Early root development of Eucalyptus pellita F. Muell. seedlings from seed and stem cutting at nursery stage

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Research

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Abstract

Background

Eucalyptus is among the important fast-growing species, and is typically managed on short rotation to sustain the production of timber, pulpwood, charcoal, and fire-wood. Macro-propagation using cutting for larger multiplying seedlings is cheaper and efficient instead of clonal seeds for uniform plant material seedling production. However, information on root growth of *Eucalyptus pellita* at early development from seed and stem cutting of *E. pellita* seedlings is still lacking. This is probably due to the difficulty in investigation belowground, and also due to methodological problems. With such information, it is useful for forest plantation company management in enhancing the understanding on strategies to optimize yield production with the appropriate agronomic or silvicultural approach in the field planting. Therefore, the objectives of this study were; to compare the root development of two different propagation seedlings of *E. pellita*; and to study the effect of various nitrogen concentration levels on two types of propagation of *E. pellita* seedlings.

Results

The study was conducted using *E. pellita* seedlings from two types of propagation, namely, seed and stem cuttings, along with three different nitrogen concentrations (0, 50, and 200 kg N ha^{-1}). Shoot biomass, root intensity (RI), total root intensity (TRI), root biomass, root length density (RLD), and specific root length (SRL) were recorded. Dried shoot biomass, RLD and SRL of *E. pellita* seedlings using stem cutting were significantly higher (P<0.05) compared to seed. Whereas, there were no significant differences (P>0.05) for root biomass, TRI and RI between the propagation types of *E. pellita* seedlings.

Conclusions:

E. pellita seedlings from stem cutting was greater in terms of root distribution compared to propagation by seeds at the nursery stage, and 50 kg N ha^{-1} was the optimal nitrogen concentration level from the considered levels to be applied to the *E. pellita* seedlings. The present study therefore provides more information and understanding on *E. pellita* for forest plantation companies in producing plant materials using stem cutting in a cost-effective and efficient manner. This would help the forest plantation companies in planning appropriate agronomic management in the future.

Background

Plantation forestry using *Eucalyptus* spp. in Sabah started in the 1970s (Harwood and Nambiar 2014) as part of a forest conservation effort (Zaiton et al. 2020). *Eucalyptus* is among the important fast-growing species that is typically managed on short rotation to sustain the production of timber, pulpwood, charcoal, and fire-wood (Zaiton et al. 2018; Zhou et al. 2018). Sabah Softwood Berhad (SSB) is the first private forest plantation company in Sabah that pioneered using fast-growing timber species, where *E. deglupta* was initially introduced during the early plantation development (Enters et al. 2002). However, it was unsuccessful, and was later replaced with other superior species such as *Acacias*, due poor growth performance (Zaiton et al. 2020) and foliar pathogens (Japarudin et al. 2015).

Since nearly three decades, *Acacia mangium* and hybrids have been the primary species planted in Sabah, especially in some forest plantation companies such as Acacia Forest Industries Sdn Bhd (AFI), Sabah Forest Development Authority (SAFODA) and SSB. However, *A. mangium* and hybrids performance are affected mainly by serious fungi *Ceratocystis* disease (Tarigan et al. 2011; Japarudin et al. 2015), wilt (Japarudin et al. 2015), and *Ganoderma philippii* (Mohammed et al. 2014), which have caused death to about 10 to 20% of the *Acacia* trees in plantations (Wong et al. 2015). Therefore, *E. pellita* is an alternative option for the fast-growing timber production industry. Since 2008, most of forest plantation companies in Sabah and Sarawak have been involved in using *Eucalyptus* species in plantations (Zaiton et al. 2020).

Eucalyptus pellita F. Muell, or red mahogany, is a medium-to-large tree that can grow up to 40 m in height and over 1 m in diameter (Harwood 1998). *E. pellita* is native to Papua New Guinea and northern Queensland, Australia (Hung et al. 2015; Yahya et al. 2020; Yew et al. 2015). It has good growth and a high survival rate because of its wider range of adaptability with sites and favourable stem form (Yahya 2020). Currently, *E. pellita* plays an important role in reforestation in countries such as Brazil, Cuba, Indonesia, Malaysia and the Philippines (Hung et al. 2015). Furthermore, *E. pellita* is used for a variety of products such as fine furniture (Clarke et al. 2009), pulp production (Eldridge et al. 1993; Poke and Raymond 2006) and high quality writing and printing paper or tissue products (Raymond 2002; Raymond and Schimleck 2002; Schimleck et al. 2006).

In order to sustain the plant material supply with efficient and cost-effective means (Kuppusamy et al. 2019), macro-propagation using cutting can be used instead of clonal seeds for uniform plant material seedling production. Cutting is the most widely used technique, and is cheaper for larger multiplying seedlings of *Eucalyptus*, due to easier handling as compared to the micro-propagation method (Sulichantini et al. 2014).

However, although there exist many studies on *E. pellita*, there is a limited amount of information on root growth of *E. pellita* at early development from seed and stem cutting of *E. pellita* seedlings. This is probably due to the difficulty in investigation belowground, and also due to methodological problems. With such information, it is useful for forest plantation company management in enhancing the understanding on strategies to optimize yield production with the appropriate agronomic or silvicultural approach. In this present study, we used two types of planting material sources from seed and stem cutting of *E. pellita*, and studied their root traits at three different nitrogen concentrations. We hypothesied that, both above and belowground, *E. pellita* seedlings from stem cutting were greater than seedlings from seed propagation. On this basis, the objectives of this study were formulated as follows: i) to compare the root development of two different propagation seedlings of *E. pellita*; and ii) to study the effect of different nitrogen concentrations on two types of propagation of *E. pellita* seedlings.

