RESEARCH ARTICLE
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Effect of Peanut Seed Orientation on Germination, Seedling Biomass, and Morphology in an Oak Tree Sawdust Cultivation System

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Abstract

We performed seed germination tests to investigate the effects of seed sowing orientation on germination viability on peanut (*Arachis hypogaea* L.) sprouts. Specifically, we assessed the influence of seed sowing orientation on germination rate, seedling weight, and seedling length, as well as the seedling vigor index. The seeds were sown in oak tree sawdust at 3.0 cm depth. Four seed orientations were tested: vertical with the hypocotyl end down, vertical with the hypocotyl end up, horizontal with the hypocotyl end down, and horizontal with the hypocotyl end up. The mean seed germination percentages of the four seed orientations were significantly different ($p < 0.01$) and ranged from 25 to 91.7%. The vertical orientation with hypocotyl-end-down and hypocotyl-end-up orientations showed the highest (91.7%) and lowest (25%) germination rates, respectively. The vertical orientation with the hypocotyl end down produced the heaviest (4.9 g) seedlings and the longest hypocotyls (4.65 cm). This orientation also produced the longest true leaf + epycotyl (2.15 cm) and had the highest seedling vigor index (197.1). The seedlings had a straight growth pattern, whereas seedlings from seeds sown with the hypocotyl up had an awkward plumular hook shape. Taken together, to produce peanut sprouts, we recommend placing the seeds vertically with the hypocotyl end down because this orientation leads to a high germination rate, high biomass production, and high overall seedling quality.

Additional key words: *Arachis hypogaea*, cotyledon, hypocotyl, morphology, sowing direction, vigor index

Introduction

Early sprout development depends on successful seed germination, hypocotyl growth, and root emergence in the direction of gravity (Ma and Hasenstein, 2006). Seeds have a physiological ability to germinate and correctly orient themselves according to gravity, a process known as gravitropism (Takakura et al., 1996). Seed orientation during sowing can affect the seed germination rate, seedling physiology, and seedling morphology (Kevin et al., 2015). The seed is composed of a cotyledon, a plumule (embryonic shoot with first leaves), and a radicle (embryonic root). The seed should be properly orientated when sown so that the radicle points downwards, since the seeds of some plant species exhibit gravitropism (Blancaflor and Masson, 2003). If the radicle points upwards, the embryonic root and stem emerge in the wrong direction and have to change orientation, which requires energy and subsequently reduces plant growth (Jha et al., 2012).

Peanut sprouts have recently become popular as a functional food material because they contain resveratrol (3,4',5-trihydroxystilbene), a potent bioactive phytochemical that helps prevent cardiovascular diseases and cancer (Sanders et al., 2000). The oak tree sawdust cultivation system has been developed to increase resveratrol contents in peanut sprouts (Lee, 2016). However, this system is costly and time consuming compared to water cultivation of peanut sprouts. Therefore, high germination rates and good morphological growth with high biomass are essential for successful sprout cultivation and the commercialization of peanut sprouts. In general, germination rate, seedling growth, and morphology often vary due to seed sowing orientation in similar environments and for the same varieties (Bosy and Aarssen, 1995; Lee et al., 2016). In this study, we investigated the effects of seed sowing orientation on germination rate, growth, and morphology in peanut under oak tree sawdust cultivation conditions in order to determine the optimal seed orientation for seed germination and to improve germination rates and seedling vigor.

Materials and Methods

Oak Tree Sawdust Cultivation System for Seed Germination

Peanut (*Arachis hypogaea* L.) seeds were sown in trays (50 × 30 × 10 cm; Namyoung Chemical, Hanam, Korea) containing oak tree saw dust and were planted in rows. The spacing between seeds was 5 cm, row spacing was 9 cm, and the sowing depth was 3 cm. All seven seeds per row had the same orientation. Fourteen seeds were sown in four different orientations as follows: vertical with the hypocotyl end down (No. 1), vertical with the hypocotyl end up (No. 2), horizontal with the hypocotyl end down (No. 3), and horizontal with the hypocotyl end up (No. 4) (Fig. 1). Oak sawdust to a depth of 3 cm was placed in a tray, and after sowing, the seeds were covered with a 3 cm layer of sawdust. The trays were transferred to a dark room and watered with a total

