Plant Prod. Sci. 12(4) : 443—448 (2009)

【Short Report】

Rhizodeposition of Mucilage, Root Border Cells, Carbon and Water under Combined Soil Physical Stresses in *Zea mays* L.

Sutharsan Somasundaram[1], Theertham P. Rao[2], Jiro Tatsumi[3] and Morio Iijima[4]

([1] Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan;
[2]International Department, Taiyo Kagaku Co., Ltd, Mie 512-1111, Japan;
[3] Center for Bioresource Field Science, Kyoto Institute of Technology, Kyoto 616-8354, Japan;
[4] School of Agriculture, Kinki University, Nara 631-8505, Japan)

Abstract : We investigated the effects of combined soil physical stresses of compaction and drought on the production of fully hydrated mucilage (mucilage) and root border cells (RBCs) in maize. The exudation of carbon and water were also estimated using stable isotopes of ^{13}C and deuterated water (D_2O) under same soil conditions. As plant age progressed during seedling stage, mucilage production increased, however, RBCs release did not. Soil compaction increased the release of D_2O, RBCs, and production of mucilage which implies the function of roots to reduce mechanical impedance during root penetration. Drying stress increased only carbon release, but reduced the others. This indicates that RBCs adhere more strongly to the root cap due to drying of mucilage, and water release may be reduced to save the water loss. The highest rhizodeposition of mucilage, RBCs and D_2O were occurred under wet compact soil condition, however, that of carbon occurred under dry compact soil condition.

Key words : Crushed cells, Deuterium, Drought, Maize, Rhizodeposition, Root exudation, Soil compaction.

Deposition of organic compounds by plant roots during the period of active growth, referred to as rhizodeposition, is an important subject which has been extensively studied so far (Paterson et al., 2007; Meier et al., 2008). The compounds released from roots influence the physical and chemical properties of the soil (Gregory, 2006), stimulate the growth of rhizosphere microbial community (Benizri et al., 2007) and affect the availability of nutrients for plant uptake (Paterson, 2003). The rhizodeposition of carbon is a process of linking plant, soil and microorganisms. This linking of plant, soil and microbial productivity become increasingly important in agricultural farming systems since, rhizosphere microorganisms exert manifold effects on the growth of plants.

Root border cells (RBCs), are group of cells at the periphery of the root cap which separate from plant roots as they move through the soil. These RBCs are traditionally known as dead cells (Driouich et al., 2007), become eminent and function as a physical and biological interface between the root and soil in the rhizosphere. RBCs reduce the frictional resistance during root penetration into soil (Iijima et al., 2000, 2003b, 2004a, 2004b). In addition, the released RBCs can survive for a relatively longer period of time (Somasundaram et al., 2008b) and interact with soil borne microflora (Hawes et al., 2000). The mucilage and RBCs form a functional entity, mucilage- border cell complex, in the rhizosphere. The rhizosphere released mucilage–border cell complex play various beneficial roles for plant growth, such as, reducing resistance root experience in soil (Iijima et al., 2008), facilitating protozoan effects towards increased lateral root growth in rice plants (Somasundaram et al., 2008a). The effects of soil compaction on the release of mucilage and RBCs were reported (Iijima et al., 2000, 2003a, 2004a). However, the production of mucilage and RBCs, under the combined environmental effects of soil compaction and drought stress has never been investigated.

Most of the published studies examined only the exudation of carbon, the major constituent of root exudates, under various physical and chemical conditions of soil. Very few studies dealt with the exudation of water except the work of McCully (1995) who proposed that water released from the root surface during night allows the expansion of root cap mucilage into the surrounding soil, however, direct evidence is lacking. Release of various compounds into the rhizosphere is affected by environmental stresses, such as soil compaction and drought stresses. Soil compaction is the result of improper and frequent use of agricultural machineries for field preparation.

Received 24 December 2008. Accepted 31 March 2009. Corresponding author: M. Iijima (iijimamorio@nara.kindai.ac.jp, fax +81-742-43-1155).

Abbreviations : ANOVA, analysis of variance; RBCs, root border cells.

