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Palm Peat in Mixed Culture Media Improves Root System Architecture (RSA) in *Cucumis sativus* Seedlings

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ABSTRACT

Different culture media have various materials that impact root system architecture (RSA) and root system quality in plants. Finding the most compatible RSA of cucumber seedlings among the eight mixed culture media is critical to plant productivity. In this experiment, cucumber seeds were sown in eight mixed culture media, including a volume ratio of 30:10:60 of 1- perlite-vermicompost-coco peat as the control group, 2 - peat moss-vermicompost-palm peat, 3- cocopeat-vermicompost-palm peat, 4- perlite-vermicompost-palm peat, and 30:70 ratios of 5- peat moss- palm peat, 6- cocopeat -palm peat, 7- perlite -palm peat and 8- vermicompost-palm peat under greenhouse conditions. RSA of the seedlings was evaluated by GiA-Roots software via imaging at the four-leaf stage. The results showed that the minimum network length, network area, and network perimeter (821.6 cm, 7598.3 cm2, and 2102 .3 cm, respectively) in the seedlings occurred on perlite-vermicompost-c oco peat (control) which lacked palm peat. The minimum network volume, number of connected components, and network depth were observed in the control. However, the maximum network length, network volume, number of connected components, network width, network area, and network perimeter (88862.8 cm, 2076390.9 cm3, 23. 2, 4567.4 cm, 332356.1 cm2, and 132068.9 cm, respectively) occurred in seedlings on peat moss-vermicompost-palm peat (30:10:60). Thus, a culture medium containing 30% peat moss, 60% palm peat, and 10% vermicompost improved RSA in cucumber seedlings. Palm peat was recommended as a sustainable resource to comprise culture media for cucumber seedlings.

Introduction

Roots are essential for plant life and many processes, including nutrient and water acquisition, anchoring, and mechanical support (Hochholdinger et al., 2004). Owing to heterogeneous environments as far as water and nutrient availabilities are concerned, soils often pose a direct influence on root systems to optimize their distribution for the healthy growth and development of plants, and accordingly, roots adopt a specific architecture (Sinha et al., 2018). Roots act as an interface between the canopy and the subterrestrial root zone, reacting to biotic and abiotic factors and signaling the aboveground organs through local and systemic signaling systems. Plants can dramatically alter their RSA to optimize growth in diverse environmental and soil nutrient conditions. Therefore, it is

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unsurprising that they play an essential role in yield and overall plant productivity (Herder et al., 2010). Root systems in most dicotyledonous plants consist of a single primary root derived from the embryonic radicle that generates a branched network of lateral roots that continuously develops during plant growth (Sinha et al., 2018). Root characteristics are influenced by the physical and biochemical composition of the root medium (Souri and Hatamian, 2019; Pourranjbari Saghayesh and Souri, 2018). Motte and Beeckman (2019) observed that boosting root branching ability was beneficial to searching the bed for water and nutrients. Soil comes into direct contact with the root system, and it is undeniable that one or more soil physicochemical parameters influence the formation and development of plant root systems (Correa et al., 2019; Wu and Qi, 2021).

Root system architecture (RSA) and morphology define root system qualities. Thus, it is critical to determine their favorable traits for crop yield increase. Root morphology involves studying the characteristics of a single root axis as an organ, such as root hairs, root diameter, and cortical senescence. Root system architecture refers to the entire root system or a substantial part of the root system and can be defined as topological or geometric metrics of root form (Bucksch et al., 2014; Nguyen and Stangoulis, 2019). Current efforts to research the structure of crop root systems have resulted in the development of several root phenotyping platforms capable of elucidating RSA under diverse circumstances, including laboratory, greenhouse, and field environments (Paez-Garcia et al., 2015).