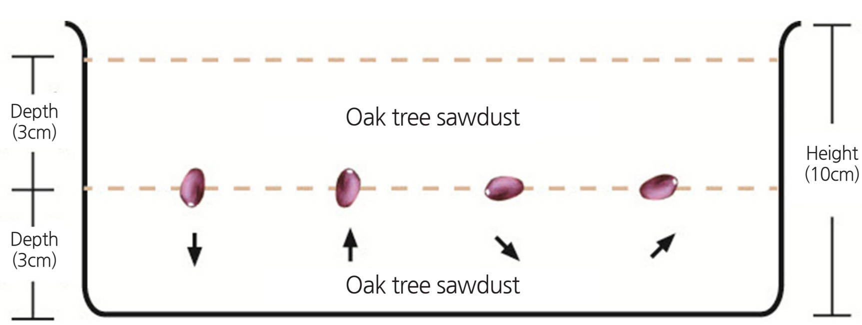

Fig. 1. Illustration of the seed orientations used in this study (from left to right: vertical with hypocotyl end down [No. 1], vertical with hypocotyl end up [No. 2], horizontal with hypocotyl end down [No. 3], and horizontal with hypocotyl end up [No. 4]). Seeds were placed in a 50 × 30 × 10 cm tray in one of the four different orientations on 3 cm of oak tree sawdust and covered with an additional 3 cm of oak tree sawdust.

of 3 L water every day in the morning and evening. Seed germination was recorded each day beginning at three days after sowing. Nine days after sowing, all seedlings in each orientation group were observed and their seed germination rate, hypocotyl, true leaf + epicotyl, root, and cotyledon length were measured, as well as the germinated seedling weight. The seedling morphology was also recorded.

Seedling Weight and Tissue Length Measurements

The peanut seedlings were removed from each tray after 9 days of culture. All seedlings were washed with tap water and blotted dry on paper towels (Yuhan Kimberly, Seoul, Korea). The fresh of the whole seedling and the lengths of the true leaf + epicotyl, hypocotyl, root, and cotyledon were measured.

Vigor Index

To optimize the conditions for peanut seedling cultivation, the four groups of peanuts (categorized by seedling orientation) were evaluated using two criteria: germination rate and the lengths of the four tissue parts mentioned above. The vigor index was calculated as follows: Germination rate of each seedling orientation group × length of each tissue part.

Morphological Observation

Freshly harvested peanut seedling samples were placed on a piece of black cotton flannel. Five individuals from each seedling orientation group were selected and photographed using a Digital Gross System camera (Humintec, Suwon, Korea). .

Results

Effect of Seed Orientation on Germination Rate

The seedling emergence time and seed germination rates were different among the seed sowing orientation groups. In the vertical with hypocotyl-end-down orientation group (No. 1), the radical began to emerge at 4 days after sowing, whereas it started to emerge after 7 days in the vertical with hypocotyl-end-up orientation group (No. 2). In the horizontal with hypocotyl end up and down orientation groups (Nos. 3 and 4, respectively), the hypocotyl began to appear after 8 and 6 days, respectively. Seeds sown vertically with the hypocotyl end downward (No. 1) emerged significantly more quickly than the others (Nos. 2, 3, and 4; data not shown). By contrast, seeds sown vertically with the hypocotyl end up (No. 2) emerged significantly later than the others (Nos. 1, 3, 4). Seed orientation group No. 1 had the highest (91.7%) germination rate, whereas seed orientation group No. 2 had the lowest germination rate (25%). Number 3 and 4 orientations had similar germination rates (41.7 and 41.7%, respectively) (Table 1 and Fig. 2). These results indicate that sowing seeds vertically with the hypocotyl end downward enhances seed emergence and the germination rate compared to sowing seeds with the hypocotyl end up and/or horizontally.

Effect of Seed Orientation on Seedling Weight

Nine days after sowing, all seedlings were uprooted and weighed. Seeds sown vertically with the hypocotyl end down (No. 1) had a significantly heavier mean weight (4.9 g) than seeds sown vertically with the hypocotyl end up (No. 2) (4.2 g). The mean weights of No. 3 and 4 seed orientations were 4.4 g and 4.7 g, respectively (Fig. 3).

