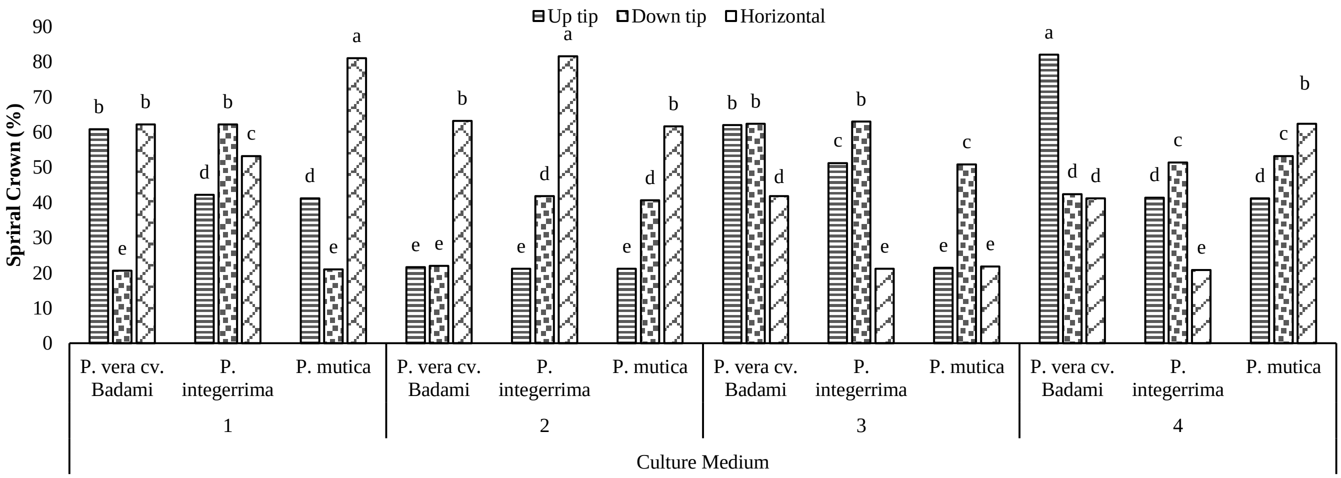

Fig. 5. The effect of seeds, seed sowing method, and planting medium on the percentage of spiral crown (1: One unit of peatmoss culture medium, one unit of perlite culture medium, and one unit of sand; 2: Two units of sand, one unit of perlite, and one unit of peatmoss; 3: Two peatmoss units, one sand unit, and one perlite unit, and 4: Control (sandy-loam soil))

Fig. 6. Some pictures of spiral crown in Pistachio

Discussion

Plant growth is typically vertical or horizontally linear (Steeves and Sussex, 1989), but it can spiral in some cases. This type of growth is classified as spiral growth (Jaffe and Galston, 1968). Simmons *et al*. demonstrate that a flat helix results from an interaction between the helical circumference of the root and the plate's impermeable surface. In some strains of wild Arabidopsis, roots are constantly tilted to the left under these conditions (Simmons *et al*., 1995). Using this approach, two genes named SPIRAL1 and SPIRAL2 were identified from mutant roots that were strongly left- or right-inclined (Furutani *et al*., 2000). SPIRAL1 mutations appear to impose abnormal helical growth on roots; they interfere with their rotation so that they show constant torsion under normal growth conditions. This torsion extends to hypocotyl and stem, and in SPIRAL2 spiral mutations, even to the petioles and petals of the leaf (Smyth, 2016). In SPIRAL1 mutants, the cellular sections of microtubules inside the root epidermal cells in the basal elongation area were abnormally spiral, pointing in the opposite direction as the epidermal cells. Studies showed that if the concentration of propyzamide is low, it can cause the microtubules to polymerize and cause root rot and plant twisting. Polymerization of microtubules alone does not control, according to the research. Treatment of wild roots with drugs creates layers of microtubules that occur in the helix instead of the usual bands, which further supports the role of microtubules (Smyth, 2016).

While plant growth typically manifests as linear or circular expansion, certain instances exhibit twists, spirals, or coils, collectively termed helical growth. In the context of root spiral growth, disruptions in microtubule dynamics were observed to induce twisted growth patterns. However, precise mechanisms underlying this phenomenon remain unclear and may involve diverse processes (Nogales, 2015). Helical growth, a characteristic feature of many plant organs, manifests in diverse forms, including circular searching movements of growing stems and other organs (circumnutation), the spiraling coils of tendrils, the unique arrangement of leaves and buds (resupination), the patterned twist of petals (contortion), and the helical shaping of leaf blades. The fundamental mechanisms of this complex growth pattern have been illuminated by recent genetic studies, which imply a connection between helical arrays of cortical microtubules and the cellulose microfibrils that overlie them. Moreover, an alternative mechanism based on differential contraction within a bilayer was discovered, contributing to various aspects of helical growth (Smyth, 2016).

Conclusions

In general, it can be concluded from the results of above study that different culture media, such as substrate and control, as well as *P.vera* cv. Badami and *P. integerrima* seeds were more effective on crowning than other treatments. *P. integerrima* had the least effect on the crown, based on previous studies, and the application of litter two with the method of sowing seeds lying down had the most effect. Besides, three control beds with *P. vera* cv. Badami seeds and a planting method with an up tip and the corm in a lying position had the highest percentage of crowns among the beds. The highest percentage of spiral crowns was related to media 1 of *P. mutica* with horizontal tip, which was similar to media 4 (control) with *P. vera* cv. Badami with up tip, which showed 82% of spiral crowns. Because no research was done on the pistachio crown, for a more comprehensive study, several factors affecting the pistachio spiral crown should be studied. Besides, all the internal events, including all the genes expressed in the seedlings with spiral crowns, should be investigated. In general, we can claim that, when planting pistachio seeds, we should pay attention that the first part that comes out of pistachio seed is actually the root, and the root should be placed towards the soil to prevent twisting of pistachio root and crown. So, the direction of the seed in cultivation is very important; however, due FFto high auxin in the pistachio seeds, the roots are very deep, and the stem has high elongation and stem due to too much growth of the stem twists. Another thing is since the root has positive geotropism so if cultivated on the contrary causes the crown to twist. Thus, we can conclude If, during seed planting, the root direction is mistakenly placed upwards in the soil, in terms of negative phototropism, and positive geotropism, the root twists again and goes back into the ground, and this causes the twisted crown in the pistachio seedling Trees which roots are twisted, their productivity is greatly reduced.

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Conflict of interst

The authors declare that they have no conflict of interest.

Ethical approval

The present Study and ethical aspect were approved by Yazd University, Yazd Iran.

Consent for publication

All authors designed the study, collected data, wrote the manuscript and revised it.

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