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Feedback effect of root exudates in cotton (*Gossypium hirsutum* L.) seedlings

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Abstract
The feedback effect of root exudates on plant growth and development was observed by measuring several growth parameters like plant height, root length, fresh weight, dry weight, SPAD meter and NDVI. All the growth parameter were observed between two set of plants ,one with regular interval of root exudates collection and another one with root exudates not collected regular interval. The result of NDVI, plant height, root length , fresh weight, shoot dry weight , root dry weight showed that statistically at 1% level of significance, there was no significant difference between collected and not collected plants .Since the measurement SPAD chlorophyll meter readings, the results showed that there was no much influence of chrophyll content. These results revealed that root exudates released into soil for many other purposes, plants can re-absorb released nutrients (recycling nutrients) in a nutrient deficit situation. This phenomenon has not reported in literature so far, thus, it is novel information and needed to further investigation. It is apparent that exudates recycling effect is an inherent property of roots, similar to absorption nutrients from the soil. Roots absorb nutrients irrespective of its source (its own or external supplied).

Keywords: Root exudates, Silica sand, Feedback effect, NDVI.

1. Introduction

Living roots secreate a wide range of compounds into the rhizosphere area of soil. These compounds can be classified into two groups based on their subsequent utilization as microbial substrates, namely low molecular weight organic compounds (such as sugars, amino acids, organic acid (OA), phenolics and various other secondary metabolites) that can be readily assimilated by soil microorganisms, and high molecular weight organic exudates (such as proteins, pigments, mucilage and miscellaneous other substances) that require extracellular enzymic activity to break them down before they can be assimilated (Meharg 1994).

Rhizodeposition refers to process of secreting organic carbon compounds such as sugars, amino acids in addition to more complex secondary metabolites from roots in the form of root exudates which are involved in nutritional, communication and regulatory functions (Bertin et al., 2003). The qualitative and quantitative composition of root exudates varies based on cultivar, plant species, plant developmental stage and various environmental factors, including soil type, pH, temperature, presence of microorganisms and in response to various predatory insects and herbivores (Badri and Vivanco, 2009; Uren, 2000). These differences generate a unique microbial communities in the rhizosphere that have a certain degree of specificity for each plant species. The interactions between the plants and microbes viz., could be both positive and negative interactions. The positive interaction, for example, includes nitrogen fixation by Rhizobium, protection against harmful microbes, modulating soil structure, mineralization, etc. and negative interactions include attraction/activation of pathogens and parasitic weeds, deflation of nutrients disproportionately, allelopathic effect, etc. Since root exudates can have diverse interactions with soil organisms, identifying a specific root exudates pattern that has positive effect appears to be a very efficient method for crop improvement. At present, lack of comprehensive knowledge on root exudates composition is another major barrier to understand rhizosphere complexicity (Baziramakenga et al., 1995; Fan et al., 2001; Gransee and Wittenmayer, 2000). Therefore,

understanding of root exudates composition and their role in rhizosphere interactions for different genotypes may be a useful technique to identify a specific genotype having desirable rhizosphere interactions.

Material and Methods

Collection of genotypes:

Collection of suitable genotypes in cotton for root exudates studies is very important for generating reliable results. Different genotypes required for this research work was obtained from germplasm maintained by cotton research unit, UAS, Dharwad.

Table1: List of cotton genotypes used along with their respective special characters

SL No.	Genotype Name	Special characters
1	Khandwa-2	Jassid resistant variety, drought tolerant variety.
2	L-761	High yielding, drought tolerant variety.
3	F-2226	Drought tolerant variety
4	JK-4	High yielding, drought tolerant variety
5	RAJ-2	Tolerant to sucking pests , drought tolerant variety.
6	AK-23	Drought tolerant variety
7	CCH1831	Drought tolerant variety.
8	543 3A2 A03 N83	Drought tolerant variety
9	MCU-5 (Susceptible)	Resistant to *Verticillum wilt*, Drought Susceptible variety
10	RHC-0811	Drought tolerant variety
11	Sahana	Tolerant to bollworm and drought.
12	RS-810	Drought tolerant variety
13	GJHV-358	Drought tolerant variety
14	Bikaneri nerma	Drought tolerant variety
15	PH1009	More tolerant to salinity stress, tolerance to sucking pests and drought ,

Silica sand as medium of growth

White silica sand with 0.03mm diameter particle size (purchased from new water technologies, Coimbatore) was used as medium of growth. The sand was thoroughly washed several times with running tap water after which it was washed with 2 to 3 times with distilled water. After complete drying of the sand was spread on a plane paper for complete drying in room temperature for 48 hours. Washed and dried sand was sterilized in autoclave at 120° C for 30 minutes.