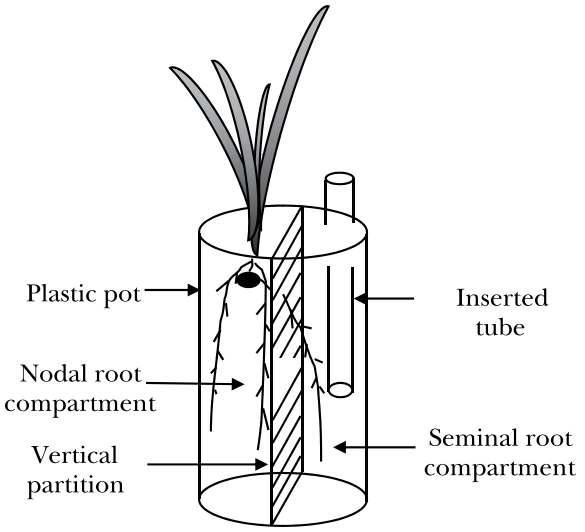

Fig. 1. Experimental setup to grow seminal and nodal roots of *Zea mays* L. Nodal and seminal compartments were separated by wax sealing so as not to allow water movement between the two compartments.

The compacted soil layers shows increased penetration resistance (Zegada-Lizarazu et al., 2006) and reduce the root extension rates (Iijima and Kato, 2007). In addition, soil compaction affects the movement and distribution of water for plant uptake and hence, creates drought stress for the plant growth. Previous studies have been investigated the independent effects of soil compaction (Barber and Gunn, 1974; Iijima et al., 2000; Tubeileh et al., 2003) or drought stress (Martin and Merckx, 1992; Palta and Gregory, 1997; Henry et al., 2007) on the exudation of carbon. However, the exudation of carbon and water under the combined environmental stresses of soil compaction and drought stress has not yet been reported. Therefore, the present study aimed to find out the rhizodeposition of mucilage, RBCs, carbon and water under the combined environmental stresses of soil compaction and drought stress.

Materials and Methods

1. Experimental set-up

Maize caryopses (*Zea mays* L. cv. Robust 30–71) were soaked in distilled water for 30 min and placed on moistened blotting paper in petri dishes at 30°C for 48 hr in the dark for germination. Germinated caryopses, each with a seminal root of 20–35 mm length, were grown in vertically partitioned plastic pots (20 cm long and 5 cm in diameter). In each tube, seedlings were raised in such a way that nodal and seminal roots were grown in separate compartments (Fig. 1). Both compartments were separated by a polyvinylchloride sheet sealed with wax to strictly avoid water movement between the compartments. Germinated seedlings were planted in soil with two soil moisture levels, either, dry (corresponding to −100 kPa) or wet (corresponding to −20 kPa) and two soil compaction levels, either, loose (corresponding to a bulk density of 1.3 g cm^{-3}) or compact (corresponding to a bulk density of 1.5 g cm^{-3}). We sieved to homogenize the loamy sand soil (Kiso river alluvial soil; particle size distribution, sand 87.0%, silt 9.6%, clay 3.4%) through a 2 mm mesh and mixed with powdered compound synthetic fertilizer (N: 12%, P_2O_5: 16%, K_2O: 14%) at the rate of 0.4 g Kg^{-1}. Soil water content was maintained throughout the experimental period by adjusting the water through weighing the pots for 1–2 times a day for both experiments. In experiment 1, plants were allowed to grow in controlled temperature chamber. During the experimental period (25 March to 15 April), the temperature inside the chamber was controlled at 30/24°C (day/night), with minimum and maximum natural day length of 12.3 and 13.1 hr, respectively. The plants in experiment 2 were grown under natural light conditions in a glass house during the Japanese summer time with average minimum and maximum temperatures of 25 and 37°C respectively. The minimum and maximum day length during this period (22 July to 12 August) was 13.6 and 14.1 hr, respectively. Both experiments were conducted at Nagoya University, Japan.