Material And Methods

Experiment description

This study was conducted from the 12th of April to the 30th of August 2019 at a greenhouse at the Forestry Complex of Faculty Science and Natural Resources, Universiti Malaysia Sabah (UMS), Kota Kinabalu, Sabah, Malaysia (6°02’ 08.4” N 116°07’ 34.4”E). According to the Malaysia Meteorological Department 2020 (www.met.gov.my), the temperature was in the range of 30 to 32 °C, while rainfall distribution was in the range of 111.76 mm (April) to 304.80 mm (June), throughout the study period. A transparent plastic pot 20 cm in height × 140 mm in inner diameter, which had a total volume of 3,079 cm^3, was used as the medium pot. The bottom of the container was created with small holes to facilitate the flow of water and air, and was covered with fine net. Each pot had four sides for grid lines, which were marked as sides A, B, C and D using a red permanent marker. The grid size was 20 × 20 mm was, and the total grid length for each side was 1.42 m. These grid lines were used to observe and count the root intensity of *E. pellita*. This involved repeatedly counting the number of intersections of the roots along the grid lines. During the experiment, the pot was always covered using non-transparent plastic to avoid light exposure of the soil and roots, and was opened only during the measurement process. Topsoil was taken from Tamparuli, Sabah (30 km from the Universiti Malaysia Sabah campus). The soil was open-dried for seven days in a greenhouse, sieved using a 2.0 mm soil mesh and filled into a pot. The moisture content of the soil sample before the experiment was 15.8%. After that, soil in the pot was washed with 5 L of water under low water pressure, to ensure all the nutrients in the soil were empty or low and homogenized.

In this experiment, seedlings of four-week-old *E. pellita* propagated from seeds, and stem cutting was supplied from Acacia Forest Industries Sdn. Bhd. (AFI). Stem cutting was produced from their superior mother clonal plants. The tip was selected for cutting; the rooting duration was four weeks in a greenhouse, prior to the experiment. The seedlings were then transferred to the pot that was filled with the topsoil. The 36 *E. pellita* seedlings from seeds and 36 *E. pellita* seedlings from stem cutting, accounting for a total of 72 experimental units, including three replications (12 replicates for each fertilizer treatment), were arranged using complete randomized design (CRD). A liquid nitrogen fertilizer (AG Leader 954) was diluted and corresponded to the three different rates of 0 (control), 50 N kg ha^{-1} and 200 N kg ha^{-1}. No watering was done as the experiment was exposed to natural conditions.

Data collection

In this experiment, dried shoot biomass, root biomass, root intensity (RI), total root intensity (TRI), root length density (RLD), and specific root length (SRL) were recorded (Hassan et al. 2019). RI data was collected based on the method by Thorup-Kristensen (2001). RI was measured by counting the number of roots crossing the lines of 20 × 20 mm grid squares placed on the container surface view sides. RI data was recorded every week, starting from when the roots started to appear on the surface of the transparent pot, until the roots reach the bottom of the pot.

Three different dates sampling procedures were carried out 4, 6 and 8 weeks after transplanting (WAT). Each sampling involved the harvesting of 12 experimental units, or four (4) replicates for each fertilizer treatment from both planting materials. Aboveground biomass was cut from the ground topsoil, washed, and placed in a labelled plastic bag. It was then kept in an oven at 70 °C for 48 hours, before being weighed. For root parameters, roots biomass was washed out from soil and organic matter using a sieve 2.0 mm mesh under low pressure water. It was then stored in 50% ethanol in a 50 ml eppendorf tube at 5 °C, before root image analysis. RLD (cm cm^{-3}) was determined using an EPSON® scanner and Winrhizo® software, and expressed in cm cm^{-3} (Hassan et al. 2019). For SRL (cm g^{-1}), the length of a sub-sample was measured, was then divided by its mass (g), before being converted to actual root biomass.

Statistical analysis

All the mean values were subject to statistical analysis using the Statistical Package Social Science (IBM SPSS Statistics 22.0). An independent sampled T-test was used to compare the TRI, RLD, SRL, root biomass, and shoot biomass between two types of plant material *E. pellita* seedlings at various nitrogen concentrations for all sampling dates. Subsequently, a one-way ANOVA followed Tukey HSD's post hoc analysis was used for RI at different nitrogen concentrations for both seed and stem cutting seedlings. In assessing the differences between the results, tests with $P < 0.05$ were considered statistically significant. Prior to statistical analyses, all data were tested for normality using the Shapiro-Wilk Normality test, and for homogeneity using Levene's test.

Results

Dried shoot biomass for E. pellita

Dried shoot biomass was harvested three times, four, six and eight weeks after transplanting (4, 6, 8 WAT), as indicated in Fig. 1. It is clear that there was a significant difference ($P < 0.05$) of dried shoot biomass of *E. pellita* seedlings between seed and stem cutting, especially at 6 WAT (Fig. 1b). At 4 WAT, there was no significant difference between seed and stem cutting for both 0 and 50 kg N ha^{-1}, but the shoot biomass of stem cutting was nearly double than seed under 200 kg N ha^{-1}. In contrast, at 6 WAT, all the treatments showed a significant difference ($P < 0.05$), where 50% of stem cutting was higher than seed. However, there was no significant difference between seed and stem cutting for all treatments at 8 WAT (Fig. 1c).

Figure 1: Dried Shoot Biomass (g) of *E. pellita* seedlings from seeds and stem cutting plant materials at different nitrogen concentrations (0, 50, 200 kg N ha^{-1}) at three selected dates of root measurement; 4 weeks after transplanting, WAT (a), 6 WAT (b), and 8 WAT (c). The mean values were tested using Independent Samples T-Test. All mean values were significant different *($P < 0.05$). Error bars denote standard deviations of the mean, ($n = 4$).

Root Biomass of E. pellita

There was no significant difference ($P > 0.05$) between the seed and stem cutting of *E. pellita* for all treatments in 4 WAT (Fig. 2a). However, in 6 WAT, only for 50 kg N^{-1}, the seeds of *E. pellita* showed a significant difference ($P < 0.05$), as compared to stem cutting (Fig. 2b). Interestingly, without fertilizer, the root biomass of *E. pellita* seeds witnessed a significant difference ($P < 0.05$), as compared to stem cutting (Fig. 2c).