Effect of Seed Orientation on True Leaf + Epycotyl (TLE), Hypocotyl, Root, and Cotyledon Length

The true leaf + epycotyl (TLE) in seedlings from group No. 1 (vertical with the hypocotyl end down) (2.15 cm) and group No. 3 (horizontal with the hypocotyl end down) (2.41 cm) were longer than those from groups No. 2 (vertical with the hypocotyl end up) (1.45 cm) and No. 4 (horizontal with the hypocotyl end up) (1.49 cm) (Fig. 4A). The average hypocotyl length of group No. 1 seeds was significantly greater (4.65 cm) than that produced by the other seed orientations (No. 2, 3.37 cm; No. 3, 3.54 cm; and No. 4, 3.74 cm) (Fig. 4B). The roots of seedlings in group No. 2 (6.25 cm) were significantly longer than those of the other groups

Table 1. Effect of seed sowing orientation on vigor index.

Orientation[z]	Germination rate (%)	Length (cm)				Vigor index (VI)[y]			
		True leaf+ Epicotyl	Hypocotyl	Root	Cotyledon	True leaf+ Epicotyl	Hypocotyl	Root	Cotyledon
#1	91.7	2.15	4.65	2.00	2.47	197.1 ± 12.7	426.3 ± 38.8	183.3 ± 16.7	226.1 ± 20.6
#2	25.0	1.45	3.37	6.25	2.64	36.1 ± 8.5	84.3 ± 28.1	156.1 ± 52.0	65.9 ± 22.0
#3	41.7	2.41	3.54	4.62	2.55	100.4 ± 14.2	147.5 ± 29.5	192.5 ± 38.5	106.3 ± 21.3
#4	41.7	1.49	3.74	4.32	2.48	62.2 ± 8.8	155.9 ± 31.2	179.9 ± 36.0	103.5 ± 20.7

[z]#1: ↓, #2: ↑, #3: ↘, #4: ↗.
[y]Germination Rate (%) ×Length (cm).

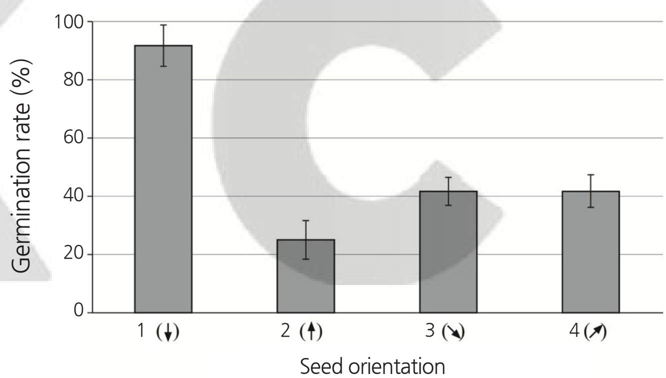

Fig. 2. Effect of seed sowing orientation (from left to right: vertical with hypocotyl end down [No. 1], vertical with hypocotyl end up [No. 2], horizontal with hypocotyl end down [No. 3], and horizontal with hypocotyl end up [No. 4]) on seed germination rate.

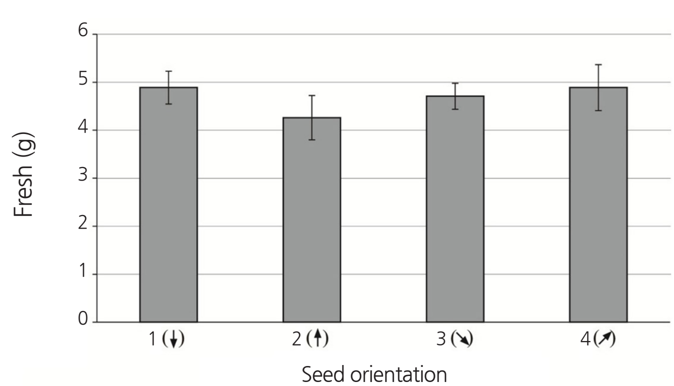

Fig. 3. Effect of seed sowing orientation (from left to right: vertical with hypocotyl end down [No. 1], vertical with hypocotyl end up [No. 2], horizontal with hypocotyl end down [No. 3], and horizontal with hypocotyl end up [No. 4]) on fresh.

(Fig. 4C). Seedlings in group No. 1 had the shortest roots (2.0 cm) (Fig. 4C). All orientations produced cotyledons that were similar in length (No. 1, 2.47 cm; No. 2, 2.64 cm; No. 3, 2.55 cm; and No. 4, 2.48 cm) (Fig. 4D).

