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Silicon Forms in Polynucleic Acids from Rice and Peanut Embryos

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Summary

Little is known about silicon as an essential nutrient in plants, despite the deficiency symptoms associated with rice reproductive structures under silicon deprivation conditions. Silicon concentrations in the embryos of rice, peanut and other plants were compared, and the presence of silicon in the polynucleic acids (PNA) extracted from embryos of rice (*Oryza sativa* L.), a siliceous plant, and peanut (*Arachis hypogaea* L.), a calciphilous plant, was demonstrated using molecular sieve chromatography. Silicon co-eluted with RNA and DNA from both rice and peanuts, and the ratio of silicon to RNA or DNA in each fraction differed between plant embryos, and between nucleic acids. The ratio of silicon to DNA was higher than to RNA in both rice and peanut, and was higher in the peanut embryo than in the rice embryo, while silicon to RNA was opposite to the result of DNA. Digestion with DNase or RNase significantly reduced the peak of silicon associated DNA or RNA. Although aluminum was not found in the embryos of plant species except thea and maize, silicon was found in the embryos of all tested plant species. These results indicate that silicon combines with DNA and RNA in the PNA of plant embryos, suggesting that silicon is essential for plant reproduction.

Key words: silicon, DNA, RNA, plant embryo

Introduction

Silicon deficiency symptoms have been observed during the reproductive stage in many plants [1, 2, 3, 4], yet silicon has not been recognized as an essential element for plant nutrition. One reason for this is the limited information about organic compounds containing silicon that play important roles in plants. Silicon deficiency during the rice reproductive stage decreases the number of spikelets, increases hull sterility, and lowers yield weights [5, 6]. These effects have been attributed to the lower rates of photosynthesis that result from ineffective light reception due to insufficient accumulation of silicon in the cuticular layer of leaves [7]. However, reduced pollen fertility in soybean and cucumber plants [2, 3], failure of pollination and malformed tomato fruit [1], and the slow growth of rice panicles under severe silicon deficiency [8] are all reproductive stage-related abnormalities that would be difficult to explain by reduced photosynthesis. Rather, these phenomena suggest a developmental role for silicon.

Organic-silicon compounds are not detectable by IR or NMR from whole ground rice plants [9,

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10], but cell wall extracts apparently contain silicon in association with lignin- or phenol- carbohydrate complexes [11, 12]. Furthermore, silicon deficiency at the panicle stage results in a decrease in spikelets and kernel weight before heading, possibly because of the suppression of cell division in the spikelets at the panicle stage due to small nuclei and low DNA content [8]. Silicon may be required in combination with the polynucleic acids (PNA) of spikelet cells during the panicle stage, but its form is unclear [8, 13, 14]. Plant embryos, which provide all of the essential elements required for growth between germination and the establishment of a root system, were used to clarify the form in which silicon is associated with PNA.

Materials and Methods

Polynucleic acids extraction and molecular sieve column chromatography

PNA were extracted from the embryos of rice and peanut seeds by the phenol-dodecylsulfate method [15]. The $Abs_{230/260}$ and $Abs_{280/260}$ values of the extract were below 0.55. The PNA solution was loaded onto a Sephacryl-400 (16 x 850mm) column and eluted with about 250 ml of 0.05M tris-buffer (pH 7.4) at 1mL min^{-1}, and 3.2mL fractions were collected in 70 test tubes. Abs_{260}, DNA, RNA and Si concentrations were determined for each fraction.

RNase and DNase digestion

50mg of either RNase or DNase was added to 1mL of the PNA solution and incubated for 20min at 37℃ and then a protease was added and incubated with the solutions for 20min at 37℃ to stop nuclease activity. The solutions were then extracted with an equal volume of 90% phenol solution and precipitated with sodium acetate and ethanol. Extracted DNA and RNA were redissolved in nuclease-free tris-buffer (pH 7.4).

General analysis

DNA concentrations were determined by the diphenylamine method, RNA concentrations were determined by the orcinol method [15] and silicon concentrations were determined by atomic absorption with a graphite furnace and metal element after wet-decomposition of embryos by atomic absorption method.

