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Influence of Different Nutrient Concentrations and Population Densities on the Root System of *Arabidopsis thaliana*

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Abstract: This study examined the modifications made to the root system architecture of *Arabidopsis thaliana* when subjected to an increase in population (biotic) and a decrease in nutrient concentration (abiotic). Population density was found to have significant effect on number of root hairs ($p<0.05$) while nutrient concentration was found to have significant effect on length of primary root and number of lateral roots ($p<0.05$). Furthermore the study showed that *Arabidopsis thaliana* modifies its root system architecture in response to different growing conditions separately but it did not show significant interaction for 'population density x nutrient concentration' in all response variables ($p>0.05$). This work could serve as a guideline when considering sowing density and fertilizer application to other crop species.

Keywords: Root System Architecture, Lateral Root, Root Hair, *Arabidopsis thaliana*, Population Density

1. Introduction

Plant Roots are important for a variety of processes, including nutrient and water uptake, anchoring and mechanical support, storage functions, as well as connections between the plant and various biotic and abiotic factors in the soil environment [1]. The root system architecture of a plant is considered a highly plastic trait where it is sensitive to several developmental and environmental factors [2]. By understanding the development and architecture of roots, it is possible to explore the potential for the exploitation and manipulation of root characteristics to help increase food plant yield and optimize agricultural land use [1].

Studies show that in presence of nutrient deficiency, root architecture could be modified, for example lateral root growth is inhibited at low concentrations of nitrate whereas nitrate-rich medium stimulates elongation of lateral roots in order to increase N uptake [1]. Studies also have shown that plants can distinguish and discriminate their own roots from those of other plants. Thus plants would overproduce roots such as more lateral roots and root hairs to maximize competition with neighboring plants [3]. However, in order not to lose to competitors, plants within the common growing space would also produce more roots. Hence by doing so, plants would take more nutrients from the soil to carry out functions such as reproduction [3]. The knowledge of a suitable population density and amount of nutrients available can help exploit and manipulate root morphology to help increase plant yield and reduce the cost of fertilizers.

The knowledge of root system architecture of plants can be vital to humans to determine optimum growth conditions for plants especially food crops but many studies [2], [4] commented that the study of root system architecture is compromised. There are difficulties such as extracting an entire root system from soil without damage. In model plant *Arabidopsis thaliana*, hair-forming cells (trichoblasts) and non-hair cells (atrichoblasts) are arranged in alternating files along the root surface so hairs are produced in a simple and invariant striped pattern. The simplicity of the patterning in hair pattern and morphology make *Arabidopsis thaliana* root a useful model for study of plant cell growth [5]. By growing *Arabidopsis thaliana* in an agar medium, its entire root system can be observed and studied. Hence using *Arabidopsis thaliana* we studied whether there is a significant interaction between these growth conditions: nutrient concentration (abiotic) and population density (biotic) on the root system architecture of *Arabidopsis thaliana*. The study proposes that differences in length of primary root hair, and number of root hairs and lateral roots are due to difference in nutrient availability and difference in population density. In this study we are interested in the combined roles of abiotic and biotic factors on the development of *Arabidopsis thaliana* lateral roots, this study would give insights on the adaptation of root system architecture towards combinational environmental variables.

2. Methods

Seeds of *Arabidopsis thaliana* ecotype Col-0 were surfaced sterilized with 70% ethanol and chlorox. Sterilized seeds were planted on agar plates containing Murashige and Skoog (MS) mineral salts. Agar plates (12cm x 12cm square petri dishes) were prepared to contain either full MS concentration, half MS concentration or quarter MS concentration. Each agar plates of different MS concentration have seeds planted in rows to come up with populations of ten, fifty and one hundred seeds. The plates were left to stand vertically and seeds were grown under 12-hour light / 12-hour dark cycles at 24°C tissue culture room.

For data collection, three seedlings from each plate were randomly chosen. Their roots were analyzed for the length of their primary roots, the number of lateral roots and the number of root hairs presents (Figure 1). The length of their primary roots was measured with Image J after acquisition of the image of the root system architecture. All lateral roots or branching roots regardless of length were counted. The number of root hairs was counted at 20X magnification and every projection from the primary root and lateral roots regardless of length was counted as root hair.