Effect of Seed Orientation on Vigor Index

We calculated the vigor index of each germinated seedling to determine which orientation led to the most efficient biomass production (Table 1 and Fig. 5). According to the calculation, the order (from highest to lowest) was No. 1 > No. 3 > No. 4 > No. 2. In general, seeds oriented with the hypocotyl end down produced seedlings with better vigor index values than those grown from seeds oriented with the hypocotyl end up. When the seeds were placed horizontally, the orientation did not appear to have a significant effect on seedling vigor. However, in the vertical orientation, the hypocotyl-end-up position had a significantly adverse effect on the vigor index (Fig. 5).

Effect of Seed Orientation on Seedling Morphology

The No. 1 orientated seeds produced an upright seedling form (Fig. 6A). The vertical hypocotyl-end-up orientation (No. 2) led to seedlings with an awkward, plumular hook shape and long roots (Fig. 6B). The No. 3 and No. 4 seed orientations produced seedlings with similar shapes (Figs. 6C and D, respectively). However, seedlings grown from horizontal seeds with the hypocotyl end downward showed slightly better emergence of true leaves than the other group.

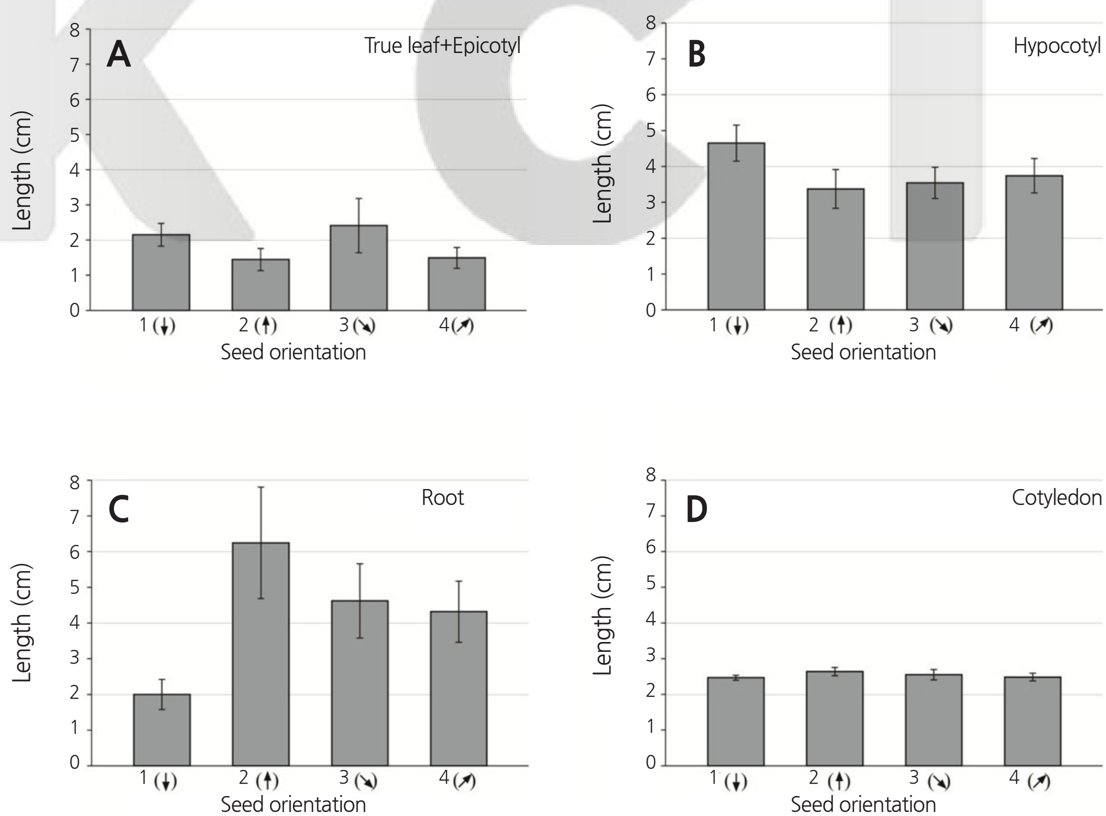

Fig. 4. Effect of seed sowing orientation (from left to right: vertical with hypocotyl end down [No. 1], vertical with hypocotyl end up [No. 2], horizontal with hypocotyl end down [No. 3], and horizontal with hypocotyl end up [No. 4]) on seedling length. A: True leaf + Epicotyl; B: Hypocotyl; C: Root; D: Cotyledon.

