Title

Induction of ultrastructural specialization for ureide metabolism in non-nodule soybean tissues cultured *in vitro*

Authors

Nayeema Bulbul, Miei Sakurai, Hisashi Matsushima, Yasuko Kaneko

Address

Graduate School of Science and Engineering, Saitama University, Saitama 338-8570, Japan.

Correspondent foot note

Corresponding author: Dr. Yasuko Kaneko

Address: Biology Section in Faculty of Education, Saitama University, Saitama 338-8570, Japan

Fax: +81-48-858-3690

Tel: +81-48-858-3218

E-mail: yakaneko@mail.saitama-u.ac.jp

Abstract

Root nodules of tropical legumes, such as beans and soybeans, produce ureides from nitrogen fixed by bacterial symbionts. Ultrastructural specialization for ureide production, including the appearance of abundant tubular ER and marked enlargement of peroxisomes, is known to occur in uninfected cells in the root nodule. Here we have investigated the capability of non-nodule tissues of soybean to specialize for ureide metabolism. Soybean embryonic axes excised from imbibed seeds were cultured *in vitro* on media with various nitrogen sources. The axes grew well on media containing urate or allantoin as the sole nitrogen source, but grew poorly on media with asparagine or glutamine as the sole nitrogen source. Root tissue and callus induced from segments of soybean embryonic axes also grew well on media containing urate or allantoin as the major nitrogen source. Numerous peroxisomes were observed in the root tissue and in proliferating callus cells growing on media containing urate as the major nitrogen source, whereas abundant ER was observed in the root tissue and callus cells growing on media containing allantoin. The capacity of non-nodule tissue cells to ultrastructurally differentiate for metabolizing externally supplied urate or allantoin was demonstrated.

Keywords:

Soybean tissue culture; Ureides; Peroxisomes; Endoplasmic reticulum (ER); TEM

Abbreviations:

BA, benzyladenine; DAB, diaminobenzidine; ER, endoplasmic reticulum; NAA, naphthaleneacetic acid; TEM, transmission electron microscope.

1. Introduction

Many leguminous plants, including important crops, produce root nodules in which biological nitrogen fixation takes place. Organic nitrogenous compounds formed by N_2 fixation can be transported to the upper parts of the plant either as amides or ureides (allantoin and allantoic acid), so that legume nodules are classified as amide exporters or ureide exporters according to the compounds used for the mobilization of fixed nitrogen [1]. Soybeans develop globular nodules and synthesize ureides from fixed nitrogen. It is known that the uninfected cells in the nodules play an important role in the final steps of ureide biogenesis. For this purpose, they undergo ultrastructural differentiation, which includes the appearance of abundant tubular endoplasmic

reticulum (ER) and a marked enlargement of peroxisomes [2-4]. Urate derived from xanthine dehydrogenase action in infected cells in the nodules moves to the uninfected cells where it is oxidized by uricase and catalase in the peroxisomes, eventually forming allantoin [5]. Allantoinase (EC 3.5.2.5) catalyzes the hydrolysis of allantoin to form allantoic acid, which is a key reaction for biogenesis and the degradation of ureides. Localization of nodule specific uricase in the peroxisomes has been shown immunocytochemically [4, 6]. Since allantoinase was found in the microsomal fraction [7], it is assumed to be localized in the abundant tubular ER in the uninfected cells. Recently, the gene coding for allantoinase has been identified in Arabidopsis and Robinia [8] and allantoinase was shown to be localized in the ER in Arabidopsis [9]. However, cellular specialization of these tissues has not been observed at the ultrastructural level.

In order to better understand the mechanisms which furnish the capacity to produce and utilize ureides and the accompanying cellular differentiation outside of nodules, callus and root cultures of soybean were initiated from embryonic axis segments grown on media containing either urate or allantoin as the major nitrogen source and the resulting ultrastructural differentiation of these tissues was investigated.

2. Materials and methods

2. 1. Culture of embryonic axes

Seeds of *Glycine max* (L.) Merr. cv. Mikawashima and *Pisum sativum* L. cv. Denko 30 nichi were disinfected in 70% (v/v) ethanol for 10 minutes, 1.2% sodium hypochlorite for 15 minutes and then washed in sterile distilled water 6 times. Embryonic axes were excised from the sterilized seeds and cultured on MS [10] based media (pH 5.7) with the respective nitrogen source and kept under continuous light (15 Wm-2, FL40S fluorescent lamps, Toshiba, Tokyo, Japan) at 25±2 °C. The media used in this experiment were MS medium, MS without nitrogen source, and MS with one of the following as the sole organic nitrogen source: asparagine, glutamine, xanthine, urate or allantoin at a concentration of 12 mM each. All media also contained 3% sucrose and 0.8% agar.

2. 2. Root culture from embryonic axes

In order to identify the cells with specialized structure for ureide metabolism, we tried to establish a simpler culture system producing only root or callus. However, as consistent results were difficult to obtain with the media for embryonic axes, we compromised and adopted the following media for this purpose. The embryonic axes were excised from the disinfected *G. max* cv. Mikawashima seeds (as described above)

and sliced into 1 mm thick segments. The segments were cultured on B5 [11] medium (pH 5.7) in which the inorganic nitrogen (KNO_3, $(NH_4)_2SO_4$) was reduced to 1/20 of its original content and the organic nitrogen sources 4 mM urate or 4 mM allantoin (modified from Shetty *et al.* [12]) with 0.01 ppm NAA, 0.001 ppm BA, 3% sucrose and 0.2% gelrite were added. The culture was maintained at 25±2°C under continuous light as above. Root segments for observation were taken 7 days after initiation of culture.