Results

Silicon concentrations in rice and peanut embryos and PNA from both embryos

Silicon concentrations were more than 7 times higher in rice embryos than in peanut embryos (table 1). However, silicon concentrations in PNA from peanut embryos were nearly equal to rice plant concentrations.

Si associated with rice and peanut nucleic acids

Abs_{260} peaks were observed in rice embryo Sephacryl-400 fractions (F.) 22, 31 and total volume (V_t), and in the void volume (V_0), F. 23 and 41 from peanut plants (figure 1). RNA peaks were also observed in F. 22 and 31 from rice and in V_0 and F. 23 from peanut, but were not observed in the rice V_t or in F. 41 from peanut. A DNA peak co-eluted with Abs_{260} peaks in the rice V_t and in F. 41 of peanut. Silicon peaks coincided with one of the Abs_{260} peaks in each plant.

In rice embryos, the ratio of silicon to RNA was 0.01 from Fs. 21 to 26, and in the range of 0.01

Table 1. Silicon contents of rice and peanut embryos, and polynucleic acids (mg kg^{-1}).

	Embryo	PNA[a] (DNA + RNA)
Rice	24.0	4.2×10^3
Peanut	3.4	4.4×10^3

[a]polynucleic acids

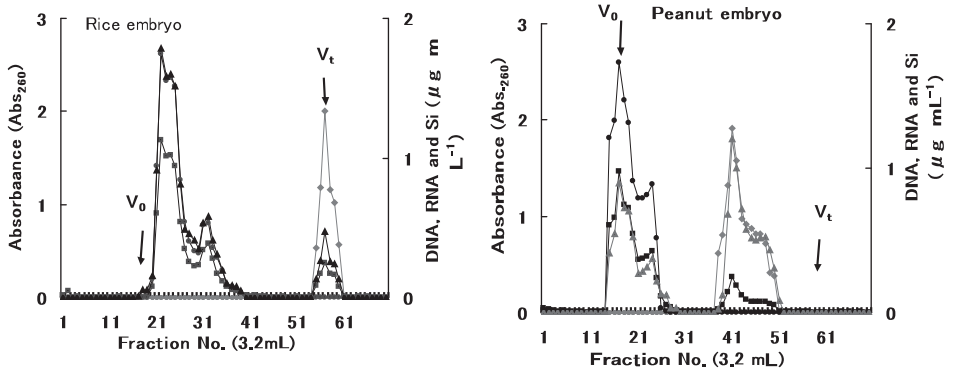

Figure 1. Molecular sieve chromatograms (Sephacryl-400) of polynucleic acids extracted from rice and peanut embryos.
V_0: void volume; V_t: total volume
■: Abs_{260}, ◆: RNA, × 0.01, ●: DNA, × 0.1, ▲: Si

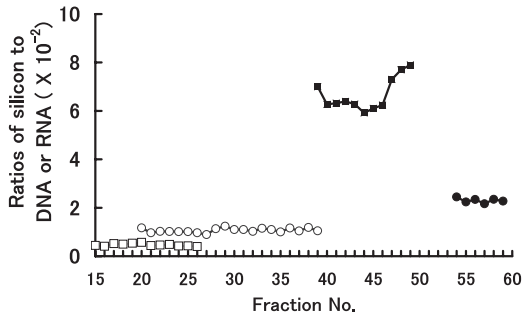

Figure 2. Silicon ratios to DNA or RNA in each fraction.
○: rice-RNA, □: peanut-RNA
●: rice-DNA, ■: peanut-DNA

to 0.012 from Fs. 28~39, and the silicon to DNA ratio was 0.022 (figure 2). In peanut embryos, the ratio of silicon to RNA was in the range of 0.005 to 0.0058 from Fs. 17 to 20 and 0.004 to 0.0005 in Fs. 21~26. The silicon to DNA ratio was 0.06.

RNase and DNase digestion

Silicon and Abs_{260} chromatograms from the Sephacryl-400 column after RNase or DNase digestion are shown in figure 3. RNase-digested PNA had Abs_{260} values that were remarkably decreased compared to Fs. 21-31 without digestion, but the silicon peak associated with DNA in the V_t remained. DNase treatment resulted in the disappearance of the V_t, Abs_{260} peak, but the two silicon peaks from F.