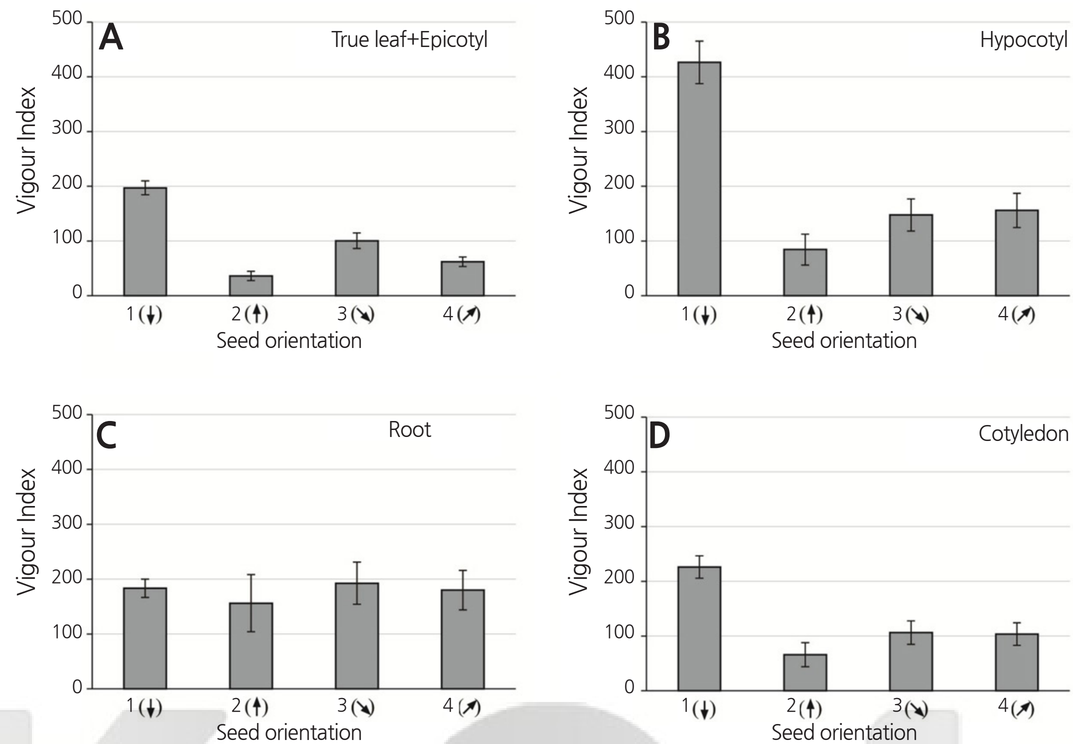

Fig. 5. Effect of seed sowing orientation (from left to right: vertical with hypocotyl end down [No. 1], vertical with hypocotyl end up [No. 2], horizontal with hypocotyl end down [No. 3], and horizontal with hypocotyl end up [No. 4]) on vigor index (VI). A: True leaf + Epicotyl; B: Hypocotyl; C: Root; D: Cotyledon.

Discussion

We investigated the effects of seed orientation on the germination rate, seedling weight, length of each seedling tissue, and morphology of peanut sprouts. The vertical with hypocotyl end downward orientation produced the highest germination rate compared to the other orientations, which indicates that there is a close relationship between seed orientation and germination (Bowers and Hayen, 1972). Indeed, the best seed germination and seedling growth in Anjan (*Hardwickia binata* Roxb) were achieved when the seed was planted in a horizontal position (Masilamani et al., 1999), whereas bean seeds planted hypocotyl end up led to significantly higher germination rates than hypocotyl end down and the horizontal position (Bowers and Hayden, 1972). Previous reports have suggested that it is essential to determine the optimal seed orientations for different plant species if efficient germination and seedling growth are to be achieved. In the current study, the hypocotyl-end-up orientations produced the lowest seedling weight compared to the vertical orientations. By contrast, Anjan (*H. binata* Roxb) seedlings grown from seeds placed in a horizontal orientation had a higher biomass than those from seeds planted in the hypocotyl end upward and downward orientations (Masilamani et al., 1999). In addition, the hypocotyl-end-up orientation led to reduced germination and seedling growth in Anjan (Masilamani et al., 1999).