2. 3. Callus induction from embryonic axes

The embryonic axes were excised from the disinfected *G. max* cv. Mikawashima seeds (as described above) and sliced into 1 mm thick segments. The segments were cultured on B5 medium (pH 5.7) with exactly the same replacement of nitrogen sources, etc, as above, but with 1 ppm NAA and 0.01 ppm BA. The culture was maintained at 25±2°C under continuous light as above. Induced calli were subcultured every two weeks with the same media. Calli for observation were taken after three successive subcultures.

2. 4. Processing for light and electron microscopy

The root segments were cut with a razor blade and the callus was divided into small pieces (less than 2 mm in diameter), which were fixed with 2% glutaraldehyde in 0.05 M potassium phosphate buffer (pH 6.8) for 2 h at room temperature and at 4°C

overnight. They were rinsed with the same buffer and post-fixed in 2% OsO_4 in 0.05 M potassium phosphate buffer (pH 6.8) for 2 h at room temperature, dehydrated in an acetone series and embedded in Spurr's resin. For light microscopy, sections about 0.5 μm in thickness were stained with a mixture of 0.05% toluidine blue and 1% sodium borate. For TEM, ultrathin sections (silver-gold) were stained with aqueous 2% uranyl acetate for 10 min followed by lead citrate for 5 min and observed with a Hitachi H-7500 TEM at an accelerating voltage of 100 kV.

2. 5. Uricase cytochemistry with DAB

We used DAB for the cytochemical localization of uricase (EC 1.7.3.3) according to the method of Yokota and Nagata [13] as modified by Kaneko and Newcomb [3]. The callus and root cultures grown on urate containing medium were fixed in 2% glutaraldehyde in 0.05 M potassium phosphate buffer (pH 6.8) for 30 min at 0°C and rinsed in the same buffer for 30 min at 0°C. The standard incubation medium consisted of 6 ml of 0.1 M Tris HCl buffer (pH 9.6), 4 ml of a solution of sodium urate (5 mg/ml) and 10 mg of DAB (3, 3' diaminobenzidine, Sigma Chemical Company). The segments were incubated for 90 minutes at 30°C in a reciprocal incubator at 60 rpm. In order to ensure aeration, the segments were placed in 30 ml beakers with a small amount (1.5 ml) of medium so that the surface of the segments made contact with air frequently

during incubation. Control incubation was carried out in medium lacking urate. Following incubation, the segments were rinsed in 0.05 M potassium phosphate buffer (pH 6.8) and then post-fixed in 2% OsO_4 in 0.05 M potassium phosphate buffer (pH 6.8) for 1 hour at room temperature, dehydrated in an acetone series and embedded in Spurr's resin. Ultrathin sections were observed with a Hitachi H-7500 TEM at an accelerating voltage of 100 kV.

2.6. Statistical Analysis

For statistical analysis, one-way ANOVA, Holm-Sidak method was performed using the SigmaStat program.

3. Results

3. 1. Effect of different nitrogen sources on the growth of soybean and pea embryonic axes in vitro

Growth of *G. max* and *P. sativum* embryonic axes cultured *in vitro* with various nitrogen sources was compared. The media used were MS based media in which nitrogen compounds had been replaced by a single organic nitrogen source, either asparagine, glutamine, urate, allantoin, or xanthine. A typical result of 5 separate experiments is presented in Fig 1. In the case of soybean, embryonic axes grew well on

MS media containing urate or allantoin as the sole nitrogen source, but grew poorly on the media containing asparagine or glutamine as the sole nitrogen source (Fig 1 a). On the other hand, pea embryonic axes grew well on MS media containing asparagine or glutamine as the sole nitrogen source and grew poorly on the media containing urate or allantoin as the sole nitrogen source (Fig 1 b).

3. 2. Induction of roots and callus tissue from segments of soybean embryonic axes on media containing various nitrogen sources

Since it was difficult to locate the cells specialized for ureide metabolism in the plantlets developed in the embryonic axis culture, we reduced the amount of tissue for observation by using simpler systems, that is root and callus cultures. Since we could not obtain consistent growth of roots or callus from the thin sliced embryonic axis segments with the media used for whole embryonic axes, we compromised and used B5 media with a much reduced amount of inorganic nitrogen and with allantoin or urate as the major nitrogen source. Root cultures (Fig 2 a-c) and calli (Fig 2 d-f) were induced successfully from segments of soybean embryonic axes with these media. The concentrations of NAA and BA in the media were determined experimentally by culturing soybean embryonic axis segments on B5 media with combinations of NAA (1, 0.1, 0.01, 0.001, 0 ppm) and BA (1, 0.1, 0.01, 0.001, 0 ppm). Roots were most

frequently produced with 0.01 ppm NAA and 0.001ppm BA, and callus production was best with 1 ppm NAA and 0.01 ppm BA. These concentrations were thus used for the subsequent experiments.

When the segments were grown on B5 media, where inorganic nitrogen had been reduced to 1/20, but which contained either 4 mM urate or allantoin in the presence of 0.01 ppm NAA and 0.001ppm BA, roots were produced in all the media mentioned above (Fig 2 a-c). However, the best growth was observed on B5 with 4 mM allantoin (Fig 2 b) and some vigorous root growth was also observed on media containing 4 mM urate (Fig 2 c). While roots grew fairly well without additional organic nitrogen sources at the early stage of culture (Fig 2 a), they stopped growing earlier (before three weeks of culture) compared to root cultures with urate or allantoin, which continued to grow almost one month (Fig 3). After 4 weeks of culture, roots grown on media containing urate or allantoin were significantly longer than those grown on media without additional organic nitrogen (one way analysis of variance test, Holm Sidak method; $P < 0.001$). The experiments were repeated five times and similar results were obtained.