2. Production of fully hydrated mucilage and RBCs as affected by combined stresses of soil compaction, drought stress and period of harvest (Experiment 1)

At one, two and three wk after planting, six replicated plants were harvested and the root tips of seminal roots, ranging from 30 to 50 mm in length were cut and immersed in distilled water for 3 hr. Root cap mucilage adhered tightly on the cap cells was expanded fully by absorbing the surrounding water (Iijima et al., 2003c) when immersed in water for more than 30 min. This state of hydrated mucilage was defined as fully hydrated mucilage (mucilage). The mucilage was collected from the tips of the root using weight known filter papers and a micro balance (METTLER MT5, Japan) with six replicates according to Iijima et al. (2003b). For the quantification of number of RBCs, the fully hydrated mucilage was transferred to a 2 mL eppendorf tube. Subsequently, it was added with 960 μL surfactant solution and 40 μL Toluidine Blue O (3×10^{-5} g g^{-1}) and the numbers of RBCs were directly counted under a light microscope at 400×magnification, after 1 hr of hydration. The number of lateral roots emerged on the seminal root axis was enumerated at one, two and three wk after planting.

3. Rhizodeposition of carbon and water as affected by combined stresses of soil compaction and drought stress (Experiment 2)

Four replicated plants were used for ^{13}C and

Table 1. Production of fully hydrated mucilage, root border cells and lateral roots in seminal root of *Zea mays* L. as affected by soil mechanical impedance, soil water status and period of harvest grown for 1, 2 and 3 wk.

		Soil stresses			
		Compact		Loose	
	Period	Wet	Dry	Wet	Dry
Mucilage	1st week	77	43	30	34
(μg root-1)	2nd week	49	45	39	35
	3rd week	100	66	56	44
Root Border	1st week	48	37	25	23
Cells (RBCs)	2nd week	38	23	23	18
(Number root-1)	3rd week	62	39	28	21
Lateral	1st week	27	41	69	45
roots	2nd week	48	44	82	48
(Number root-1)	3rd week	93	87	118	67
Three-way ANOVA		Mucilage	RBCs	Lateral roots	
	Mechanical impedance (M)	***	***	***	
	Soil water status (W)	**	***	***	
	Period (P)	***	***	***	
	M×W	*	**	***	
	M×P	ns	**	*	
	W×P	ns	ns	*	
	M×W×P	ns	ns	ns	

***, **, and * indicates statistically significant at $P<0.001$, $P<0.01$ and $P<0.05$, respectively by three- way ANOVA. ns indicates significantly no different at $P>0.05$.

deuterium analysis. Three wk after planting, pots were transferred to a transparent chamber (25 cm wide×40 cm long×100 cm high). The shoots were exposed to ^{13}C-labelled carbon dioxide, generated by addition of hydrochloric acid (HCl) to ^{13}C-labelled barium carbonate (99 atom % of $Ba^{13}CO_3$) for 8 hrs. During this period soil surfaces were sealed by the polyvinylchloride film to strictly avoid ^{13}C-labelled carbon intrusion to the soil as well as unlabelled carbon extrusion from the soil. During ^{13}C feeding the light intensity of the shoot was 450–550 μmol s^{-1} m^{-2}. Prior to the ^{13}C feeding, deuterated water (1 atom% of D_2O), which is the same amount of water expired previous day, was applied via the inserted tube (Fig. 1) for three consecutive days into the seminal root compartment. The same volume of water (H_2O) was applied to the nodal root compartment. After 24 hr from ^{13}C feeding, the rhizosheath soil in nodal root compartment was collected for ^{13}C and deuterium analysis where care was taken to minimize evaporation and, therefore, isotopic fractionation. Rhizosheath soil is defined as the soil firmly adhered to roots after the removal of remaining soil.

For the ^{13}C analysis, soil samples were macerated using mortar and pestle. The enrichment of ^{13}C (atom % excess) of the samples was calculated from the atom % of the samples subtracted by natural abundance of ^{13}C values measured for control soil. For the deuterium analysis, water contained in the soil sample was extracted by centrifugation (Zegada-Lizarazu and Iijima, 2004). The isotopic ratios of deuterium water are presented in standard delta notation (δD) in parts per thousand (‰) relative to standard (SMOW: Standard Mean Ocean Water) as expressed by the following equation, stated by Araki and Iijima (2005).

$$\delta D = [(R_{sample}/R_{standard})-1] * 1000‰$$

where R is the molar ratio of heavy to light isotope (D/H) with D being deuterium and H, hydrogen. These values were converted into concentration. The deuterium atom % excess was calculated from the atom % of the samples subtracted by natural abundance of control soil. For this experiment, the ^{13}C and deuterium were measured using the isotope ratio mass spectrometer (DELTA^{plus}, Finnigan MAT instruments, Inc.).