The root system architecture also refers to the complex physical connectivity belowground of plant parts (i.e., first-order, second-order, i-th-order, and primary roots) that connects the root tips of different root branching and serves as a networked channel for the circulation of plant matter, energy, and information (Karlova et al., 2021). The RSA is necessarily modified by many stress variables in the environment, resulting in significant phenotypic plasticity within and across plant species (Correa et al., 2019). RSA in plants would often differentiate in response to a single stimulus. Nonetheless, the RSA's phenotypic plasticity in different culture media has yet to be empirically clarified, owing to antagonistic, synergistic, or complementary effects among interacting environmental factors. Correa et al. (2019) stated that soil compaction was strongly and positively connected with the topological index; that is, the dichotomous branching pattern of RSA was a mechanism for plants to adapt to increased soil compaction strength. Increasing the length of the root system's links is an important approach for plants to increase the distribution range of their roots in the soil and maximize resource absorption (Yildirim et al., 2018).

The branching rate is a particularly sensitive RSA parameter, reflecting the adaptability of root systems to varied site circumstances (Duque and Villordon, 2019).

Every year, on average, 15 dried leaves are harvested from 105 million date palm trees worldwide, resulting in 1.6 million tons of date palm leaf material (El Janati et al., 2022). According to Benabderrahim et al. (2018), palm peat compost improved mineral content in alfalfa and soil properties, hence soil fertility. Composting is recognized as an attractive agricultural practice as well as an economical and sustainable approach to organic waste product management due to its simplicity and adaptability to a wide range of farming systems (Robin et al., 2018).

Moreover, it has many environmentally friendly effects (Najarian and Souri, 2020; Ebrahimi et al., 2021). Water, media components, and air supply are the three basics of a culture medium in horticultural plants, including cucumbers, and help to establish optimal growth conditions for the roots as well as physical support for plants (Farrokhi et al., 2021).

Cucumber (*Cucumis sativus* L.) is one of the most popular vegetables, ranking third in production and second in yield, accounting for 7.5% of the total vegetable production (FAOSTAT, 2018). The changing of RSA to different culture media represents the strategy of plant adaptation (He et al., 2022). The components of culture media influence the changes in cucumber RSA. Cucumber would take a series of strategies, such as increasing network length, network volume, the number of connected components, network depth, network width, network area, average root diameter, and network perimeter, to adapt to specific culture media.

This research aims to find the most compatible RSA of cucumber seedlings among eight mixed culture media and optimal compositions of palm peat as a natural and local bioresource in culture media.

Materials and Methods

Experimental site and plant materials

This experiment was carried out in the research greenhouse at the University of Jiroft, Iran, in 2021 using a completely randomized design with eight treatments and three replications. The average day/night temperature was 30/25±2 °C, the relative humidity was 85±5%, and the light intensity was around 10,000 Lux. The culture media included a 30:10:60 (V:V:V) mixture of 1- perlite-vermicompost-coco peat (p-vc-co), without palm peat (the control group); 2- peat moss-vermicompost-palm peat (pm-vc-pp); 3- coco peat-vermicompost-palm peat (co-vc-pp); 4- perlite- vermicompost- palm peat (p-vc-pp); and a 30:70 (V:V) mixture of 5- peat moss-palm peat (pm-pp); 6- coco peat-palm peat (co-pp); 7- perlite-palm peat (p-pp) and 8- vermicompost-palm peat (vc-pp). The chemical parameters of materials in the culture media for cucumber cultivation appear in Table 1.

Table 1. Chemical properties of the materials used in the culture media for cucumber cultivation.

Culture media	EC (ms m^{-1})	pH	N (%)	K (%)	P (%)	Mn (ppm)	Cu (ppm)	Fe (ppm)	Zn (ppm)
Peat moss	0.754	6.73	0.11	1.26	0.86	0.20	0.16	0.30	0.066
Vermicompost	2.66	6.58	0.16	1.33	0.44	0.36	0.13	0.66	0.074
Palm peat	1.73	6.78	0.12	0.86	0.39	0.22	0.18	0.33	0.029
Coco peat	1.10	6.92	0.10	0.44	0.26	0.16	0.11	0.31	0.020
Perlite	0.155	7.70	0.06	1.11	0.06	0.10	0.04	0.03	0.008

Cucumber seeds were planted in pots (10 cm in height and 8 cm in diameter). The pots were filled with the culture media. During seedling growth, all treatments had equal irrigation plans, temperatures, humidity, light, and paste management. During the growth period, to avoid damage caused by white flies, the plants were placed inside the chambers protected by insect nets and were supplied the nutrient solution weekly (half-strength Hoagland). The seedlings were gently removed from the pots after 30 days after starting the experiment when they had three fully-expanded leaves. After washing the roots with water, the plant shoots were separated from the roots.