Figure 2: Root Biomass (g) of *E. pellita* seedlings from seeds and stem cutting plant materials at different nitrogen concentrations (0, 50, 200 kg N ha^{-1}) at three selected dates of root measurement; 4 weeks after transplanting, WAT (a), 6 WAT (b), and 8 WAT (c). The mean values were tested using Independent Samples T-Test. All mean values were significant different *($P < 0.05$). Error bars denote standard deviations of the mean, ($n = 4$).

Total Root Intensity of E. pellita **from seed and stem cutting**

In comparison, the total root intensity (TRI) of *E. pellita* stem cutting was significantly higher ($P < 0.05$) compared to seed cutting for all measurement dates. Despite the large variations observed in stem cutting treatment, the TRI remained to be nearly double that of seed cutting, especially at 6 and 8 WAT.

Figure 3: Total Root Intensity (Intersections m^{-1} gridline) of *E. pellita* seedlings from seeds and stem cutting plant materials at three selected dates of root measurement (4, 6 and 8 weeks after tranplanting, WAT). The mean values tested using Independent Samples T-Test and statistically the mean values were different, ($P < 0.05$). Bars represent standard deviations of the mean, $n = 36$ (4 WAT), $n = 24$ (6 WAT) and $n = 12$ (8 WAT).

Root Intensity of E. pellita **at different nitrogen concentrations**

Figure 4 shows a comparison of root intensity (RI) of *E. pellita* from seed cutting (Fig. 4a) and stem cutting (Fig. 4b), at various measurement dates and nitrogen concentrations. According to the findings, there was no significant difference ($P > 0.05$) for treatments and types of plant material for each measurement date. However, despite the large variations of RI, *E. pellita* stem cutting was clearly higher, and increased with the measurement dates, as compared to seed cutting (Fig. 4b).

Figure 4: Root Intensity (intersections m^{-1} gridline) of *E. pellita* seedlings from seeds (a) and stem cutting (b) plant materials at different nitrogen concentrations (0, 50, 200 kg N ha^{-1}) at three selected dates of root measurement (4, 6 and 8 weeks after tranplanting, WAT). The mean values were tested using ANOVA followed by Tukey HSD's post hoc Test. The mean values were not significant different between the different N concentrations for each dates ($P > 0.05$). Bars represent standard deviations of the mean, $n = 12$ (4 WAT), $n = 8$ (6 WAT) and $n = 4$ (8 WAT).

Root Length Density of E. pellita

Figure 5 showed the root length density (RLD) of *E. pellita*, both from seed and stem cutting, taken at three independent harvest times. Based on the results, stem cutting of *E. pellita* was significantly higher at 200 kg N^{-1} than at the control and at 50 kg N^{-1} (Fig. 5a). At 6 WAT, all RLDs of stem cutting of *E. pellita* were significantly higher ($P < 0.05$) compared to seed cutting, for all N concentrations (Fig. 5b). However, the RLD of stem cutting of *E. pellita* was significantly higher compared to seed cutting under the control and high N concentrations, on the final measurement date (Fig. 5c). It was also found that the RLD of *E. pellita* for both seed and stem cutting under fertilizer treatment decreased with the measurement dates.

Figure 5: Root Length Density (cm cm^{-3}) of *E. pellita* seedlings from seeds and stem cutting plant material at different nitrogen concentrations (0, 50, 200 kg N ha^{-1}) at three selected dates of root measurement; 4 weeks after transplanting, WAT (a), 6 WAT (b), and 8 WAT (c). The mean values were tested using Independent Samples T-Test. All mean values were significant different *($P < 0.05$). Error bars denote standard deviations of the mean, ($n = 4$).

Specific Root Length (SRL) of E. pellita

The specific root length (SRL) of *E. pellita* was significantly higher ($P < 0.05$) for all treatments and measurement dates (Fig. 6). At 4 WAT, SRL of stem cutting was significantly higher ($P < 0.05$) compared to seed cutting, almost by three-fold, especially at high N concentrations. Similar findings were also found at 6 WAT, which was almost 50% higher ($P < 0.05$) than seed cutting for all fertilizer treatments (Fig. 6b). However, at 8 WAT, SRL was found to be significantly higher for approximately 50% of stem cutting, as compared to seed cutting, at zero and 50 kg n ha^{-1}. No difference in SRL was found between stem cutting and seed cutting of *E. pellita* at 200 kg N ha^{-1} (Fig. 6c).

Figure 6: Specific Root Length (cm g^{-1}) of *E. pellita* seedlings from seeds and stem cutting plant materials at different nitrogen concentrations (0, 50, 200 kg N ha^{-1}) at three selected dates of root measurement; 4 weeks after transplanting, WAT (a), 6 WAT (b), and 8 WAT (c). The mean values were tested using Independent Samples T-Test. All mean values were significant different *($P < 0.05$). Error bars denote standard deviations of the mean, ($n = 4$).

Discussion

The findings demonstrate that shoot biomass of stem cutting of *E. pellita* seedlings was greater compared to seedlings from seed cutting. The shoot biomass is connected with root distribution in the soil, especially the fine roots from stem cuttings. The larger the fine root density, the more water and nutrients are taken up, expressed by higher shoot biomass. This is confirmed by Rostamza et al. (2013), who reported that greater root length in millet is connected to an increased shoot biomass. However, in this present study, seedlings from stem cutting were significantly higher with an increase in nitrogen levels, as compared to seedlings from seed cutting (see Fig. 1b). However, seedlings from seed cutting were not affected under different nitrogen concentrations. At the final harvest, there was no significant difference between stem cutting and seed cutting for all fertilizer treatments (Fig. 1c). Therefore, biomass is influenced by species, and specific silviculture such as irrigation (Toky et al. 2011), fertilization and water availability (Ares et al. 2009).

