Auxin Polar Transport is Essential for the Early Growth Stage of Etiolated Maize (Zea mays L. cv. Honey Bantam) Seedlings

Junichi Ueda¹, Miyako Sakamoto-Kanetake¹, Yuta Toda¹, Kensuke Miyamoto², Eiji Uheda¹ and Hiroyuki Daimon³

¹Graduate School of Science, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai 599-8531, Japan;
²Faculty of Liberal Arts and Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai 599-8531, Japan;
³Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai 599-8531, Japan)

Abstract: The important role of auxin polar transport in the early growth stage of etiolated maize (Zea mays L. cv. Honey Bantam) seedlings was intensively studied. Rapid growth in the basal region of coleoptile and the apical region of mesocotyl was observed in etiolated maize seedlings in the dark. Indole-3-acetic acid (IAA) applied to the top of coleoptile substantially promoted the growth of coleoptile and mesocotyl dose-dependently, suggesting that endogenous IAA is responsible for the growth of coleoptile and mesocotyl in etiolated maize seedlings. Polar transport of radiolabeled IAA ([¹⁴C]IAA) in the coleoptile segment was significantly higher than that in the mesocotyl segment from etiolated maize seedlings. Auxin polar transport in the coleoptile and mesocotyl segments from etiolated maize seedlings increased with the growth of the seedlings from which the segments were excised. Maximum expression of the gene (ZmPIN1a) closely related to auxin polar transport was observed just prior to maximum levels of auxin polar transport in the coleoptile and mesocotyl segments. In addition, the growth rate was well correlated with the auxin polar transport and the expression of ZmPIN1a gene in coleoptile and mesocotyl of etiolated maize seedlings. These results strongly suggest that auxin polar transport regulated by ZmPIN1a protein is essentially required for the growth of coleoptile and mesocotyl in the early growth stage of etiolated maize seedlings.

Key words: Auxin polar transport, Gene expression, Growth, Indole-3-acetic acid, Maize, Zea mays, ZmPIN1a.

Auxin, a plant hormone, functions as an important factor in plant growth and development such as cell elongation and tropism. Its function is connected with its unique polar movement from cell to cell. This kind of polar movement designated as auxin polar transport is characteristic of auxin and has not been recognized in other plant hormones (Thimann, 1977). Auxin polar transport has been shown to regulate not only plant growth and development but also a gravireponse in plants. The space experiment to clarify the relationships between growth and development, with auxin polar transport in etiolated Alaska pea seedlings using a space shuttle named Discovery in NASA (STS-95 space experiments in 1998) revealed that auxin polar transport in etiolated Alaska pea epicotyls grown in space conditions was reduced to ca. 50% of that on the ground (1 g conditions) (Ueda et al., 1999; Ueda et al., 2000). In addition, auxin polar transport in etiolated epicotyls of agravitropic pea mutant, ageostropum, was also reduced to ca. 60% of that in etiolated Alaska pea epicotyls (Hoshino et al., 2007; Ueda et al., 2012).

Coleoptiles and mesocotyls are characteristic organs in young Poaceae (Gramineae) seedlings. Coleoptile joining to the mesocotyl has a tapered cylindrical structure enclosing the young leaves. Coleoptile and mesocotyl are important structures for seedling emergence. Seedlings with long coleoptiles and mesocotyls may be able to emerge through the soil better than those with short ones. The growth of coleoptile and mesocotyl is genetically controlled and is also influenced by seeding depth and environmental factors (Turner et al., 1982). The combined force of coleoptile and mesocotyl elongation is essential for successful seedling emergence (Turner et al., 1981; Dilday Mgouja et al., 1988; Luo et al., 2007).
Auxin production in the top region of coleoptile and its basipetal transport has been considered to be essential for the growth of seedlings. Recent studies on auxin polar transport showed the presence of some membrane proteins controlling auxin polar transport which function as influx and efflux facilitators of auxin (Miyamoto et al., 2011; Ueda et al., 2011, 2012), but it is obvious that PIN1 protein is essential for auxin polar transport (Okada et al., 1991; Gülweiler et al., 1998). The growth of rice coleoptiles and mesocotyls was significantly stimulated by exogenous application of brassinolide (Chon et al., 2000). Exogenously applied ABA stimulated mesocotyl elongation but inhibited coleoptile growth (Takahashi et al., 1983; Takahashi and Kaufman, 1992). These results indicated that the growth of coleoptile and mesocotyl was substantially under hormonal regulation. Little is known, however, about the effect of auxin polar transport on the growth and development of coleoptile and mesocotyl in etiolated cereal seedlings.