When the segments were grown on the same media as above but with different concentrations of 1 ppm NAA and 0.01 ppm BA, calli developed in each case (Fig 2 d-f). Calli proliferated well on media containing allantoin (Fig 2 e) or urate (Fig 2 f) as

the major nitrogen source. Callus was induced on the media without additional nitrogen source (Fig 2 d), although the color remained brownish and it did not grow vigorously. The experiments were repeated five times and similar results were obtained.

3. 3. TEM observation

The ultrastructure of the parenchyma cells in the vascular cylinder encircled by endodermis in root cultures grown on media with different nitrogen sources is shown in Fig 4 a-c. Proliferation of ER was prominent in the cytoplasm of the cells grown on allantoin containing media (Fig 4 b). In the soybean root culture grown on urate containing media, numerous peroxisomes were observed in these cells as shown in Fig 4 c. Rarely, peroxisomes were observed in root culture cells on other media (Fig 4 a, b).

The typical ultrastructure of calli grown on media with different nitrogen sources is shown in Fig 4 d-f. Calli grown on media with allantoin developed abundant endoplasmic reticulum, both rough and tubular (Fig 4 e). We encountered many single cells in which several regions of transition from rough to tubular ER could be observed (Fig 4 e asterisk). On the other hand, callus grown on urate developed numerous peroxisomes (Fig 4 f, arrows) and the ER frequently appeared to be in close association with the peroxisomes.

3. 4. Cytochemical localization of uricase in the cultured tissue

Uricase activity in the cultured tissue was investigated by incubating root and callus segments in a medium containing DAB and urate [3]. The action of uricase on urate produces H_2O_2, which serves as the substrate for catalase in the oxidation of DAB and concomitant precipitation of osmium black. This resulted in electron opaque staining in the peroxisomes that had developed in root and callus cells grown on urate containing media (Fig 5 a and c) and thus demonstrated simultaneously the presence and localization of uricase and catalase activities. This electron opaque staining was not seen in peroxisomes incubated in the control medium lacking urate (Fig 5 b and d). Deposits of electron opaque material were frequently seen in the mitochondrial cristae of both the control and the standard uricase reaction, which are assumed to be artifacts irrelevant to uricase activity.

4. Discussion

The availability of reduced nitrogen is an important determinant in the growth and development of plants. Tropical and subtropical legumes like cowpea (*Vigna unguiculata)*, soybean (*Glycine max*) and French bean (*Phaseolus vulgaris*) transport large amounts of nitrogen in the form of ureides such as allantoin and allantoic acid. In other legumes, amides are exported (reviewed by Todd *et al.* [14]). Our comparison of

nitrogen sources revealed that soybean tissue was more efficient in using external ureides than in using external amides (Fig 1 a). We confirmed that the growth differences were not caused by the higher nitrogen content of ureides (twice that of amides), by performing experiments with increased amounts of amides in the media (up to threefold), in which no enhanced growth of soybean embryonic axes occurred (data not shown). It has previously been observed that when non-nodulated soybean plants were supplied KNO_3 nutrient solution, asparagine and nitrate content in the xylem sap was much higher than allantoic acid content [15]. Our result is consistent with this, since soybean embryonic axes grew well on MS medium where inorganic N consists of KNO_3 and NH_4NO_3 as seen in Fig 1 a. Conversely, pea embryonic axes did not grow well on urate or allantoin (Fig.1 b). It has previously been shown that a portion of ureide N is released as urea in nitrogen fixing soybean plants [14]. Absence of Ni in the media may have suppressed urease activity since the enzyme requires Ni, resulting in a toxic accumulation of urea that hampered growth in the case of the pea cultures. It is conceivable that soybeans are able to process allantoin without accumulating urea [16, 17]. The allantoin transporter PvUPS1 has recently been isolated from French bean [18]. PvUPS1 expression was also detected in the roots of plants grown with allantoin as the sole nitrogen source, indicating a role of PvUPS1 in allantoin uptake and

translocation to root or shoot. PvUPS1 mRNA has been localized in the endodermis surrounding the vasculature as well as in the phloem [18]. Although no similar transporter has been described in soybeans until now, such a transporter may exist in soybean root tissue. Similarly, there might be specific amide transporters involved in taking up amides by root tissue of pea.

We looked for cellular differentiation accompanying the specialization for ureide production in the soybean tissue, since ultrastructural specialization for ureide production, including marked enlargement of peroxisomes and proliferation of tubular ER in uninfected cells of soybean root nodules, has been demonstrated previously [2]. Uricase was immunocytochemically localized in peroxisomes of soybean cotyledons using antibodies against nodule uricase [19]. However, localization of uricase in non-nodule root tissues has never been demonstrated. Proliferation of tubular ER in response to nitrogen metabolism has not been seen outside of nodule tissue either.

Our goal was to develop a simple system where the ultrastructural specialization for ureide metabolism could be demonstrated easily. For that purpose, whole embryonic axes generating both shoot and root seemed to be overly complicated. Therefore, we chose tissue culture material for ultrastructural observation. We decided to use B5 media with a reduced amount of inorganic nitrogen to look for the effect of the extra

supply of organic nitrogen [12], since we had initiated a series of experiments to determine the most appropriate combination of various NAA and BA concentrations for callus induction and root culture using B5 based media. B5 based media have been used extensively for experiments on organogenesis from soybean embryonic tissue (e.g. Yoshida [20]). Although both root and callus grew on the media without addition of organic nitrogen, we conclude that they are utilizing allantoin or urate when these are added to the media for the following reasons: 1) root cultures with extra allantoin or urate grow for a longer period of time and 2) calli grow significantly better when allantoin or urate are supplied.