In root biomass, most of the mean values did not differ for seed and stem cutting, for all treatments, except for 50 N kg ha^{-1} at 6 WAT, and for zero nitrogen at 8 WAT (Fig. 2). However, Coleman et al. (2004) reported that root biomass tends to increase with fertilizer rate, but the proportion of root biomass tends to decrease with more fertilizer application. This argument is associated with the current findings, especially for higher nitrogen concentrations, showing that there is no increase with fertilization rate. Another explanation of the results is that seedlings from seed cutting were higher compared to stem cutting, and had no significant difference, because seed cutting produces tap root and high root mass, while stem cutting produces fibrous and fine roots.

Without any statistical significance, the total RI for both propagation types of *E. pellita* seedlings was not different, although there was a higher distribution under stem cutting (Fig. 3). Looking at RI, at different nitrogen concentrations for both seed and stem cutting, the distribution of RI was higher in stem cutting compared to seed cutting, although there was no significant difference. This shows that there are more fine roots in stem cutting than in seed cutting, which and increased with the measurement date (Fig. 4). The findings of this study are in agreement with prior work that has proven that more root distribution, especially for fine roots, is closely associated to soil water and nutrients (Zhang et al. 1995; Wang 1990; Zhao et al. 1990). This explains the presence of more fine roots after stem cutting on the soil.

In RLD, stem cutting was also significantly higher at high nitrogen concentrations, compared to seed cutting. In the subsequent measurement date, all of the nitrogen levels under stem cutting increased significantly compared to seed cutting; this also applies on the final measurement date, except under 50 kg N ha^{-1} (Fig. 5). However, the values decreased with the measurement dates. The RLD of seedlings from seed cutting were neither affected by different nitrogen levels, nor by measurement dates. Similarly, in SRL, stem cutting was also significantly higher compared to seed cutting, as found in RLD.

Despite stem cutting being higher compared to seed cutting, especially in shoot biomass, in SRL and RLD, these growth parameters were not affected by different nitrogen levels. Especially at high nitrogen concentrations (Fig. 6c), the SRL did not differ between the propagation types. Previous work reported various responses of fertilizer rates against *Eucalyptus* in Brazil (Goncalves et al. 2004; Goncalves et al. 2008; Stape et al. 2010), in Australia (Smethurst et al. 2004; Mendham et al. 2008) and in South Africa (du Toit et al. 2010). Nevertheless, fertilizer responses varied, depending on the species and sites considered (Halomoan et al. 2015). *Eucalyptus* in Brazil and South Africa responded to fertilization when water was available (Stape et al. 2010; du Toit et al. 2010). Stape et al. (2010) reported that the application of very high rates and excessive nitrogen levels in Brazil did not show any significant effects on *Eucalyptus* productivity (Halomoan et al. 2015). Fertilizer rates from 50 to 100 kg N ha^{-1} increased biomass, but then biomass decreased at a rate of 200 kg N ha^{-1} (Halomoan et al. 2015); this was also reported in the present study. Graciano et al. (2006) reported that P applications affected *E. grandis* biomass more than N applications in Argentina. However, we cannot validate this argument, since this present study tested *E. pellita* seedlings at a nursery scale.

In this case, a rate of 50 kg N ha^{-1} could be cheaper and more efficient to absorb by *E. pellita*, as opposed to 200 kg N ha^{-1}. Chen et al. (2011) reported that moderate nitrogen fertilizer increased the root intensity in soil layers. As explained above, the root distribution from propagation seeds is less compared to stem cutting. With the short period for the experiment under a small pot, we cannot observe the difference between the nitrogen concentrations. This needs to be done in a larger field.

In the comparison between seed and stem cutting, as the above findings, propagation by stem cutting of *E. pellita* was proved to be viable and productive in terms of root performance at the nursery stage. Although there are works in the related literature that have proved that seed cutting is still the better propagation method (Kiragu et al. 2015), producing plant material using stem cutting is not only more efficient and faster, but would also be able to reduce the production costs and time spent for upkeep and maintenance in the nursery. Partelli et al. (2014) also supported the fact that the cuttingpropagated method for coffee is more productive than the seedpropagated method. Furthermore, Naidu and Jones (2015) also suggested a superior initial survival and growth of *E. dunnii* minicuttings compared to seedlings based on early indications. This finding has also proven that secondary branches as semi-hard wood cuttings could be the most effective propagation material of *Jatropha curcas* (Santoso and Parwata, 2014).

Notwithstanding this, rooting ability of cuttings from woody or perennial plants declined with an increase in the age of the mother plants (Santoso and Parwata 2014). Root ability of cutting formation becomes more difficult with a farther position from the apical shoot (Hartmann et al. 2002: Wilson 1993), due to differences in the type and number of carbohydrates and other stored materials (Hartmann et al. 2002; Leakey 1999). Therefore, root system characteristics are known to differ according to species, genotype, plant age, physiological status of mother plant (Henning 2003), season, climate, plant density, root diameter, biotic stresses, and soil texture and structure (Lynch 1995). Also, the growth rate of stem cutting depends on age variation, position in stem, and diameter of stem (Kraiem et al. 2010).

The present study therefore provides more information and understanding on *E. pellita* for forest plantation companies in producing plant materials using stem cutting in a cost-effective and efficient manner. Further research is required on the root aspect, especially in real field conditions, as the soil is more heterogenous and exhibits different environmental conditions. Such findings will help these companies take agronomic measures and a silvicultural approach in the future.

Conclusions

To conclude, *E. pellita* seedlings from stem cutting were greater in terms of root distribution compared to propagation by seed cutting, at the nursery stage. In addition, aboveground biomass of stem cutting was also higher in *E. pellita* seedlings than of seed cutting. The 50 kg N ha^{-1} was the optimal nitrogen concentration to be applied to the *E. pellita* seedlings. This is because excessive fertilizer application not only increases fertilizer costs, but may also not necessarily result in an increased volume yield or shoot biomass. Moreover, it is harmful to the soil. Research on the root distribution of these two types of propagation in real field soil merits further investigation, as different environmental factors may affect the growth performance of *E. pellita*. Thus, this would help the forest plantation companies in planning appropriate agronomic management in the future.