Sowing of seeds in cups filled with sterilized silica sand

Transparent plastic cups with diameter of – 7cm filled with sterilized sand up to one third volume of plastic cup height (40 grams of weight). Seeds were dibbled into half inch depth and watered. The numbers of seeds per cup sown were varied according to the experiment. The cups were watered two times a day with sterilized distilled water with enough to saturate the soil at the same time not to lose water by percolating out of the cups.

Experimental setup

The experiment was carried out in IABT glass house with dry and optimum temperature of 28 ± 2 °C. The cups were arranged in a rows and columns to accommodate replication and genotypes in a manner of experimental design.

Collection of the root exudates

At the time of collection (different intervals of time), the cups were watered to saturation point and allowed to release the exudates into the solution. About 30 minutes later, exudates were collected by washing off by adding 8 ml of water (plate 1 C) and collected the percolating solutions (water + root exudates) though the holes into sterile a 15 ml centrifuge tube. The centrifuge tubes with exudates were centrifuged at 9000 rpm for 10 min, to get rid of minute sand particles and any cell debris or sloughed off cells. After centrifugation, the samples were decanted into fresh 15 ml centrifuge tubes.

Identification of recycling phenomenon (feedback effect) of root exudates

In order to find the possibility of recycling of the root exudates on plant growth , an experiment was designed in a such way that two set of experiment was carried out simultaneously with regular interval collection of root exudates in one set and no collection of root exudates in other set. As mentioned previously, the exudates were collected in 3 days interval regularly till 12th DAS. In second set of experiment, the experiment, the exudates were collected only on 12th DAS. On 12th DAS the plants were observed for overall health condition by measuring 6 agronomic characters such as plant height, root length, fresh weight, dry weight, chlorophyll content and NDVI.

1 Plant height: Plant height was measured and expressed in cm from the base of the plant to tip of the apical bud

2 Root length: Root length was measured and expressed in cm from base of the plant tip of the primary root

3 Fresh weights: Seedling were kept in weighing balance and weight measured in gm.

4 Dry weights: After complete drying of the seedlings in the hot air oven at 50^{0}C for 24 hours, the dry weights of the seedlings were measured in gm.

5 SPAD Chlorophyll meter Readings (SCMR): The chlorophyll content of the seedling is measured using SPAD (Soil Plant Analyses Development) meter. The reading was taken in three regions of the leaf and the final values obtained were the average of the three reading.

6. Normalized Difference Vegetation Index (NDVI): which is an important indicator of chlorophyll content in plants**?** One indicator of plant stress is light absorption and reflectance.

Investigation on possibility of seedlings revival after continuous root exudates collected plants and assessment of its desirable characters

After collection of exudates continuously for define period of time , seedlings grown in silica cups, were revived by feeding plants first with half strength Hoagland nutrient

solution (two days) and given full strength after another two days. About 5 days later, seedlings were supplied with vermicompost, and then transplanted to soil get open field condition.

Statistical analysis

The physiological observations taken to find significance difference between root exudates collected and not collected plants were statistically analyzed using paired t test .All the experiment were replicated thrice.

Results and Discussion

Feedback effect of root exudates on plant growth and development

The feedback effect of root exudates on plant growth and development was observed by measuring several growth parameters like plant height, root length, fresh weight, dry weight, SPAD meter and NDVI. All the growth parameter were observed between two set of plants ,one with regular interval of root exudates collection and another one with root exudates not collected regular interval. The result of NDVI, plant height, root length , fresh weight, shoot dry weight , root dry weight (Table 2) showed that statistically at 1% level of significance, there was no significant difference between collected and not collected plants .Since the measurement SPAD chlorophyll meter readings, the results showed that there was no much influence of chrophyll content.

While other parameter like plant height, root length, fresh weight, root and shoot dry weight and NDVI (Table 2) showed significant difference between the collected and non-collected plants. There was significant difference in root length, because the root received nutrient supply by means of root exudates only. Thus, the roots in not collected plant showed higher growth compared to the regular interval collected plants. The NDVI, fresh weight, shoot and root dry weight represents the active photosynthesis activity in the not collected plants compared to the collected plants. Thus, it can be concluded that from above results revealed that root exudates released into soil for many other purposes, plants can re-absorb released nutrients (recycling nutrients) in a nutrient deficit situation (Plate 1 E). This phenomenon has not reported in literature so far, thus, it is novel information and needed to further investigation. It is apparent that exudates recycling effect is an inherent property of roots, similar to absorption nutrients from the soil. Roots absorb nutrients irrespective of its source (its own or external supplied).