Here, we show that auxin polar transport is a factor essential for the growth and development of etiolated maize (*Zea mays* L. cv. Honey Bantam) seedlings based on the time-course of auxin polar transport in etiolated coleoptiles and mesocotyls. The time-course of the expression of the gene closely related to efflux facilitator protein (*ZmPIN1s*) in auxin polar transport (Carraro et al., 2006; Gallavotti et al., 2008; Nishimura et al., 2009; Skirpan et al., 2009) is also reported.

**Materials and Methods**

1. **Plant materials and growth conditions**

An acrylic chamber similar to a Plant Growth Chamber (PGC, Krikorian and Levine, 1991) used in a space experiment in NASA was used. According to the STS-95 space experiments (Ueda et al., 1999, 2000), 32 dry seeds of maize (*Zea mays* cv. Honey Bantam) were set in dry rockwool (Nippon Rockwool Co., Tokyo, Japan) and allowed to germinate after irrigating 180 ml of distilled water. The axis of the radicle of dry seed was oriented perpendicular to the rockwool surface. The PGC was kept in a plastic bag (Ziploc) and the seedlings were grown in PGC at 23ºC in the dark for the designated time. The time courses of growth in maize coleoptiles and mesocotyls were investigated. To clarify which zone in the coleoptiles and mesocotyls is responsible for total growth, each organ was divided into 3 and 4 zones of the same length marked with India ink at 3 days after imbibition. Experiments were made in triplicate and the increments in growth were determined for individual plants. The means with standard errors were calculated ($n = 3$).

2. **Application of IAA to the top of etiolated maize coleoptile**

Lanolin paste containing containing 30% water (w w$^{-1}$) and designated dose of IAA were prepared, and lanolin was applied to the top of the maize coleoptile just after germination in the dark (2 days after imbibition). Five to seven etiolated maize seedlings with the lanolin tips were set in a tall-beaker containing 2 layers of filter paper moistened with small amount of distilled water. Beakers were covered with plastic films and kept at 23ºC in the dark. Maize seedlings with lanolin tips without IAA were used as a control. After incubation for a designated period, coleoptile and mesocotyl lengths were measured.

3. **Determinations of auxin polar transport**

Auxin polar transport was determined according to the method reported previously (Oka et al., 1995; Ueda et al., 1999, 2000, 2012, 2013; Hoshino et al., 2007). The segments (10 to 20 mm in length) of coleoptile and mesocotyl of etiolated maize seedlings were prepared. Agar medium (0.9%, w v$^{-1}$, 30 μl) containing radiolabeled IAA
(\[^{14}\text{C}]\text{IAA}, 1 \mu\text{Ci ml}^{-1}, \text{American Radiolabeled Chemicals Inc., St. Louis, Mo.}) \text{ was prepared in 1.5 ml Eppendorf tubes, and was incorporated into the apical or the basal side of the segments in the tube. After the designated incubation period, the radioactivity on the opposite sides (1 to 2 mm) of the segment was directly determined using a liquid scintillation counter and auxin polar transport was shown by the radioactivity. This method has been considered to be suitable for the determination of auxin polar transport (Okada et al. 1991; Oka et al. 1995, 1998, 1999; Ueda et al., 2012). Since radioactivities transported from basal to apical side of the segments were extremely small (Okada et al. 1991; Oka et al. 1995, 1998, 1999; Ueda et al., 2012), only \[^{14}\text{C}]\text{IAA} from the apical side of the segments was shown as a result. Experiments were made in triplicate, and the results were expressed as the mean with standard errors (n = 3).

4. Semi-quantitative RT-PCR for determination of the expression of \(\text{ZmPIN1a} \) gene

Total RNA was isolated from the coleoptiles without young leaves and the mesoycotyls of etiolated maize seedlings using Isogen (Nippon Gene Corp., Tokyo, Japan) according to the manufacture’s instructions with a minor modification. An AMV reverse transcriptase XL and an oligo-dT adaptor primer of RNA PCR Kit (TaKaRa RNA PCRTM Kit (AMV) Ver.3.0, Takara Bio Inc., Shiga, Japan) were used to synthesize the first-strand cDNA. To obtain the central regions of cDNA encoding putative auxin efflux facilitator (ZmPIN1a) in maize seedlings, we used primers, 5'-CACCGACGAGCGTGATGA-3' (forward) and 5'-TCGTGTTCGCCAAGGAGTAC-3' (reverse) designed from ZmPIN1a gene in maize seedlings (Carraro et al., 2006; Gallavotti et al., 2008; Nishimura et al., 2009; Skirpan et al., 2009, Accession No. DQ836239).