Evaluation of root system architecture

At the end of the experiment, the roots were washed and photographed. The camera was fixed at 40 cm above the roots to conduct imaging on A4 paper (29.7×21 cm). The images were numbered and analyzed by the GiA Roots software to convert the images into data. The RSA characteristics enabled assessments of root growth rate. The measurable characteristics included network length, network length distribution, network volume, specific root length, maximum root count, media number of roots, network bushiness, number of connected components, network depth, network width, network width-to-depth ratio, network surface area, network convex area, network solidity, average root width diameter, network area, network perimeter, ellipse aspects ratio, and minor ellipse axis, which were outputs of the GiA Roots software.

Statistical analysis

SAS software version 9.4 was used for analyzing variance, and the data were compared using Duncan’s multiple range test.

Results

According to ANOVA (Table 2), the culture medium treatment significantly ($P \leq 0.01$) affected the eight parameters of root system architecture (RSA) in cucumber seedlings, including network length, network volume, network area, network perimeter, network width, number of connected components, average root diameter, and network depth. However, the culture medium did not significantly affect ($P \leq 0.05$) the other parameters related to the RSA in cucumber seedlings, including network length distribution, maximum root count, media root count, network bushiness, network width-to-depth ratio, network convex area, network solidity, ellipse aspects ratio, and minor ellipse axis (data not shown).

According to the comparison of mean values (Fig. 1), minimum network length, network area, and network perimeter were observed in seedlings grown on culture medium No. 1 (perlite-vermicompost-coco peat), which were 822 cm, 7598 cm2, and 2102 cm, respectively. Furthermore, network volume, number of connected components, and network depth in seedlings grown on culture medium No. 1 were in the lower statistical group, compared to culture media No. 2, 3, 4, and 7. Seedlings grown on culture medium No. 2 had the maximum network length, network volume, network area, network perimeter (Fig. 1), network width, and number of connected components (Table 3), which were

88863 cm, 2076391 cm3, 332356 cm2, 132069 cm, 4567 cm, and 23, respectively. The maximum network depth occurred in seedlings grown on culture medium No. 4. The minimum average root diameter occurred in seedlings grown on culture medium No. 5.

Table 2. Analysis of variance (ANOVA) for the effect of culture medium on root architecture of cucumber seedlings.

Variation sources	DF	Network length	Network volume	Network area	Network perimeter
Culture medium	7	4112282820**	2.00**	51646302456**	10209899637**
Block	2	57267726^{ns}	10534780639^{ns}	306504529.41^{ns}	235836601^{ns}
Error	14	105630373	11419173516	407425113.49	118912315
C.V.	-	22.29	13.69	16.22	19.12

Variation sources	DF	Network width	Number of connected components	Average root diameter	Network depth
Culture medium	7	7535569.59**	119.23**	10.44**	5648424.58**
Block	2	162073.38^{ns}	3.200^{ns}	0.49^{ns}	383233.11^{ns}
Error	14	156784.86	6.63	0.52	97927.96
C.V.	-	13.39	21.94	13.58	16.36

^{ns} and ** indicate non-significance and significance (P≤0.01), respectively, based on Duncan's multiple range test.

Network length, network volume, network area, and the number of connected components of seedlings grown on culture medium No. 2 increased 108, 40, 44, and 4 times compared to seedlings grown on culture medium No. 1 (Fig. 1 and Table 3). According to Pearson's correlation coefficient (Table 4), a significantly negative correlation occurred among the average root diameter and network volume (-0.506*), number of connected components (-0.522**), network depth (-0.549**), network width (-0.374*), network area (-0.520**), and network perimeter (-0.543**). However, there was a positive and significant correlation among network length and network volume (0.829**), number of connected components (0.701**), network depth (0.781**), network width (0.795**), network area (0.834**), and network perimeter (0.844**).