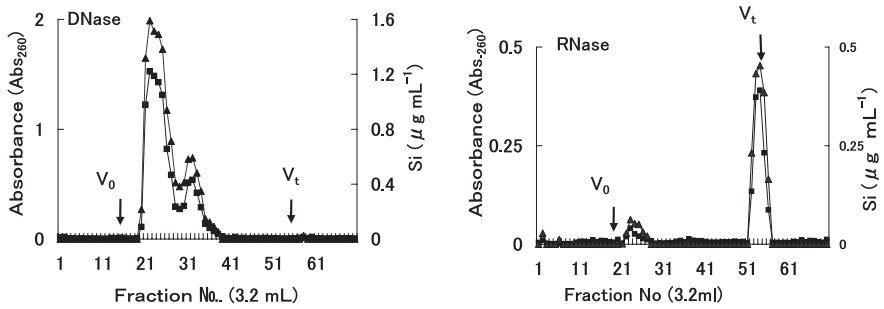

Figure 3. Molecular sieve chromatograms (sephacryl-400) after DNase or RNase digestion of polynucleic acids extracted from rice embryos.
V_0: void volume, V_t: total volume
■ : Abs260, ▲ : Si

Table 2. Some element contents in embryo of 10 plants.

Family	Species	K	Mg	Ca	Mn	Zn	Fe	Al	Si
		(g kg^{-1})			(mg kg^{-1})				
Graminales	Rice	20.9	2.5	4.1	114.1	68.4	5.2	0.1	24.1
	Wheat	22.1	4.2	16.6	228.2	95.3	22.6	0.3	7.5
	Maize	9.3	3.1	4.2	28.1	5.1	3.9	1.1	0.1
Legume	Soybean	20.1	3.2	1.7	18.4	27.7	5.2	0.1	9.4
	Peanut	16.1	8.9	3.2	25.1	59.3	2.5	0.2	5.4
	Broad bean	28.6	1.3	1.5	30.1	88.4	12.1	0.1	3.3
Cucurbitaceae	Pumpkin	15.9	4.6	2.7	20.1	35.7	7.3	0	2.1
	Sechum	14.2	3.8	4.3	21.1	58.6	9.2	0	10.9
Theaceae	Thea	20.8	2.6	1.1	28.1	5.1	3.2	2.2	7.7
Ginkgoalea	Ginglo nut	22.2	1.5	2.1	19.4	0.8	8.2	0	1.4

20 and F 39 remained.

Some elements in embryos from 10 plants

The metal ion contents of embryos collected from the seeds of rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), soybean (*Glycine max* L.), peanut (*Arachis hypogea* L.), broad bean (*Vicia faba* L.), pumpkin (*Cucurbita moschata* L.), sechum (*Momordica charanta* L.), thea (*Camellia sinesis* L.) and ginkgo nut (*Ginkgo biloba* L.) indicate that potassium concentrations are the highest among the determined elements in every embryo (Table 2). Calcium concentrations exceeded magnesium, and manganese concentrations were higher than zinc in the *Graminales*, whereas in legumes and the *Cucurbitaceae*, calcium concentrations were lower than magnesium, and manganese concentrations were lower than zinc. In all cases, iron was at lower concentrations than manganese and zinc. All of the embryos contained silicon, but its concentrations were always lower than manganese or zinc, at about the same level as iron. Aluminum, being necessary for the growth of tea plant, was only found in tea and maize.

Discussion

In plants, the essential elements needed for seedling growth are concentrated in the embryo. As shown in table 1, some silicon was also in peanut embryos, a calciphilous plant. Silicon has thus far been considered unnecessary for calciphilous plants. We previously reported that there are significant concentrations of silicon in the PNA of growing rice spikelets [8, 14]. It is interesting that silicon concentrations in the PNA of peanut embryos are essentially the same as in rice embryos (table 2).

Generally, poly-DNA eluted to the forward of RNA in the molecular sieve chromatography. We observed that when loading PNA immediately after extraction onto the Sephacryl-400, RNA eluted with DNA, and that DNA decreased during reservation as PNA precipitation in the freezer at -20℃ (did not show as data). As shown in figure 1, RNA eluted to the forward of DNA, suggesting that a piece of DNA might result by decomposition of poly-DNA during reservation at -20℃ for any days.