In the current study, the true leaf, cotyledon, hypocotyl, and root length were affected by seed orientation. The hypocotyl-downward direction, regardless of whether the seed position was horizontal or vertical, produced better true leaf growth, and the hypocotyl end downward with vertical orientation produced the longest leaves. However, the hypocotyl end downward with vertical orientation produced the shortest roots, whereas the hypocotyl end upward with vertical orientation produced the

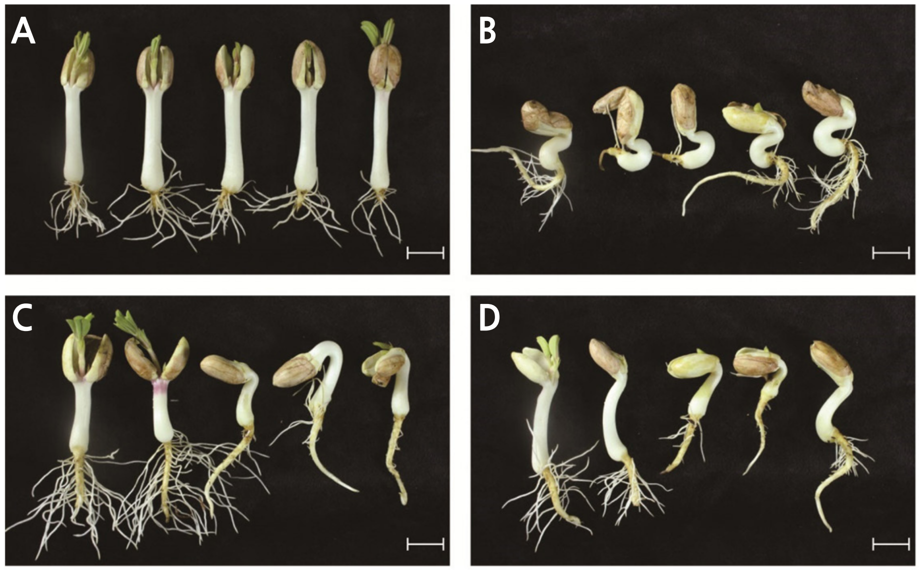

Fig. 6. Effect of seed sowing orientation on the phenotype of germinated seedlings. A: (↓, vertical with hypocotyl end down); B: (↑, vertical with hypocotyl end up); C(↘, horizontal with hypocotyl end down); D:(↗, horizontal with hypocotyl end up). Scale bar=2 cm.

longest roots. The root length was shorter in group No. 1 seedlings than in the others because the tray depth was limited to 3 cm. Seed orientation had no significant effect on cotyledon length. Overall, proper seed orientation optimizes seedling growth in many plant species (Bosy and Aarssen, 1995; Sharma and Purohit, 1980).

Peanut sprouts have become a popular fresh vegetable. Therefore, morphological quality as well as weight are important factors. The relationship between seed sowing orientation and seed germination physiology and morphology can be explained by the previous finding that the inversion of the seedling sometimes produces abnormal chemical or physiological conditions, resulting in an abnormal morphology (Bennetclark et al., 1959). However, the current results suggest that sowing peanut seeds in a vertical hypocotyl end downward orientation led to a higher germination rate and uniform seedling shape compared to the other orientations. In this study, seedling morphology was significantly influenced by orientation. The hypocotyl upwards seed position adversely affected seedling growth and shape (Masilamani et al., 1999). Shape abnormalities are closely related to auxin allocation to specific tissue positions (Thapliyal, 1979). In addition, the seed germination rate also decreased and the hypocotyl growth of the germinated seedling was suppressed under this orientation. The deleterious effect of the vertical hypocotyl end upward orientation is due to the extra energy required for germination and seedling growth (Masilamani et al., 1999). The greater energy requirement for germination can reduce seed germination, emergence, and seedling growth (Thapliyal, 1979). Seedling germination, vigor index, and shape are influenced by seed orientation, including the distribution of plant hormones (Phat et al., 2015).

The present study demonstrates that seed-sowing orientation affects germination efficiency and seedling growth in peanut sprouts in an oak tree sawdust cultivation system. Thus, it is essential to consider proper sowing orientation when attempting to successfully grow peanut seedlings with a good shape. The hypocotyl end upward seed orientation adversely affected the germination rate and altered the seedling growth pattern (Bosy and Aarssen, 1995). In addition, seed orientation has varying effects, which are dependent on plant species, variety, and environmental conditions. Therefore, all of these factors should be carefully considered in order to achieve good seedling growth and shape.

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