When roots or calli were grown on media with 4 mM urate, numerous peroxisomes were observed in vigorously proliferating callus cells as well as in root tissue, especially in parenchyma cells in vascular cylinders. Uricase and catalase activities in these peroxisomes could be demonstrated by cytochemistry with DAB and urate. It seems that soybean tissue is able to respond to exogenously supplied urate by producing peroxisomes to process urate to allantoin. There has been significant progress recently in the elucidation of the mechanism of peroxisome biogenesis (for a review see Titorenko *et al* [21]). It has become clear that the peroxisomal membrane

derives from the ER. Urate taken up by cells may be able to serve as a signal inducing biogenesis of peroxisomes specialized for oxidation of urate.

A cell fractionation study by Hanks *et al.* [7] has indicated that allantoinase is localized in the endoplasmic reticulum (ER). In most plant cells, rough ER predominates and tubular ER is a minor component. However, unusual development of tubular ER was observed in the uninfected cells of root nodules of soybean [2], cowpea [4], and black locust [22]. This tubular ER is believed to play a role in the conversion of allantoin to allantoic acid in determinate nodules. Similar tubular ER was observed in the root culture and calli grown on allantoin in our study (Fig 4 b and e), which may possess allantoinase to process exogenously supplied allantoin. Fluorescent fusion protein of allantoinase was localized in the endoplasmic reticulum indicating that after the generation of allantoin in the peroxisome, plant purine degradation continues in the endoplasmic reticulum [9]. Our results support this finding.

The present study indicates that soybean cultured tissue has the capability to adapt to the available ureide or urate (but not amide) nitrogen source, and develop specialized ultrastructure with accompanying functions. When urate is the major nitrogen source, cells develop numerous peroxisomes to produce allantoin, whereas if allantoin is the major nitrogen source, they develop prominent tubular ER, which

harbors allantoinase. In the case of cultured root tissue, cellular specialization for ureide metabolism was mostly observed in vascular parenchyma cells, which makes sense if the putative allantoin transporter in soybean also localizes in the endodermis, as in the case of the French bean [18]. It would be interesting to determine the localization of the allantoin transporter and allantoinase in the soybean at the ultrastructural level, in order to better understand the significance of ER proliferation and elucidate the associated cellular mechanisms.

We conclude that soybean tissues, even in the absence of root nodules and nitrogen fixation, but if provided with an appropriate organic nitrogen source, are capable of ultrastructural specialization similar to that of the uninfected cells of determinate root nodules to metabolize ureides. We further speculate that in ureide metabolizing root nodules, the presence of urate may be a signal to develop peroxisomes for metabolizing urate and the presence of allantoin may be a signal to induce the proliferation of allantoinase containing tubular ER.

References

[1] K.R. Schubert, Products of biological nitrogen fixation in higher plants: synthesis, transport and metabolism, Ann. Rev. Plant Physiol. 37 (1986) 539–574.

[2] E.H. Newcomb, S.R. Tandon, R.R. Kowal, Ultrastructural specialization for ureide production in uninfected cells of soybean root nodules, Protoplasma 125 (1985) 1–12.

[3] Y. Kaneko, E.H. Newcomb, Cytochemical localization of uricase and catalase in developing root nodules of soybean, Protoplasma 140 (1987) 1–12.

[4] M.A. Webb, E.H. Newcomb, Cellular compartmentation of ureide biogenesis in root nodules of cowpea (*Vigna unguiculata* (L) Walp.), Planta 172 (1987) 162–175.

[5] M.C. Smith, C.A. Atkins, Purine biosynthesis: Big in cell division, even bigger in nitrogen assimilation, Plant Physiol. 128 (2002) 793–802.

[6] K.A. VandenBosch, E.H. Newcomb, Immunogold localization of nodule specific uricase in developing soybean root nodules, Planta 167 (1986) 425–436.

[7] J.F. Hanks, K.R. Schubert, N.E. Tolbert, Localization of enzymes of ureide biosynthesis in peroxisomes and microsomes of nodules, Plant Physiol. 68 (1981) 65–69.

[8] J. Yang, K.H. Han, Functional characterization of allantoinase genes from Arabidopsis and a nonureide-type legume Black Locust, Plant Physiol. 134 (2004) 1039–1049.

[9] A.K. Werner, I.A. Sparkes, T. Romeis, C.P. Witte, Identification, biochemical characterization and subcellular localization of allantoate amidohydrolases from Arabidopsis and Soybean, Plant Physiol. 146 (2008) 418–430.

[10] T. Murashige, F. Skoog, A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol. Plant. 15 (1962) 473–497.

[11] O.L. Gamborg, R.A. Miller, K. Ojima, Nutrient requirements of suspension cultures of soybean root cells, Exp. Cell Res. 50 (1968) 151–158.

[12] K. Shetty, Y. Asano, K. Oosawa, Stimulation of in vitro shoot organogenesis in Glycine max (Merrill) by allantoin and amides, Plant Sci. 81 (1992) 245–251.

[13] S. Yokota, T. Nagata, Urate oxidase, In: M.A. Hayat (ed) Electron microscopy of enzymes, vol 5, Van Nostrand Reinhold, New York, 1977, pp. 72–97.

[14] C.D. Todd, P.A. Tipton, D.G. Blevins, P. Piedras, M. Pineda, J.C. Polacco, Update on ureide degradation in legumes, J. Exp. Bot. 57 (2006) 5–12.

[15] P.R. McClure, D.W. Israel, Transport of nitrogen in the xylem of soybean plants, Plant Physiol. 64 (1979) 411–416.

[16] R.W. Stahlhut, J.M. Widholm, Ureide catabolism by soybean [*Glycine max* (L.) Merrill] cell suspension cultures I. Urea is not an intermediate in allantoin degradation, J. Plant Physiol. 134 (1989) 85–89.