Abbreviations

N
Nitrogen; RI:Root intensity; TRI:Total root intensity; RB:Root biomass; RLD:Root length density; SRL:specific root length; WAT:Weeks after transplanting.

Declarations

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Authors' contributions

AH designed the study, supervised data collection and contributed to and edited manuscripts. PB and KRK collected literatures, prepared field experiments, data collection, laboratory analysis, and prepared all figures. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests

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Figures

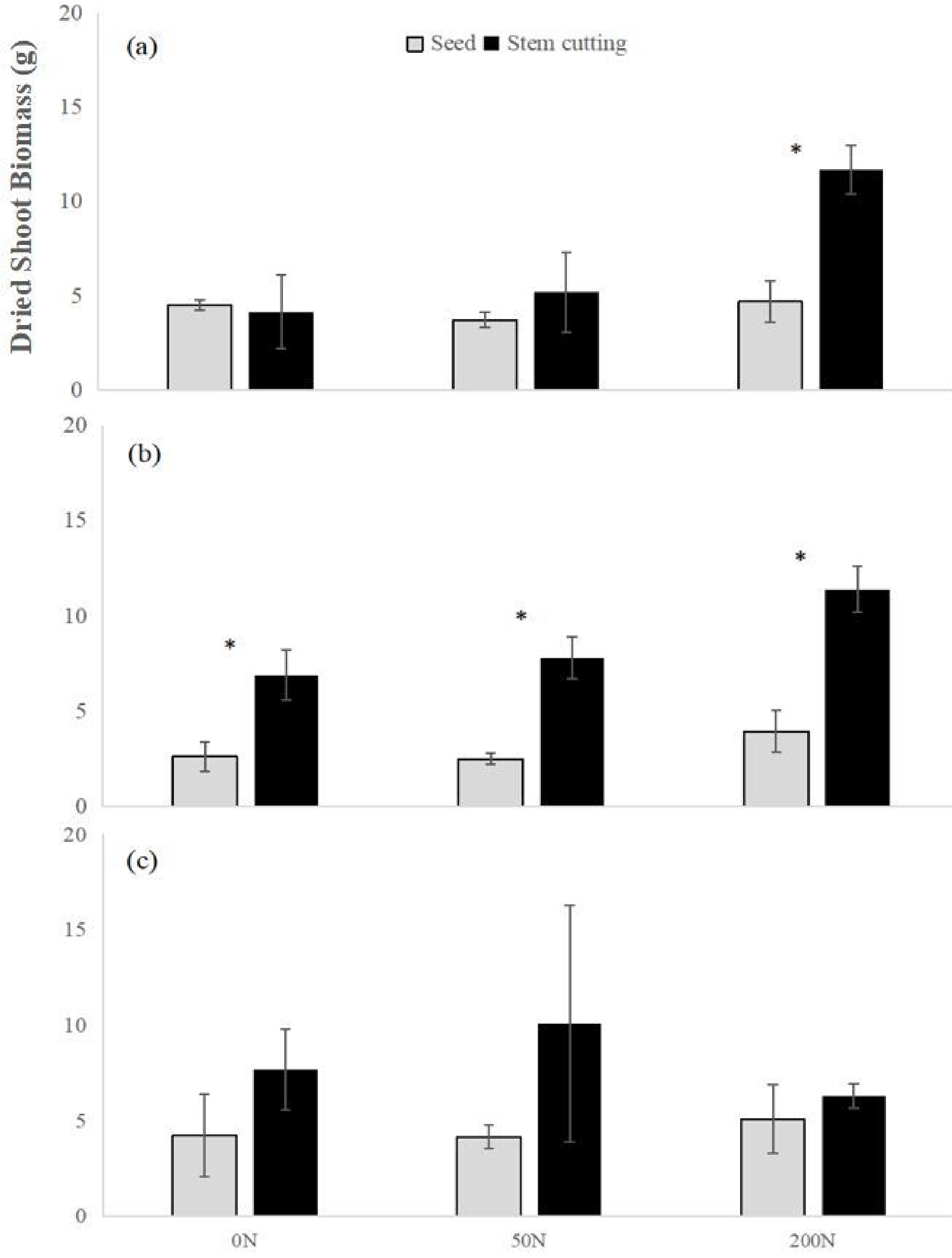

Figure 1

Dried Shoot Biomass (g) of E. pellita seedlings from seeds and stem cutting plant materials at different nitrogen concentrations (0, 50, 200 kg N ha-1) at three selected dates of root measurement; 4 weeks after transplanting, WAT (a), 6 WAT (b), and 8 WAT (c). The mean values were tested using Independent Samples T-Test. All mean values were significant different *(P< 0.05). Error bars denote standard deviations of the mean, (n= 4).