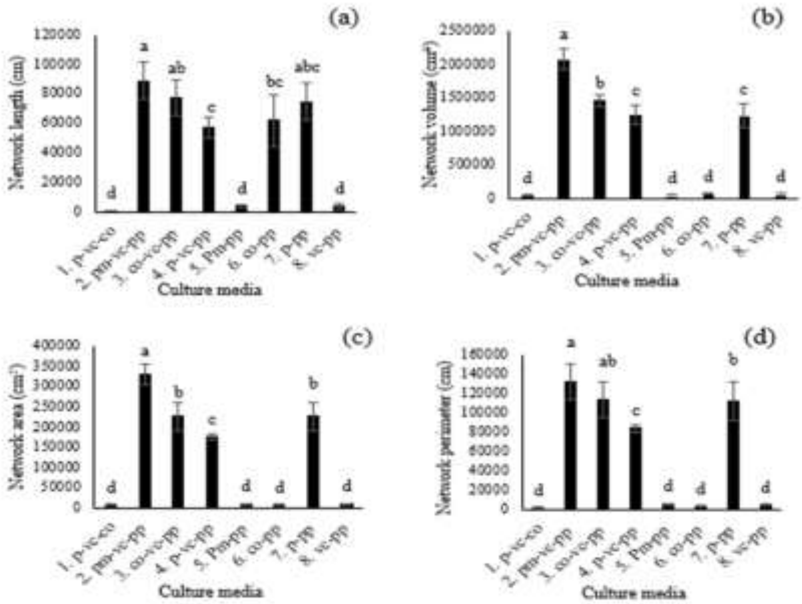

Fig. 1. Comparison of mean values regarding the effect of culture media on (a) network length, (b) network volume, (c) network area, and (d) network perimeter of cucumber seedlings. Different letters in a column indicate significant differences at P≤0.05 among the culture media, based on Duncan's multiple range test. co: coco peat, p: perlite, pm: peat moss, pp: palm peat and vc: vermicompost.

Table 3. Comparison of mean values regarding the effect of culture media on root architecture of the cucumber seedlings.

No.	Culture media	Network width (cm)	Number of connected components	Average root diameter (cm)	Network depth (cm)
1	p-vc-co (30-10-60)	2210.89^{b}	5.56^{c}	8.42^{a}	680.68^{b}
2	pm-vc-pp (30-10-60)	4567.40^{a}	23.15^{a}	4.45^{b}	3016.33^{a}
3	co-vc-pp (30-10-60)	4449.63^{a}	15.93^{b}	3.95^{b}	3131.11^{a}
4	p-vc-pp (30-10-60)	4508.67^{a}	14.67^{b}	4.33^{b}	3471.00^{a}
5	Pm-pp (30-70)	580.47^{c}	4.64^{c}	3.87^{b}	606.72^{b}
6	co-pp (30-70)	1772.30^{b}	6.03^{c}	8.19^{a}	624.96^{b}
7	p-pp (30-70)	3939.74^{a}	12.56^{b}	4.47^{b}	3142.56^{a}
8	vc-pp (30-70)	1622.79^{b}	11.40^{b}	4.96^{b}	626.41^{b}

co: coco peat, p: perlite; pm: peat moss, pp: palm peat and vc: vermicompost. Different letters in a column indicate significant differences at P≤0.05 among the culture media, based on Duncan's multiple range test.

	NWLN	NWVL	NOCC	NWDP	NWWI	NWAR	AVRD
NWVL	0.829**						
NOCC	0.701**	0.882**					
NWDP	0.781**	0.902**	0.756**				
NWWI	0.795**	0.901**	0.805**	0.940**			
NWAR	0.834**	0.986**	0.873**	0.895**	0.892**		
AVRD	-0.268^{ns}	-0.506*	-0.522**	-0.549**	-0.374*	-0.520**	
NWPM	0.844**	0.831**	0.935**	0.905**	0.905**	0.983**	-0.543**

^{ns}, *, and ** indicate non-significance and significance at P≤0.05 and P≤0.01, respectively, based on Duncan's multiple range test. Network length (NWLN), network volume (NWVL), number of connected components (NOCC), network depth (NWDP), network width (NWWI), network area (NWAR), average root diameter (AVRD), network perimeter (NWPM).