4. Statistical analysis

Mean values of a given variable together with SE of the mean, were estimated. A three-way analysis of

Table 2. Shoot dry weight of *Zea mays* L. as affected by soil mechanical impedance and soil water status grown for 3 wk.

		Soil stresses		
Dry weight	Soil water status	Compact	Loose	Mean
Shoot	Wet	0.597	0.753	0.675
(g)	Dry	0.170	0.176	0.173
				***
	Mean	0.384	0.465	*

Values are means of four replicates. *** and * indicates statistically significant at $P<0.001$ and $P<0.05$ respectively by two-way ANOVA. The two factor interaction was not significant.

variance (ANOVA) was used to compare the effects of soil compaction, drought stress and period of harvest for the parameters of mucilage, RBCs and lateral roots production. Two-way ANOVA was used to compare the effects of soil compaction and drought stress on the exudation of carbon and water.

Results

1. Production of fully hydrated mucilage and RBCs as affected by combined stresses of soil compaction, drought stress and period of harvest (Exp. 1)

The overall effects of soil compaction, drought stress and period of harvest on the production of mucilage, RBCs and lateral roots emergence were summarized in Table 1. In general, production of mucilage was greater in compact than loose soil ($F=34.17$, $P<0.001$), higher in wet than dry soil treatment ($F=11.54$, $P=0.002$) and affected by period of harvest ($F=15.57$, $P<0.001$). In general, an increasing trend of mucilage production was observed with aging of plants. The highest mucilage production was observed in roots grown under compact wet condition and showed significant difference ($F=15.70$, $P<0.001$) compared to other soil conditions according to Tukey multiple comparison test. Roots grown in compact wet condition produced 1.8 times more mucilage compared to roots in loose wet soil condition at three wk after planting. Further the production of mucilage increased with soil moisture content in the compact soil treatment (Table 1, significant interaction between soil mechanical impedance x soil water status).

The production of RBCs was also significantly greater in compact than loose soil ($F=121.67$, $P<0.001$), higher in wet than dry soil treatment ($F=41.29$, $P<0.001$). The period of harvest also affected the production of RBCs ($F=21.68$, $P<0.001$). The time course production of RBCs showed no difference under loose soil condition, however, differences were observed between second and third wk after planting under compact soil environment. At three wk after planting, the production of RBCs was 2.2 times higher in roots grown under compact wet condition compared to loose wet soil environment. The significant interaction between soil mechanical impedance x soil water status ($F=11.8$, $P=0.001$) revealed that the number of RBCs increased with soil moisture content in compact soil treatment. Production of lateral roots was significantly affected by soil mechanical impedance ($F=32.94$, $P<0.001$), soil water status ($F=47.6$, $P<0.001$) and period of harvest ($F=114.58$, $P<0.001$). Increased number of laterals was observed in loose compared to compact soil at each time of harvest. In general, the lateral root production increased with increasing soil moisture content in both soil bulk densities.

2. Rhizodeposition of carbon and water as affected by combined stresses of soil compaction and drought stress (Exp. 2)

The soil compaction and drought stress significantly affected the plant growth (Table 2). Shoot growth was significantly affected by soil compaction ($F=5.72$, $P=0.041$) and drought stress ($F=218.29$, $P<0.001$). Soil compaction reduced the shoot biomass by 21% in wet soil condition.