After the collection of data and assuming that resources such as nutrients and space per individual remain constant as the number of individuals increases. A two-way Analysis of Variance (ANOVA) was used to analyze each response variables: number of root hairs, primary root length and number of lateral roots, crossed with the factors: population density and nutrient concentration. A Tukey's test was performed on interactions analyzed from two-way ANOVA to check for significance and to derive the conditions that cause a change in root morphology of *Arabidopsis thaliana*. These tests were carried out using Minitab 17 Statistical Software.

3. Result

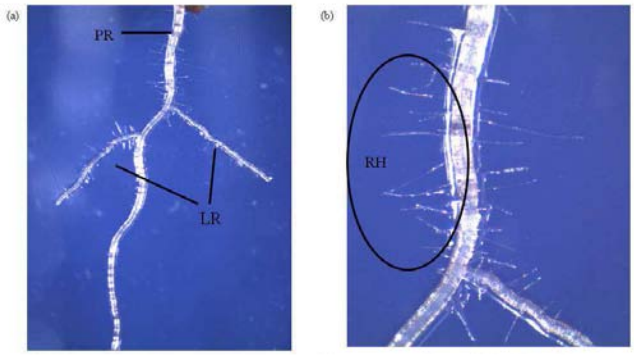

Figure 1. *Microscope scan of root architecture of Arabiopsis thaliana and key parameters for measurement. (a) The identification of primary root and lateral roots. (b) Tiny hair-like projections are identified as root hairs under magnification. PR, primary root; LR, lateral roots; RH, root hairs.*

Table 1. *Results of two-way ANOVA for the response variable, number of root hair, crossed with factors: species. n. s., not significant; sig., significant at p = 0.05.*

	df	SS	MS	F	P
Population density	2	670.52	335.259	6.01	0.010 sig.
Nutrient concentration	2	15.63	7.815	0.14	0.87 n. s
Population density X Nutrient concentration	4	348.81	87.204	1.56	0.227 n. s.

Table 2. *Results of two-way ANOVA for the response variable, length of primary root, crossed with factors: species and time. n.s., not significant; sig., significant at p = 0.05.*

	df	SS	MS	F	P
Population density	2	83.19	41.59	1.92	0.176 n. s.
Nutrient concentration	2	312.96	156.48	7.21	0.005 sig
Population density X Nutrient concentration	4	48.37	12.09	0.56	0.697 n. s.

Table 3. *Results of two-way ANOVA for the response variable, number of lateral roots, crossed with factors: species and time. n. s., not significant; sig., significant at p = 0.05.*

	df	SS	MS	F	P
Population density	2	1.407	0.7037	1.19	0.328 n. s.
Nutrient concentration	2	27.185	13.5926	22.94	0.000 sig
Population density X Nutrient concentration	4	1.926	0.4815	0.81	0.534 n. s.

From the results of the two-way ANOVA in (Table 1), (Table 2) and (Table 3), interaction between the factors: population density and nutrient concentration were not significant ($p>0.05$). Thus, there is no significant interaction between these factors with regards to all variables measured. From (Table 1), there is a significant effect of population density on the number of root hair ($p<0.05$) and Tukey's test was performed for further analysis. The test shows that the number of root hairs for population density of one hundred plants deviates slightly from the population density of ten and fifty. On the other hand, there is a significant effect of nutrient concentration on both the length of primary root and the number of lateral roots as seen in (Table 2) and (Table 3). Tukey's test shows that plants grown on quarter concentration of MS salts for both response variables deviate slightly away from the other two concentrations. Therefore, the number of root hair is seen to be affected by population density while nutrient concentration affects the length of primary root and the number of lateral roots.

Table 4. *Summary of descriptive statistics for all response variables with relation to factors: population density and nutrient concentration.*