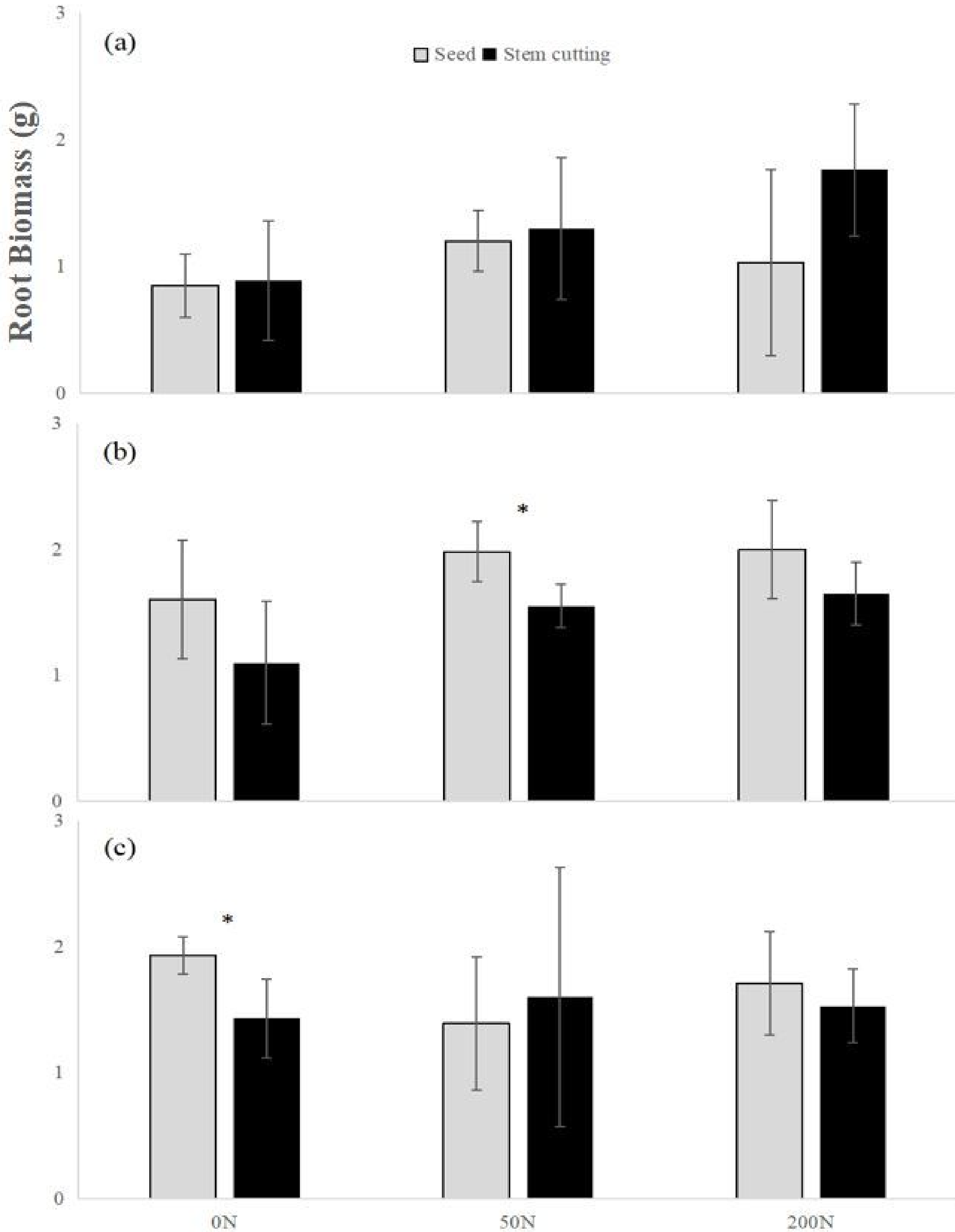

Figure 2

Root Biomass (g) of E. pellita seedlings from seeds and stem cutting plant materials at different nitrogen concentrations (0, 50, 200 kg N ha-1) at three selected dates of root measurement; 4 weeks after transplanting, WAT (a), 6 WAT (b), and 8 WAT (c). The mean values were tested using Independent Samples T-Test. All mean values were significant different *(P< 0.05). Error bars denote standard deviations of the mean, (n= 4).