A PTC-200 thermal cycler (MJ Research Inc., MA, USA)
was used for semi-quantitative RT-PCR. PCR was performed for 26 to 40 cycles (30 s at 94°C, 30 s at 52°C and 1.5 min at 72°C). Obtained cDNA was size-fractionated on 1.0% agarose gels.

Density of bands in agarose gel electrophoresis was estimated using CS Analyzer ver 3.0 for Windows system (ATTO Densitograph Software Library, ATTO Co., Japan). The amount of ribosomal RNA was used as a control. Experiment was made in duplicate and repeated three times. Results were expressed as relative values of 18S ribosomal RNA. Values are the means with standard errors (n = 3).

Results

1. Coleoptile and mesocotyl growth

As shown in Fig. 1, coleoptiles of etiolated maize seedlings grew rapidly from 2 days after imbibition and then stopped growing at 5 days after imbibition. The final length of the coleoptiles was 52 mm. A similar growth pattern was found in mesocotyls. The final length of the mesocotyls was 54 mm at 6 days after imbibition. These results suggest that auxin transported from the top of coleoptiles contributed to the growth of coleoptiles and mesocotyls similarly in etiolated maize seedlings under the experimental conditions. The most elongating zone of coleoptile was the base and that of mesocotyl was the top (Fig. 2). Slightly or almost no growth was observed in other zones of coleoptiles and mesocotyls.

Fig. 3 shows the effect of exogenously applied IAA on the growth of etiolated maize coleoptiles (upper) and mesocotyls (lower). IAA at various concentrations was applied to the top of coleoptile just after germination (2 days after imbibition). Bars indicate standard errors of the mean (n = 3).

2. Auxin polar transport in coleoptiles and mesocotyls

The presence of leaves in coleoptile segments did not affect auxin polar transport in coleoptiles (data not shown). As shown in Fig. 4, auxin polar transport in coleoptile segments excised from etiolated maize seedlings at 6 days after imbibition was ca 5 times greater than that in mesocotyl segments. In such coleoptile segments, auxin polar transport increased almost linearly after the excision for 12 hours and then was kept constant until 24 hours (Fig. 5). Almost the same pattern of transport was observed in mesocotyl
mesocotyls were almost the same as those in coleoptiles. In mesocotyls, the expression of \textit{ZmPIN1a} gene rapidly increased from 2 to 3 days after imbibition and then decreased. Thus, \textit{ZmPIN1a} gene expression preceded auxin polar transport in coleoptiles and mesocotyls, suggesting strongly that auxin polar transport is dependent on the expression of \textit{ZmPIN1a} gene.

**Discussion**

Because the time-course pattern of the expression of \textit{ZmPIN1a}, the gene encoding IAA efflux facilitator proteins (Fig. 7) closely related to that of auxin polar transport (Fig. 6) in coleoptiles and mesocotyls of etiolated maize (\textit{Zea mays} L. cv. Honey Bantam), we consider that auxin polar transport is essential for the early growth and development of etiolated maize seedlings.

Growth stimulation induced by exogenous IAA was...
observed for only a short time even when IAA was continuously supplied (Hall et al., 1985; Carrington and Esnard, 1988). In addition, the level of auxin in the tissue increased by exogenous IAA also declined after a few hours (Iino, 1996). The growth of maize coleoptiles has been reported to be greatly suppressed by decapitation or ringing at the position above the growing zone and by applying the auxin transport inhibitor \(N\)-1-naphthylphthalamic acid (NPA) together with IAA lanolin (Haga and Iino, 1998). Based on these results, Haga and Iino (1998) concluded that growth of maize coleoptile is controlled by IAA supplied from the apical region. Mori et al. (2005) also provided definite evidence that the apical 0 – 2 mm region of etiolated maize coleoptiles has the potential to produce IAA de novo, which leads to a constant flow of IAA towards the lower parts. In addition, the sensitivity of maize coleoptile to IAA increased when the endogenous level of IAA was reduced. The present study (Figs. 1, 2, 3) clearly showed that endogenous levels of IAA in the elongating zone probably supplied from the apical meristem are substantially responsible for the early growth of the coleoptile and mesocotyl in etiolated maize seedlings. Based on the data shown in Figs. 1 and 6, correlation coefficients (\(R\)) between the growth rate (mm day\(^{-1}\)) and auxin polar transport were calculated to be 0.980 and 0.997 in etiolated maize coleoptiles and mesocotyls, respectively. Strong positive correlations, \(R = 0.958\) and \(R = 0.998\), were also observed between the growth rate and gene expression of ZmPIN1a as calculated from the data in Figs. 1 and 7 in coleoptiles and mesocotyls of etiolated maize seedlings. These results together with the data in Fig. 3 suggest that a certain level of auxin polar transport is essential for maintaining endogenous levels of IAA and for the elongation of coleoptiles and mesocotyls in etiolated maize seedlings.