Variable	Nutrient concentration	Population density	n	Mean ± SD	Minimum	Maximum
Number of root hairs	Full MS salt	10	3	34.33 ± 11.06	23.27	45.39
		50	3	29.33 ± 2.08	27.25	31.41
		100	3	36.00 ± 3.46	32.53	39.46
	Half MS salt	10	3	30.33 ± 9.29	21.04	39.62
		50	3	30.33 ± 7.64	22.70	37.97
		100	3	38.67 ± 9.87	28.80	48.53
	Quarter MS salt	10	3	21.67 ± 9.50	12.16	31.17
		50	3	28.67 ± 2.08	26.59	30.75
		100	3	44.33 ± 5.13	39.20	49.46
Length of primary root	Full MS salt	10	3	22.00 ± 6.56	15.44	28.56
		50	3	19.67 ± 2.52	17.15	22.18
		100	3	20.33 ± 3.21	17.12	23.55
	Half MS salt	10	3	26.33 ± 7.57	18.76	33.91
		50	3	22.33 ± 4.93	17.40	27.27
		100	3	25.00 ± 3.46	21.54	28.47
	Quarter MS salt	10	3	33.33 ± 4.93	28.40	38.26
		50	3	28.33 ± 2.89	25.45	31.22
		100	3	25.33 ± 3.06	22.28	28.39
Number of lateral roots	Full MS salt	10	3	0	0	0
		50	3	0	0	0
		100	3	0.33 ± 0.33	- 0.24	0.91
	Half MS salt	10	3	1.00 ± 0	1.00	1.00
		50	3	0.67 ± 0.58	0.09	1.24
		100	3	1.67 ± 0.58	1.09	2.24
	Quarter MS salt	10	3	2.00 ± 1.73	0.27	3.73
		50	3	3.00 ± 0	3.00	3.00
		100	3	2.67 ± 1.15	1.51	3.82

The mean number of root hairs for plants was seen to be higher when grown in a population of one hundred *Arabidopsis thaliana* in full, half and quarter concentration of MS salts (36.00 ± 3.46, 38.67 ± 9.87 and 44.33 ± 5.13 respectively) (Table 4). Mean length of primary roots was seen to be longer in a population of ten *Arabidopsis thaliana* when subjected to full, half and quarter concentration of MS salts (22.00 ± 6.56, 26.33 ± 7.57 and 33.33 ± 4.93 respectively).

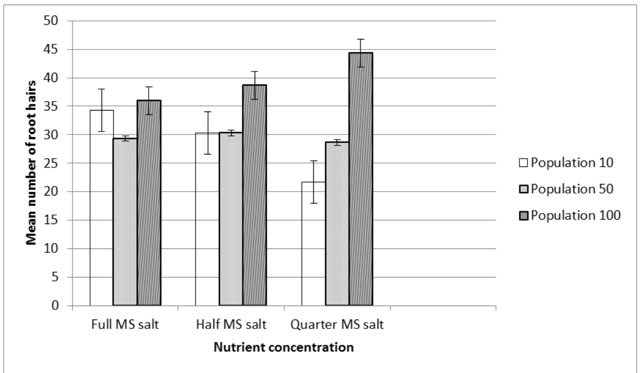

Figure 2. *Mean number of root hairs at different nutrient concentration and population density (error bars indicate S. D.).*

The mean number of root hairs was higher in population of one hundred *Arabidopsis thaliana* throughout all concentration of MS salts (36.00 ± 3.46, 38.67 ± 9.87 and 44.33 ± 5.13) as compared to a population of ten *Arabidopsis thaliana* (34.33 ± 11.06, 30.33 ± 9.29 and 21.67 ± 9.50) (Table 4 and Figure 2). Thus an increase in population of *Arabidopsis thaliana* leads to more root hairs developed.

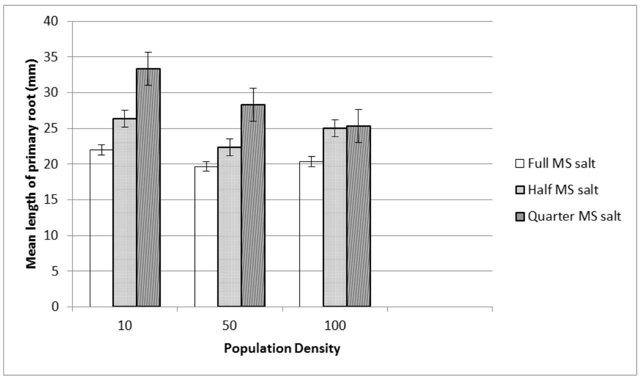

Figure 3. *Mean length of primary root at different nutrient concentration and population density (error bars indicate S. D.).*

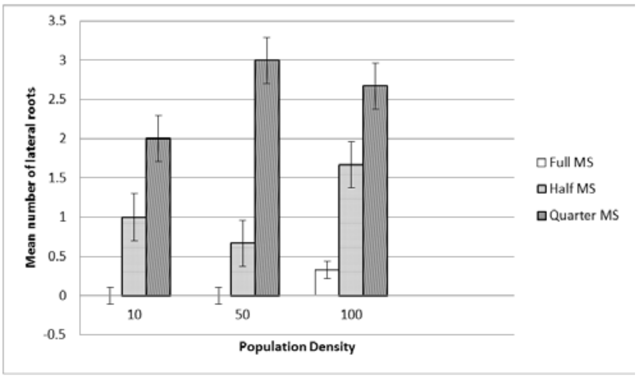

Figure 4. *Mean number of lateral roots at different nutrient concentration and population density (error bars indicate S. D.).*