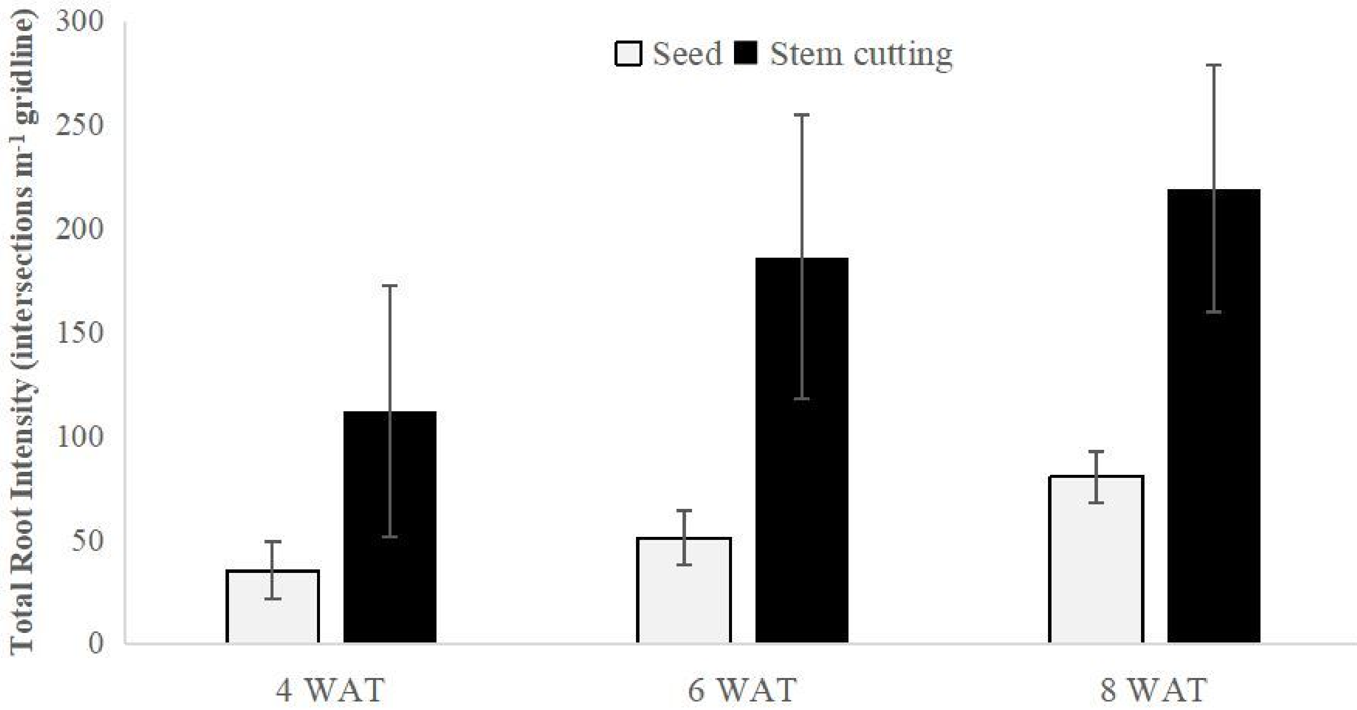

Figure 3

Total Root Intensity (Intersections m-1 gridline) of E. pellita seedlings from seeds and stem cutting plant materials at three selected dates of root measurement (4, 6 and 8 weeks after tranplanting, WAT). The mean values tested using Independent Samples T-Test and statistically the mean values were different, (P<0.05). Bars represent standard deviations of the mean, n=36 (4 WAT), n=24 (6 WAT) and n= 12 (8 WAT).

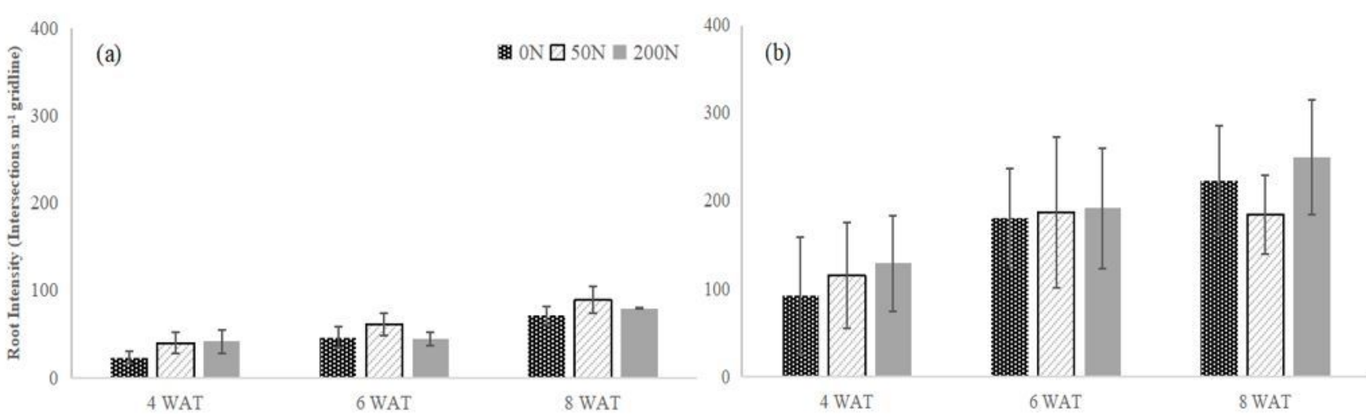

Figure 4

Root Intensity (intersections m-1 gridline) of E. pellita seedlings from seeds (a) and stem cutting (b) plant materials at different nitrogen concentrations (0, 50, 200 kg N ha-1) at three selected dates of root measurement (4, 6 and 8 weeks after tranplanting, WAT). The mean values were tested using ANOVA followed by Tukey HSD's post hoc Test. The mean values were not significant different between the different N concentrations for each dates (P>0.05). Bars represent standard deviations of the mean, n=12 (4 WAT), n=8 (6 WAT) and n= 4 (8 WAT).