Auxin polar transport in the coleoptile and mesocotyl of etiolated maize seedling increased accompanied with subsequent growth promotion until 5 and 3 days after imbibition, respectively, and then decreased gradually (Fig. 6). Auxin polar transport has been considered to be regulated by several functional proteins. In recent molecular studies on auxin polar transport some proteins that function as influx and efflux facilitators of auxin were identified. The auxin polar transport proteins located on plasma membrane were classified into three families: AUXIN RESISTANT1/LIKE AUX1 (AUX1/LAX) as an auxin influx facilitator which is auxin uptake symporters with two protons (Marchant et al., 1999; Swarup et al., 2001), PIN-FORMED (PIN) as efflux carriers and/or facilitators (Okada et al., 1991; Gälweiler et al., 1998; Chen and Masson, 2006), and P-GLYCOPROTEIN (MDR/PGP/ABCB) as efflux and/or conditional transporters (Noh et al., 2001, 2003; Santelia et al., 2008). Phospho-glycoproteins (PGPs) belong to the ATP-binding cassette protein subfamily B (ABCB) subgroup of the ATP-binding cassette (ABC) transporter superfamily. The PINOID gene encodes a protein-serine/threonine (Ser/Thr) kinase (Friml et al., 2004). The Arabidopsis Gnom gene has also been known to encode an ARF GDP/GTP exchange factor involved in embryonic axis formation and polar localization of the auxin efflux regulator PIN1 protein (Geldner et al., 2004). Among them, PIN proteins which were located on the polar side of plasmamembrane are substantially essential for regulating auxin polar transport (Okada et al., 1991; Gähweiler et al., 1998; Chen and Masson, 2006; Petrášek and Friml, 2009). Three families of cellular transport proteins can independently and co-ordinately transport auxin in plants. Regulation by these proteins involve intricate
and co-ordinated cellular processes, including protein-protein interactions, vesicular trafficking, protein phosphorylation, ubiquitination, and stabilization of the transporter complexes on the plasma membrane (Titapiwatanakun and Murphy, 2009). The expression of the genes encoding PIN proteins was substantially affected by various gravistimulations (Hoshino et al., 2006). These results strongly suggest that auxin polar transport regulated by PIN proteins is important for the subsequent growth and development on plants. In maize plants, ZmPINs proteins have already been reported to control the growth and development (Carraro et al., 2006), especially in branching (Gallavotti et al., 2008), inflorescence development (Skirpan et al., 2009) and a gravitropic response (Nishimura et al., 2009).

Auxin is known to alter gene expression rapidly. Several mRNA species have been demonstrated to increase or to decrease in plant cells within a few minutes to several hours after the application. Numerous sequences that are up-regulated or down-regulated by auxin have also been described (Hagen and Guilfoyle, 2002; see Chapman and Estelle, 2009). *PsPINs* and *PaUX1* genes have also been shown to be induced by auxin (Hoshino et al., 2005). Results in this study revealed that the expression of *ZmPIN1a* gene preceded auxin polar transport in coleoptiles and mesocotyls (Fig. 7), suggesting strongly that auxin polar transport is dependent on the expression of the *ZmPIN1a* gene. Transported auxin might function to enhance the expression of *ZmPIN1a* gene.

The time-course patterns of auxin polar transport in the coleoptile and mesocotyl (Fig. 6), were similar to those of gene expression of *ZmPIN1a* (Fig. 7). These results together with the growth rate, auxin polar transport and the expression of *ZmPIN1a* gene substantially confirm the idea that the expression of genes encoding auxin efflux facilitator (*ZmPINs* proteins) is important for the growth of coleoptile and mesocotyl in etiolated maize seedlings. Since functional proteins related to auxin polar transport involved intricate and co-ordinated cellular processes as described above (Muday and Murphy, 2002; Kleine-Vehna et al., 2008; Titapiwatanakun and Murphy, 2009) and ZmPIN1 protein determined by immunohistochemistry are localized in the leaves, the primary root and the shoot apical meristem (Carraro et al., 2006; Gallavotti et al., 2008), further intensive studies on the mode of actions of efflux facilitator proteins in the early growth stage of maize coleoptiles and mesocotyls using antibodies of ZmPINs proteins are required.

**References**

Carraro, N., Forestan, C., Canova, S., Tras, J. and Varotto, S. 2006. *ZmPIN1a* and *ZmPIN1b* encode two novel putative candidates for polar auxin transport and plant architecture determination of maize. *Plant Physiol.* 142: 254-264.