Table 2: Feedback effect of root exudates in growth and development

I. No.	Parameter	Mean	Variance	Observations	Pearson Correlation	df	t Stat	P(T<=t) one-tail	t Critical one-tail	P(T<=t) two-tail	t Critical two-tail
1	Spad RE collcted set	44.9200	47.05	15.0	0.330	14.00	-0.327	0.374	1.761	0.749	2.14
	Spad RE not collcted set	45.5089	23.86	15.0							
2	NDVRE collected set	0.1579	0.00	15.0	0.162	14.00	-2.614*	0.010	1.761	0.020	2.14
	NDVI RE not collected set	0.2433	0.01	15.0							
3	Plant height RE collected set	8.6906	0.46	15.0	0.180	14.00	-13.026*	0.000	1.761	0.000	2.14
	Plant height RE non collected set	12.2250	0.87	15.0							
4	Root length RE of collected set	4.9822	1.70	15.0	0.230	14.00	-6.160*	0.000	1.761	0.000	2.14
	Root length RE non collcted set	7.4622	1.46	15.0							
5	Fresh weight RE collected set	0.4700	0.01	15.0	0.544	14.00	-10.093*	0.000	1.761	0.000	2.14
	Fresh weight RE non collected set	0.8557	0.03	15.0							
6	Shoot dry weight RE collected set	0.0645	0.00	15.0	0.502	14.00	-4.571*	0.000	1.761	0.000	2.14
	Shoot dry weight RE non collected set	0.0807	0.00	15.0							
7	Root dry weight RE collcted set	0.0112	0.00	15.0	0.425	14.00	-1.863*	0.042	1.761	0.084	2.14
	Root dry wt non RE	0.0166	0.00	15.0							

*- significantly different at 1% level of significance ($p<0.01$)

A: White silica sand

B: plastic cups with silica sand

C: Method of collection of root exudates in cotton seedlings

D: General view of experiment 12th day old cotton seedlings

E: Feedback effect of root exudates on plant growth a) root exudates not collected (heathy plant) b) root exudates collected plant (weak plant)

F: Revival of seedlings

Plate-1

4.1.3. Revival of seedlings

After collection of exudates, the seedlings were grown further in silica sand with half strength Hoagland solution nutrient supplements and increased the concentration gradually for 5 days. The seedling uses the nutrient supplement provided and shows energetic growth compared to the later where no nutrient supplement was available. The seedlings were then supplied with vermicompost for getting acclimatized to natural environment. The seedlings after few days were transplanted to soil and then exposed to open-field condition. This method is very useful, when plants analysed for root exudates showed difference between them and presence of some compound is correlated to particular agronomic trait. By this method it is possible to grow the seedlings to entire plant for further morphological character analysis. (Plate 1 F)

Discussion

Plant root exudates having diverse roles in the soil have reported by a number of scientists (Flores *et al.*, 1999). Even though it is well known that root exudates released into the soil by the plants are mainly meant for enhanced mineralization and attracting microorganisms (Ryan and Delhaize, 2001) plants absorbing its own root exudates is not reported. The experiments conducted during this study showed a phenomenon that plants re-absorbs the released nutrients back (recycling) in a nutrient deficit conditions (Plate-1E) (Table2). The experiments conducted by growing two sets of seedlings, one with root exudates collected in regular intervals and another batch without collection of root exudates, clearly indicated that there is a significance difference for overall health condition of seedlings, which was assessed based on measurements taken for six parameters (plant height, chlorophyll content, root fresh weight and root dry weight, NDVI). Root

exudates are believed to have important functions in regulation of plant growth (both directly or indirectly) (Bertin *et al.*, 2003; Walker *et al.*, 2003). These results revealed that root exudates released into soil for many other purposes, plants can re-absorb released nutrients (recycling nutrients) in a nutrient deficit situation. This phenomenon has not reported in literature so far, thus, it is novel information and needed to further investigation. It is apparent that exudates recycling effect is an inherent property of roots, similar to absorption nutrients from the soil. Roots absorb nutrients irrespective of its source (its own or external supplied).

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