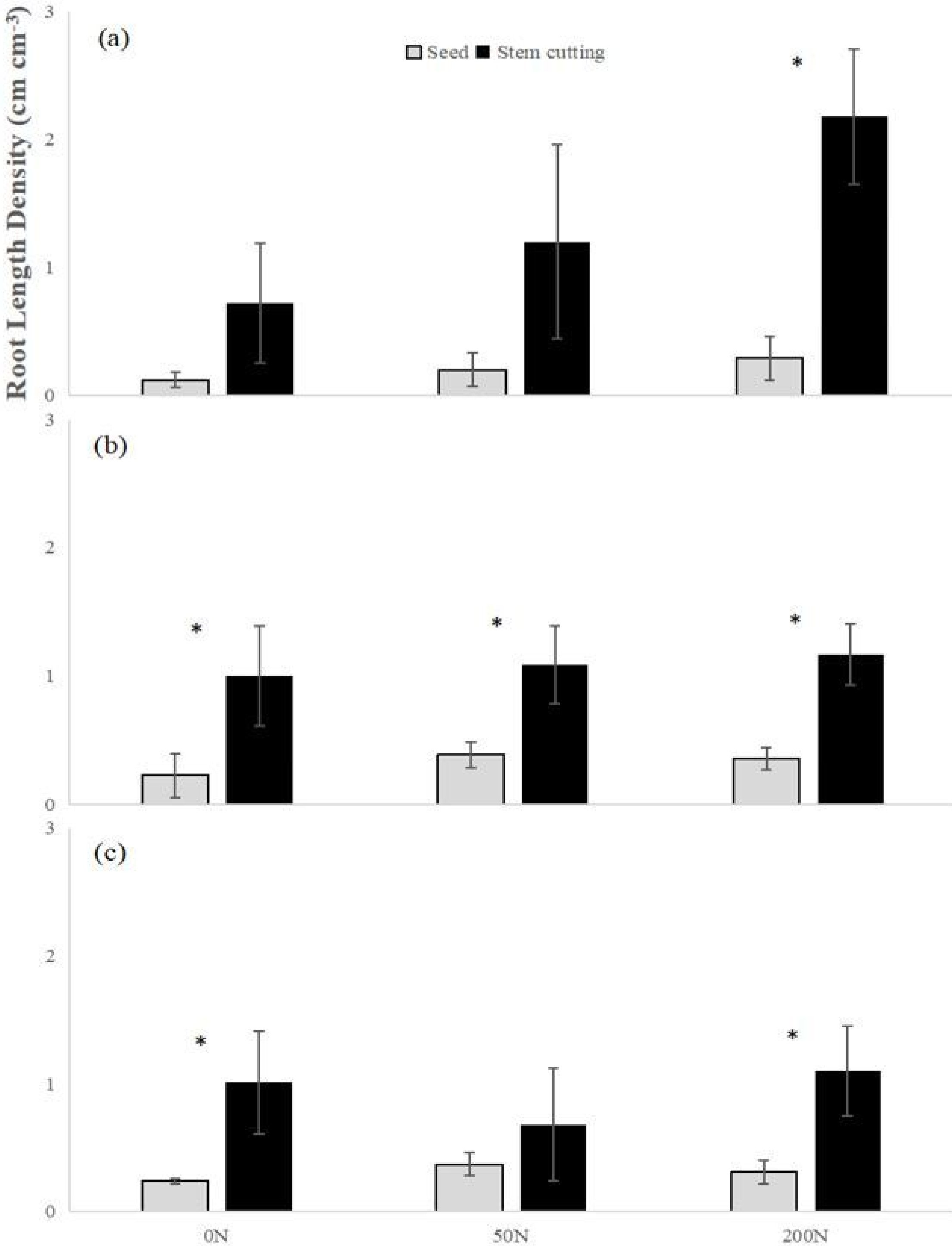

Figure 5

Root Length Density (cm cm-3) of E. pellita seedlings from seeds and stem cutting plant material at different nitrogen concentrations (0, 50, 200 kg N ha-1) at three selected dates of root measurement; 4 weeks after transplanting, WAT (a), 6 WAT (b), and 8 WAT (c). The mean values were tested using Independent Samples T-Test. All mean values were significant different *(P< 0.05). Error bars denote standard deviations of the mean, (n= 4).

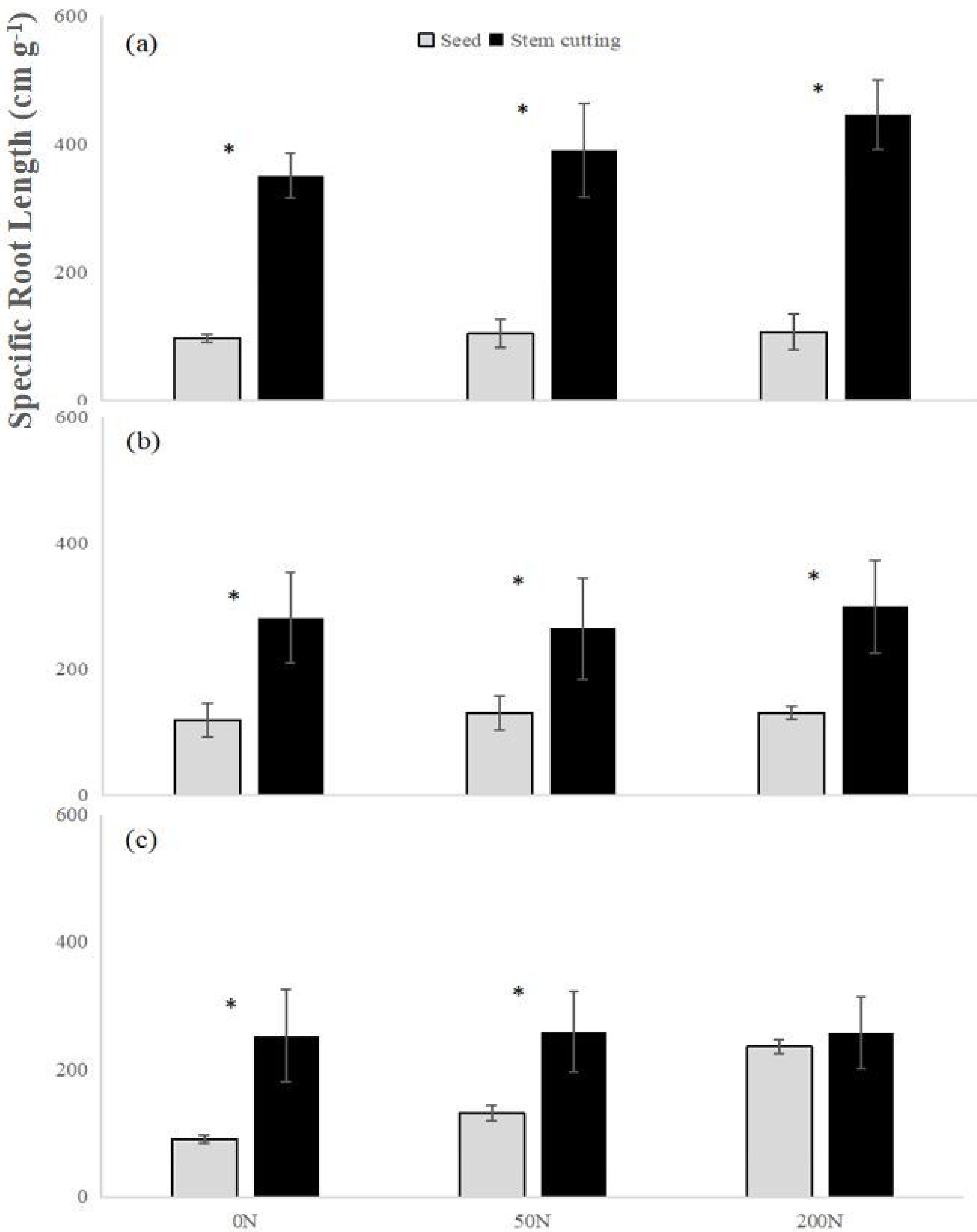

Figure 6

Specific Root Length (cm g-1) of E. pellita seedlings from seeds and stem cutting plant materials at different nitrogen concentrations (0, 50, 200 kg N ha-1) at three selected dates of root measurement; 4 weeks after transplanting, WAT (a), 6 WAT (b), and 8 WAT (c). The mean values were tested using Independent Samples T-Test. All mean values were significant different *($P<0.05$). Error bars denote standard deviations of the mean, (n= 4).