PNA from both rice and peanut eluted in the same fractions as silicon, and peak Abs. In both chromatograms, two Abs_{260} peaks consisted of RNA, and a later peak contained DNA (figure 1). The ratios of silicon to RNA or DNA were different between both embryos and nucleic acids, but each had almost constant values among the fractions (figure 2). Furthermore, digestion with RNase or DNase reduced the presence of silicon to nearly undetectable amounts in fractions corresponding to RNA or DNA, suggesting that silicon combines with DNA and RNA in the PNA of plant embryos.

Silicon was present in higher concentrations in association with DNA than with RNA. Kinrade et.al. demonstrated that silicon forms a complex with ribose [16], and that in diatom silicon was coordinated with at least one nitrogen [17]. A bathochromic effect in UV spectra, shifting to a longer wavelength by the insertion of a metal ion into an H-bond in the double chain of DNA, and absorbance bands at 1208, 1137 and 810 cm^{-1} in the IR spectra, thus indicating the presence of $Si\text{-}OCH_3$, was observed in the PNA from spikelets of rice 5 days before heading in a silicon treatment [14]. This suggested that silicon may not only play a role in the stability of DNA structure, but also that silicon may associate with the nucleoside moiety of DNA and RNA.

Under severe silicon deprivation conditions during the reproductive stage, pollen fertility was lower in soybean and cucumber, and tomato plants failed to pollinate, or produced malformed fruit [1, 2, 3]. Rice plants cultured under conditions of silicon deficiency have decreased amounts of DNA, and nucleus formation is depressed in spikelets [8, 14]. Although peanut embryos contained less silicon than rice, the DNA in its embryos contained more than 3 times as much as rice embryo DNA. If silicon is a generally essential element for plant growth, it is likely to be concentrated in the embryos of other plants. There are, however, large differences in silicon concentrations and the concentrations of other metal and transition metals among families or species (table 2). Although there was no aluminum in embryos except for thea and maize, silicon was found in the embryos of all 10 species, suggesting that physiological injuries observed in the reproductive organs of calciphilous plants may be due to silicon deficiency [1. 2. 3. 4] because of abnormal cell division.

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水稲およびラッカセイの胚から抽出された高分子核酸中のケイ素の存在形態

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要　約

生殖成長期にケイ素が不足すると，植物には不稔，果実の奇形などが発生する。水稲では幼穂形成期にケイ素が欠如すると小穂のDNA，核画分が低下し，幼穂形成期にケイ素は核画分の高分子核酸（PNA）に多く存在した。

本研究では，水稲およびラッカセイの胚から抽出されたPNAに含まれるケイ素の存在形態を分子篩クロマトグラフィ法を用いて調べると共に，ケイ酸植物，石灰植物，好アルミニュウム植物など10種の胚中に含まれる数種の元素含有量を比較検討した。

水稲およびラッカセイ胚から抽出されたPNAに含まれるケイ素濃度はほぼ等しかった。両種のPNAをSephacryl-400で分画すると，Abs_{260}のピークが高分子画分と低分子画分に観察され，ケイ素のクロマトグラムはAns_{260}のそれらと一致した。前者のピークはRNA，後者はDNAであった。各フラクション中のRNAまたはDNAに対するケイ素の比はほぼ一定の値を示し，両種ともSi/DNAがSi/RNAよりも高い値を示した。Si/DNはラッカセイが，Si/RNAは水稲が高かった。水稲胚からのPNAをRNなaseまたはDNase処理すると，ケイ素のピークはAbs_{260}とともに小さくなるかまたは消失した。

植物の初期成育に必要な無機養分が濃縮されている胚の数種の元素の分析結果，アルミニュウムはトウモロコシと好アルミニウム植物の茶の胚に含まれたがそれ以外の植物胚にはほとんど含まれなかったのに対し，ケイ素はケイ酸植物の水稲が著しく高かったが，他の植物胚にも含まれた。

これらの結果は，植物胚ではケイ素の一部は，DNA，RNAと結合して存在することを示スト共に，生殖成長聞に観察されるケイ素欠如による不稔，奇形果などの発生は，不十分なDNA形成に伴う細胞分裂の抑制こ起因していることを示唆している。

キーワード：ケイ素，DNA，RNA，植物胚

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