The exudation activity of labeled ^{13}C and deuterium from roots to the rhizosphere soil was shown as the values of atom % excess in Table 3. Exudation of ^{13}C was significantly affected by soil moisture conditions ($F=12.67$, $P=0.004$), where the exudation of ^{13}C was higher in dry than wet condition. Further, the exudation of ^{13}C showed increased trend in compact than loose soil condition. The highest ^{13}C exudation was observed in roots grown under compact dry condition compared to other soil conditions. Roots grown in compact dry condition produced 5.5 times more ^{13}C exudation compared to roots in loose wet soil condition. Deuterated water exudation was mainly affected by drought stress ($F=5.15$, $P=0.049$). In addition, soil compaction also exerted an effect on its exudation ($F=4.35$, $P=0.067$) in which exudation was higher in compact than loose soil condition. The highest deuterium exudation was observed in roots grown under compact wet condition compared to other soil conditions ($F=3.53$, $P=0.062$ by Turkey multiple comparison test). Roots grown in compact

Table 3. Enrichment of carbon (C) and deuterium (D) in rhizosheath of *Zea mays* L. as affected by soil mechanical impedance and soil water status grown for 3 wk.

		Soil stresses		
	Soil water status	Compact	Loose	Mean
^{13}C atom % excess				
	Wet	0.160	0.076	0.118
	Dry	0.420	0.352	0.386
				**
	Mean	0.290	0.214 ns	
D atom % excess				
	Wet	0.251	0.184	0.218
	Dry	0.181	0.165	0.173
				*
	Mean	0.216	0.175 †	

**, *, and † indicates statistically significant at $P<0.01$, $P<0.05$ and $P<0.10$ respectively by two-way ANOVA. ns indicates significantly no different at $P>0.05$. The two factor interaction was not significant.

wet condition exuded 1.4 times more deuterated water compared to roots in loose wet soil condition.

Discussion

This paper describes the release of not only carbon but also water, mucilage, and detached cells, most of the major constituents of plant rhizodeposition with emphasis on the combined soil physical stresses which has never been reported to the best of our knowledge. Our results showed that the release of deuterated water, mucilage and RBCs were increased by soil compaction stress and the highest amount of these rhizodeposits was observed under compact wet soil condition (Tables 1 and 3). The release of these rhizodeposits will help the root to reduce soil frictional resistance during root penetration into soil (Iijima and Kono, 1992; Iijima et al., 2000, 2003a, 2004a, 2008) and facilitate to prevent the root tips from desiccation (Watt et al., 1994). Furthermore, the released mucilage-border cells complex may act as an energy source for soil microorganisms in the rhizosphere (Knee et al., 2001; Somasundaram et al., 2008a) and increase the uptake of plant nutrients. In the meantime, with the increase of soil compaction, an increased trend of carbon exudation was also observed. This agrees with the former studies (Iijima et al., 2000; Tubeileh et al., 2003). In addition, Boeuf-Tremblay et al. (1995) and Groleau-Renaud et al. (1998) also indicated that mechanical impedance promoted the root exudation under hydroponic conditions.

Soil drying increased only the exudation of carbon to the rhizosphere especially under compact dry soil condition. However, soil drying reduced the rhizodeposition of deuterated water, RBCs and production of mucilage. The possible explanation for the increased carbon under compact dry soil condition will be the increased rate of crushed RBCs. As reported previously (Somasundaram et al., 2008b), some of the RBCs were crushed by abrasion with soil particles during the root penetration. We estimated that about four-fifths of the cap cells were crushed during the first day of root elongation. The number of crushed cells was estimated by the quantification of cell production rate in the root cap meristem, changes in the cap cell number, and the number of RBCs released to rhizosphere soil (Somasundaram et al., 2008b). Under dry soil condition, the abrasion with soil particles increases, and hence, the rate of crushed cells may have increased. More crushed cells will increase the carbon in the rhizosphere soil, and this could be the possible reason for the enhanced carbon under dry soil conditions. On the other hand, release of RBCs and mucilage were reduced under the soil drying condition. Because the RBCs would be strongly adhered to the root cap due to more dehydrated state of mucilage, this would lead to the reduction in mucilage and RBCs (Iijima et al., 2004b). Further, under drying soil condition the protective tissue such as cortical schlerenchyma tissues often develop in the outer layer of cortex (Galamay et al., 1991), and diameter of both central cylinder and xylem vessels will be usually reduced (Iijima and Kato, 2007). Therefore, these may be the reason for the reduction of deuterated water release under dry soil condition. This may be a protective mechanism of plants to reduce the water loss. Deutrated water was exuded in the soil through root axis in different soil region although the soil moisture in both compartments is similar. Therefore, hydraulic lift (Zegada-Lizarazu et al., 2005) should not occur under this experimental condition. Water soluble compounds should be released together with the root exudates. McCully (1995) has observed

similar phenomenon in five wk old maize plants grown under field condition. Further study is necessary to discuss this phenomenon in detail.