[17] N.E. Stebbins, J.C. Polacco, Urease is not essential for ureide degradation in soybean, Plant Physiol. 109 (1995) 169–175.

[18] H.C. Pelissier, A. Frerich, M. Desimone, K. Schumacher, M. Tegeder, PvUPS1, an allantoin transporter in nodulated roots of French bean, Plant Physiol. 134 (2004) 664–675.

[19] B. Damsz, J.M. Dannenhoffer, J.A. Bell, M.A. Webb, Immunocytochemical localization of uricase in peroxisomes of soybean cotyledons, Plant Cell Physiol. 35 (1994) 979-982.

[20] T. Yoshida, Adventitious shoot formation from hypocotyls sections of mature soybean seeds, Breed. Sci. 52 (2002) 1–8.

[21] V.I. Titorenko, R.T. Mullen, Peroxisome biogenesis: the peroxisomal endomembrane system and the role of the ER, J. Cell Biol. 174, 1, (2006) 11–17.

[22] Y. Kaneko, E.H. Newcomb, Specialization for ureide biogenesis in the root nodules of black locust (*Robinia pseudoacacia* L.) an amide exporter, Protoplasma 157 (1990) 102–111.

Figure legends

Fig 1. (a) Soybean embryonic axes cultured for 12 days and (b) Pea embryonic axes cultured for 18 days on MS, MS without inorganic nitrogen, MS without inorganic nitrogen but with either glutamine, asparagine, xanthine, urate, or allantoin (From left to right).

Bar, 1 cm.

Fig 2. Soybean root cultures grown for 7 days (a-c) and soybean calli (d-f) on B5 with 1/20 of inorganic nitrogen: with none (a, d); with 4 mM allantoin (b, e); with 4 mM urate (c, f). Bar, 1 cm.

Fig 3. Growth of the roots from soybean embryonic axis segments cultured on different media. Control: 1/20 B5 N (1.2 mM KNO_3 +1 mM NH_4NO_3); other media contain 1/20 B5 N plus either 4 mM allantoin or 4 mM urate.