The mean length of primary root is longer in *Arabidopsis thaliana* grown in quarter concentration of MS salts of all population density (33.33 ± 4.93, 28.33 ± 2.89 and 25.33 ± 3.06) as compared to full concentration of MS salts (22.00 ± 6.56, 19.67 ± 2.52 and 20.33 ± 3.21) and half concentration of MS salts (26.33 ± 7.57, 22.33 ± 4.93 and 25.00 ± 3.46) (Table 4 and Figure 3). Similarly the mean number of lateral roots is higher when *Arabidopsis thaliana* is grown in quarter concentration of MS salts of all population density (2.00 ± 1.73, 3.00 ± 0 and 2.67 ± 1.15) (Figure 4). Therefore, length of primary root and number of lateral roots are dependent on concentration of MS salts.

4. Discussion

In this study, the effects on population density and nutrient concentration on the root system architecture of *Arabidopsis thaliana* were examined. Our data didn't support strong interaction between population densities with nutrient concentration (Table 1, 2 and 3). This shows that the two factors can be analyzed as independent factors. *Arabidopsis thaliana* modifies its root system architecture in response to population density and nutrient concentration separately.

Research done on other plants has shown that by sharing a common space with other plants, plants grow more roots per individual [6] as they are able to identify competition through root recognition [7]. Through this study, it can be seen that *Arabidopsis thaliana* was able to recognize competition with other roots of their type grown in common space. It modifies its root system by increasing the number of root hairs in their primary and lateral roots. This result is consistent with other studies done where Smith et al. (2012) [1] mentioned that by increasing the number of root hair, it increases surface area for *Arabidopsis thaliana* to tap on more distant reserves and Messier et al. (2009) [8] noted the increase in fine root hairs is a strategy to optimize uptake of resources below ground.

Research also has shown that nutrient concentration in the soil plays an important role in root architecture [9]. In this study, there was a statistical difference in the length of primary root and the number of lateral roots with regards to the level of MS salt concentration *Arabidopsis thaliana* was subjected to. The length of primary root was recorded to be longer and more lateral roots were observed when *Arabidopsis thaliana* was subjected to only quarter concentration of MS salt. Therefore it shows that as the availability of nutrients is limited, *Arabidopsis thaliana* modifies its root architecture by increasing primary root length and develop more lateral roots. The findings for the length of primary root did not concur with a study showing root length increased in response to increase in N availability [10]. On the other hand, other literature reviews [1], [2], [9] state that roots are actively acquiring nutrient for plant growth. Hence to acquire them effectively, roots modify its architecture by branching out – creating more lateral roots to areas where root densities of competing neighbors are low [9].

In this study, population density and nutrient concentration were concluded to be independent factors affecting root architecture of *Arabidopsis thaliana.* It can be seen that there were statistically significant results to conclude that *Arabidopsis thaliana* modifies its root system architecture when subjected to abiotic (MS salt concentration) and biotic (population density) factors to increase stress tolerance and optimize plant growth in given environmental conditions. Tests on fitness could be done to determine best condition for *Arabidopsis thaliana* to be grown in.

The study assumed that each individual plant has a constant amount of nutrients even as the number of individuals increases. Therefore it is hard to conclude if modification of root system architecture due to presence of neighboring roots as each individual may be able to obtain different amount of nutrients. However, Messier et al. (2009) [8] brought up that plants may have genetically pre-disposed architectural rules to decrease self-interference among same species. As such, Hess et al. (2007) [3] suggests more research to be done without confound factors to conclude if modification of root system architecture takes place in presence of neighboring roots.

More conclusive studies can be done following this to determine more factors that affect the root system architecture of *Arabidopsis thaliana.* A follow-up study could be introducing a different genotype of *Arabidopsis thaliana* to determine if there is a difference in root architecture with the presence of a 'sibling' or as a competitor.

5. Conclusion

This study examined the biotic factor of population density and abiotic factor of nutrient concentration using model plant *Arabidopsis thaliana*. Population density significantly affected the number of root hairs while nutrient concentration significantly affected the length of primary roots and number of lateral roots. However *Arabidopsis thaliana* modifies its root system architecture in response to different growing conditions separately in absence of significant interaction for 'population density x nutrient concentration'.

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