In conclusion, our results elucidated that the rhizodeposition of mucilage, RBCs, carbon and water were differently affected by the combined soil environmental stresses of compaction and soil drying. Soil compaction increased the release of mucilage-border cells complex and effectively reduced the soil frictional resistance roots will experience during penetration into field soil (Iijima et al., 2008). Soil drying increased the carbon exudation but reduced other components released into the rhizosphere. Therefore, these could be a protective mechanism of the plants to overcome the soil environmental stresses and to facilitate the growth of roots in the rhizosphere soil.

Acknowledgements

We thank Ms. Junko Tsueda, former member of the Graduate School of Bioagricultural Sciences, Nagoya University for her helpful assistance. This work was funded by the grant-in-aid (B2-15380014) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

References

Araki, H. and Iijima, M. 2005. Plant Soil 270 : 147-157.
Barber, D.A. and Gunn, K.B. 1974. New Phytol. 73 : 39-45.
Benizri, E. et al. 2007. Soil Biol. Biochem. 39 : 1230-1233.
Boeuf-Tremblay, V. et al. 1995. Plant Soil 172 : 279-287.
Driouich, A. et al. 2007. Trends Plant Sci. 12 : 14-19.
Galamay, T.O. et al. 1991. Jpn. J. Crop. Sci. 60 : 184-190.
Gregory, P.J. 2006. Eur. J. Soil Sci. 57 : 2-12.
Groleau-Renaud, V. et al. 1998. Plant Soil 2001 : 231-239.
Hawes, M.C. et al. 2000. Trends Plant Sci. 5 : 128-133.
Henry, A. et al. 2007. J. Environ. Qual. 36 : 904-912.
Iijima, M. and Kono, Y. 1992. Ann. Bot. 70 : 207-212.
Iijima, M. et al. 2000. New Phytol. 145 : 477-482.
Iijima, M. et al. 2003a. New Phytol. 160 : 127-134.
Iijima, M. et al. 2003b. J. Exp. Bot. 54 : 2105-2109.
Iijima, M. et al. 2003c. Plant Soil 255 : 399-407.
Iijima, M. et al. 2004a. Ann. Bot. 94 : 473-477.
Iijima, M. et al. 2004b. Soil Biol. Biochem. 36 : 1517-1519.
Iijima, M. and Kato, J. 2007. Plant Prod. Sci. 10 : 451-459.
Iijima, M. et al. 2008. Plant Prod. Sci. 11 : 17-27.
Knee, E.M. et al. 2001. Mol. Plant Microbe Interact. 14 : 775-784.
Martin, J.K. and Merckx, R. 1992. Soil Biol. Biochem. 24 : 1147-1156.
McCully, M.E. 1995. Physiol. Plant 95 : 217-224.
Meier, C.L. et al. 2008. J. Ecol. 96 : 421-430.
Palta, J.A. and Gregory, P.J. 1997. Soil Biol. Biochem. 29 : 1395-1403.
Paterson, E. 2003. Eur. J. Soil Sci. 54 : 741-750.
Paterson, E. et al. 2007. New Phytol. 173 : 600-610.
Somasundaram, S. et al. 2008a. Plant Prod. Sci. 11 : 344-351.
Somasundaram, S. et al. 2008b. Plant Prod. Sci. 11 : 440-446.
Tubeileh, A. et al. 2003. Soil Tillage Res. 71 : 151-161.
Watt, M. et al. 1994. Plant Physiol. 106 : 179-186.
Zegada-Lizarazu, W. and Iijima, M. 2004. Plant Prod. Sci. 7 : 427-434.
Zegada-Lizarazu, W. et al. 2005. Plant Prod. Sci. 8 : 433-440.
Zegada-Lizarazu, W. et al. 2006. Plant Prod. Sci. 9 : 123-132.