Root cultures were grown for one month and the length of the roots grown on media with different nitrogen sources was recorded. Addition of organic nitrogen caused a significant increase in root length (one way analysis of variance, Holm-Sidak method, $P < 0.001$). Values shown represent the mean $\pm$ SE.

Fig 4. Transmission electron micrographs of vascular parenchyma cells of the soybean root cultures grown for 7 days (a-c) and calli (d-f) on B5 with 1/20 of inorganic nitrogen: without organic nitrogen (a, d); with 4 mM allantoin (b, e); or with 4 mM urate (c, f). Occasional peroxisomes (arrows) and some ER (*) are observed in cells cultured on control media (a, d). Proliferation of tubular ER (*) is prominent in cells cultured with allantoin (b, e). Abundant peroxisomes (arrows) are generated in cells cultured with urate (c, f). G, Golgi body; L, lipid body; M, mitochondria; N, nucleus; P, plastids; V, vacuole; *, endoplasmic reticulum (ER); arrows, peroxisomes. Bars, 1 μm.

Fig 5. Cytochemical localization of uricase and catalase with DAB in soybean (a, b) root cultures and (c, d) calli grown on B5 with 1/20 inorganic nitrogen supplemented with 4 mM urate. Electron opaque reaction product is seen in peroxisomes (arrows) in cells incubated with urate and DAB (standard incubation) (a, c). The precipitate is not seen in peroxisomes (arrows) in cells incubated with DAB without urate (control incubation) (b, d). Electron opaque precipitates occur in mitochondrial cristae in cells after both standard and control incubation. M, mitochondria. Bars, 1 μm.

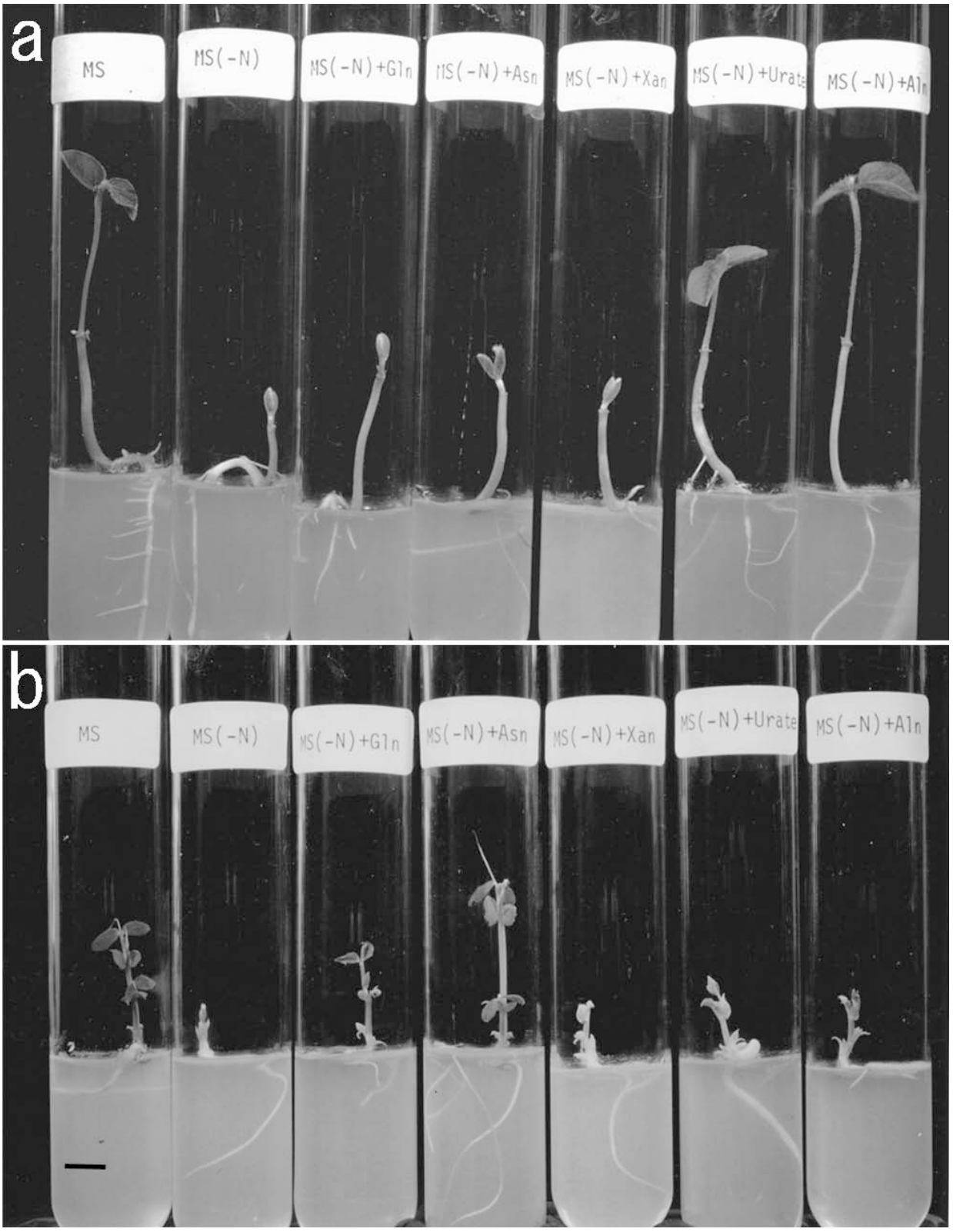
a
MS
MS(-N)
MS(-N)+Gln
MS(-N)+Asn
MS(-N)+Xan
MS(-N)+Urate
MS(-N)+Aln
b
MS
MS(-N)
MS(-N)+Gln
MS(-N)+Asn
MS(-N)+Xan
MS(-N)+Urate
MS(-N)+Aln