Discussion

A suitable culture medium can provide sufficient anchorage for plants, serving as a store of water and nutrients. It promotes oxygen passage to the roots and enhances gas exchange between roots and the atmosphere (Barrett et al., 2016; Mohammadi Ghehsareh, 2013). Organic wastes have high apparent density, porosity, and water-holding capacity (Mohammadi Ghehsareh, 2013). Roots are among the first tissues that detect environmental signals and respond physiologically, influencing the development of shoots. Essential morphological characteristics that may help to evaluate root growth quality are main root length, lateral root number, root fresh mass, and root uniformity (Lu et al., 2019). According to Lu et al. (2019), lateral roots and the overall root system affect the specific surface, activity, and root volume. Thus, increasing the number of lateral roots can improve the total surface and volume of the root system, water absorption and nutrients by the roots, and root strength activity.

Deeper roots can absorb water and nutrients from the soil. Lateral roots are the most essential part of the root system, influencing the overall surface area and root system activity (Lu et al., 2019). Meier et al. (2020) demonstrated that accessible nitrogen stimulated lateral root growth and development, resulting in a more organized root branching system. As shown in Table 1, the chemical properties of palm peat and vermicompost culture media have more nitrogen than other culture media. Seedlings grown on culture medium No. 1 (perlite-vermicompost-coco peat) had the thickest roots and the lowest growth of RSA. However, root growth improved in seedlings with minimal average root diameter, resulting from more absorption of nutrients by narrower roots.

Palm peat has several properties that improve growth media quality, especially when mixed with other culture media. Therefore, the seedlings grown on culture medium No. 2 (peat moss-vermicompost-palm peat) had the best RSA than in other types of culture media, showing optimal values of network length, network volume, connected components, network width, network area, network perimeter network depth, and average root diameter. By providing nutrients (Table 1), vermicompost probably increases the activity of microorganisms, humic acid, and growth regulators. In culture media No. 2, peat moss (30%), vermicompost (10%), and palm peat (60%) provided a favorable culture medium for greenhouse cucumber roots. According to Table 1, potassium deficiency in cocopeat formed 60% of culture medium No. 1 and decreased RSA in seedlings.

This finding agrees with Mohammadi Ghahsareh et al. (2013) and Soltani and Naderi (2015) that the physicochemical properties of the culture medium affect the growth of seedlings. Kawa et al. (2016) found that an inorganic phosphate shortage reduced the root length while increasing the number of lateral roots. A deep root system is advantageous for the uptake of nitrogen and water in deep layers, whereas a shallow system with many adventitious roots is advantageous for the uptake of relatively immobile nutrients such as phosphorus (Lynch, 2011; He et al., 2017). Therefore, phosphorous deficiency in perlite (Table 1) in culture medium No. 1 can be the reason for shorter root length in seedlings. Indeed, competition for carbon between roots and nodules can restrict plant growth and development while affecting functionality (Voisin et al., 2003; Maslard et al., 2021). According to Luo et al. (2020), high temperatures can enhance branching intensity while decreasing average root diameter. According to Shao et al. (2018), increased competitive pressure can reduce nodal root count, lateral root density, and average lateral root length.

Conclusion

Changing the root system architecture (RSA) in different culture media shows how plants can adapt to a given culture medium. Our research results emphasize that the cucumber root system can respond variedly to a newly applied culture medium. It involved increasing the network length, network volume, number of connected components, network depth, network width, network area, average root diameter, and network perimeter. However, it decreased the average root diameter in culture media containing palm peat compared to culture medium No. 1, the control (which lacked palm peat). Among the eight culture media, the mixture of peat moss-palm peat-vermicompost (30:10:60), with water and nutrients, could better develop RSA in cucumber seedlings. Deficiency in nutrients, especially potassium in cocopeat and phosphorus in perlite in culture medium No. 1 (control), may primarily explain the significant difference and decrease in the RSA compared to other culture media. Our results showed how RSA has plasticity in response to heterogeneity in the culture medium, which helps better understand solutions to culture medium changes. Therefore, in addition to sustainable production, it is possible to create employment and reduce expenses by using local biological resources such as composted palm waste, known as palm peat in agriculture, for greenhouse seedling production.

Conflict of Interest

The authors indicate no conflict of interest for this work.

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