Fig.1

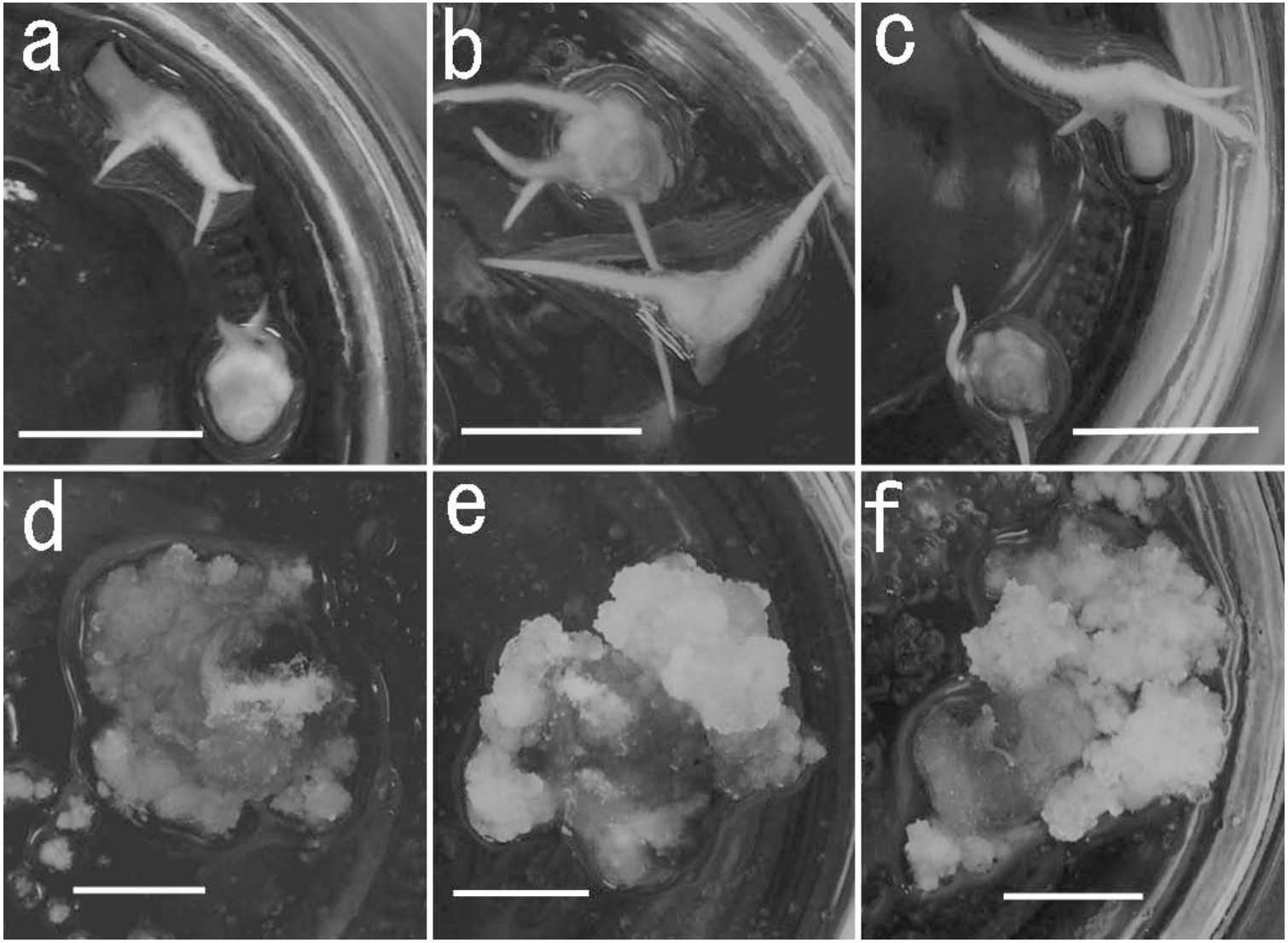

Fig.2

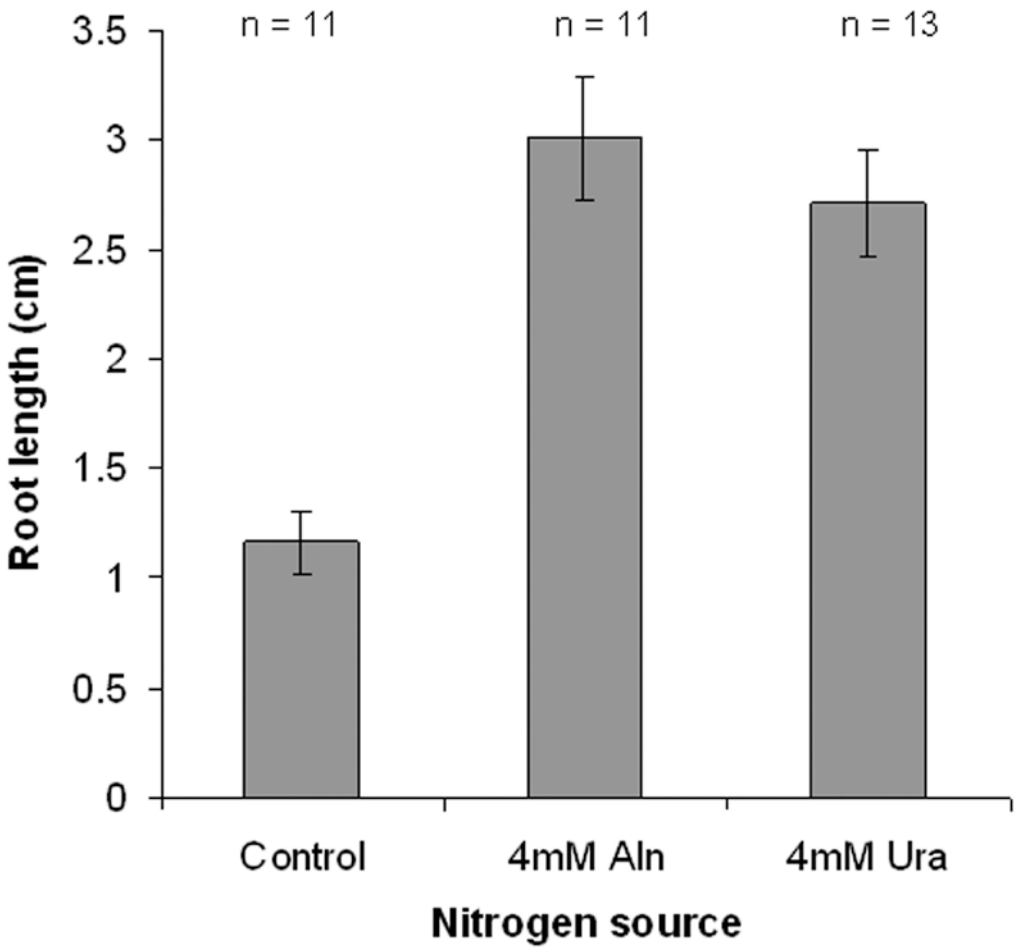
n = 11
n = 11
n = 13
3.5
3
2.5
2
1.5
1
0.5
0
Root length (cm)
Control
4mM Aln
4mM Ura
Nitrogen source

Fig.3

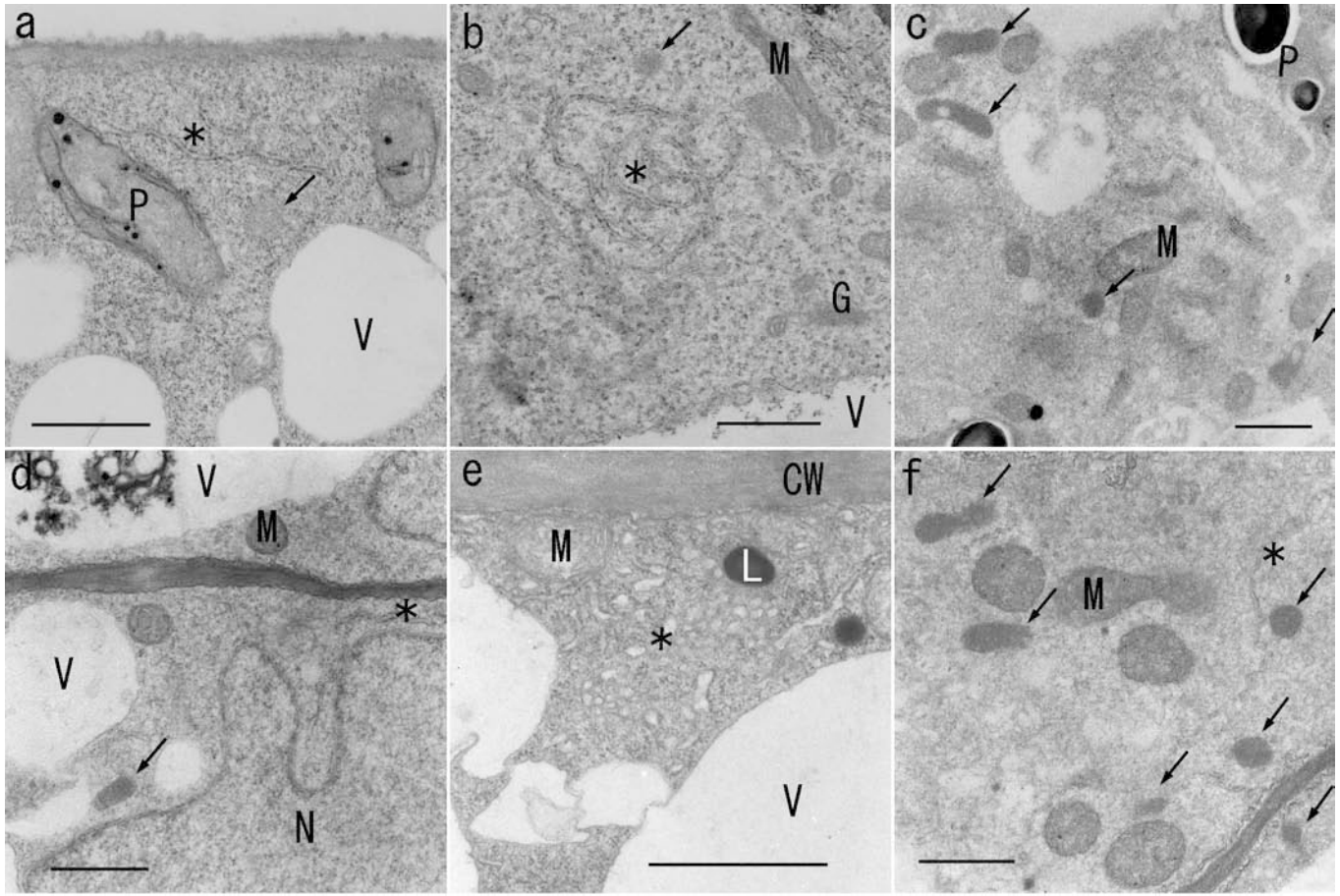

Fig.4

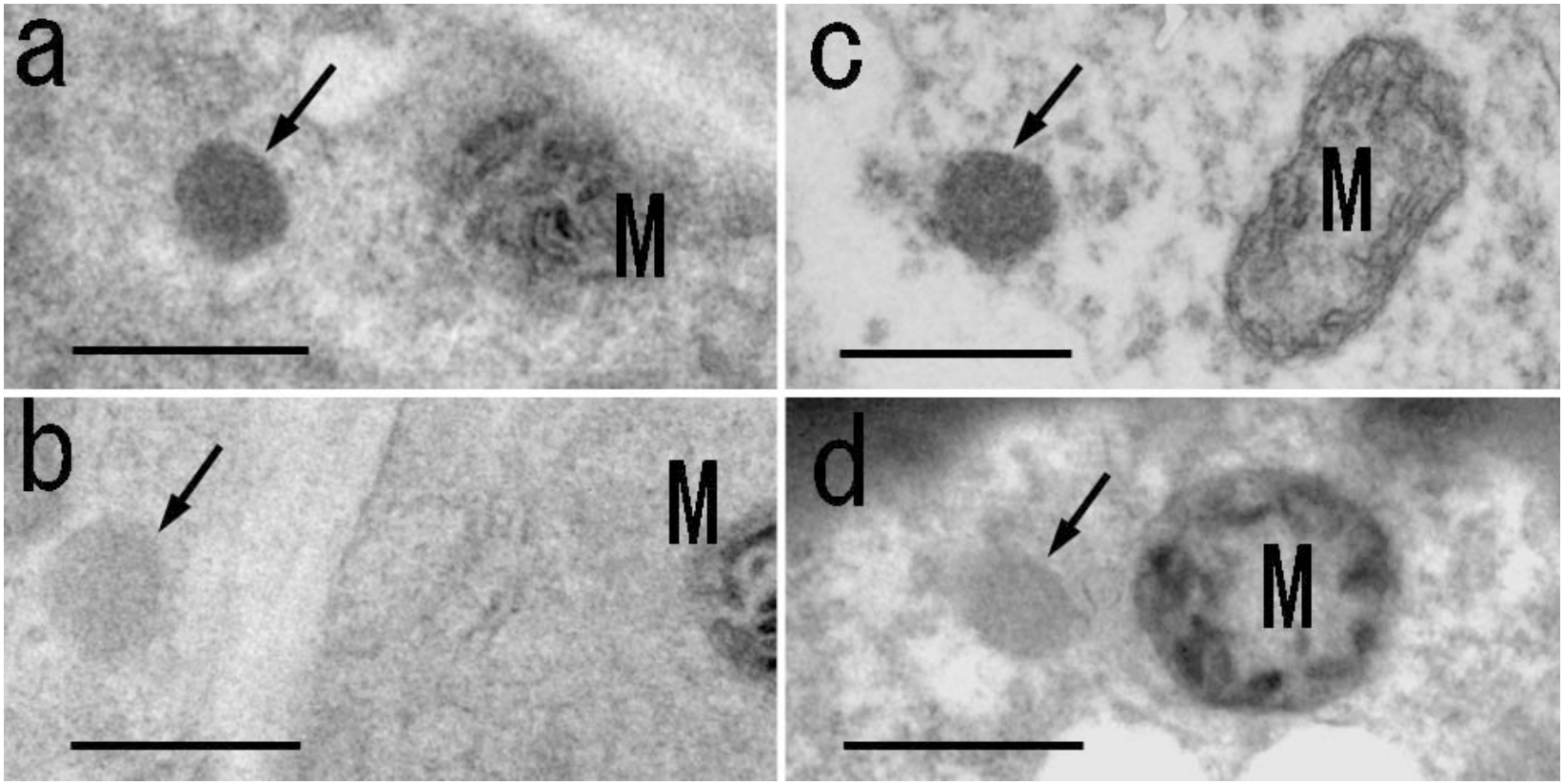

Fig.5