Carrington, C.M.S. and Esnard, J. 1988. The elongation response of watermelon hypocotyls to indole-3-acetic acid: a comparative study of excised segments and intact plants. *J. Exp. Bot.* 39: 441-450.

Chapman, E.J. and Estelle, M. 2009. Mechanism of auxin-regulated gene expression in plants. *Annu. Rev. Genet.* 43: 265-285.

Chen, R. and Masson, P.H. 2006. Auxin transport and recycling of PIN proteins in plants. *In J. Šamája, F. Baláška, D. Menzel eds., Plant Endocytosis. Springer-Verlag, Berlin, Heidelberg. 139-157.

Chon, N.M., Nishikawa-Koseki, N., Hirata, Y., Saka, H. and Abe, H. 2000. Effects of brassinolide on mesocotyl, coleoptile and leaf growth in rice seedlings. *Plant Prod. Sci.* 3: 360-363.

Friml, J., Yang, X., Michniewicz, M., Weijers, D., Quint, A., Tietz, O., Benjamins, R., Ouwerkerk, P.B.F., Ljung, K., Sandberg, G., Hooykaas, P.J.J., Palme, K. and Offringa, R. 2004. A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* 306: 862-865.

Gallavotti, A., Yang, V., Schmidt, R.J. and Jackson, D. 2008. The relationship between auxin transport and maize branching. *Plant Physiol.* 147: 1913-1923.

Gälweiler, L., Guan, C., Müller, A., Wisman, E., Mendgen, K., Yephremov, A. and Palme, K. 1998. Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science* 282: 2225-2230.

Geldner, N., Richter, S., Vieten, A., Marquardt, S., Torres-Ruiz, R.A., Mayer, U. and Jurgens, G. 2004. Partial loss-of-function alleles reveal a role for *GNOM* in auxin transport-related, post-embryonic development of *Arabidopsis*. *Development* 131: 389-400.

Haga, K. and Iino, M. 1998. Auxin-growth relationships in maize coleoptiles and pea internodes and control by auxin of the tissue sensitivity to auxin. *Plant Physiol.* 117: 1473-1486.

Hagen, G. and Guilfoyle, T. 2002. Auxin-responsive gene expression: genes, promoters and regulatory factors. *Plant Mol. Biol.* 49: 373-385.

Hall, J.L., Brummell, D.A. and Gillespie, J. 1985. Does auxin stimulate the elongation of intact plant stems? *New PhytoL* 100: 341-345.

Hoshino, T., Hitotsubashi, R., Miyamoto, K., Tanimoto, E. and Ueda, J. 2005. Isolation of *PsPIN2* and *PaUX1* from etiolated pea epicotyls and their expression on a three-dimensional clinostat. *Adv. Space Res.* 36: 1284-1291.

Hoshino, T., Miyamoto, K. and Ueda, J. 2006. Requirement for the gravity-controlled transport of auxin for a negative gravitropic response of epicotyls in the early growth stage of etiolated pea seedlings. *Plant Cell Physiol.* 47: 1496-1508.

Hoshino, T., Miyamoto, K. and Ueda, J. 2007. Gravity-controlled asymmetrical transport of auxin regulates a gravitropic response in the early growth stage of etiolated pea seedlings. *Plant Cell Physiol.* 47: 1496-1508.

Iino, M. 1996. Short-term stimulation of growth induced by the apical application of IAA to intact maize coleoptiles. *Plant Cell Physiol.* 37: 27-33.

Kleine-Vehna, J., Langowski, L., Wiśniewska, J., Dhommske, P., Brewer, P.B. and Friml, J. 2008. Cellular and molecular requirements for polar PIN targeting and transcytosis in plants. *Mol. Plant.* 1: 1056-1066.

Kriticorian, A.D. and Levine, H.G. 1991. Development and growth in space. *In R.G.S. Bidwell ed., Plant Physiology: A Treatise. Vol. X. Academic Press, Orlando, Florida. 491-555.

Luo, J., Tang, S., Hu, P., Louis, A., Jiao, G. and Tang, J. 2007. Analysis on factors affecting seedling establishment in rice. *Rice Sci.* 14: 27-32.

Marchant, A., Kargul, J., May, S.T., Muller, P., Delbarre, A., Perrot-
Rechenmann, C. and Bennett, M.J. 1999. AUX1 regulates root gravitropism in Arabidopsis by facilitating auxin uptake within root apical tissues. *EMBO J.* 18: 2066-2073.

Mgonja, M.A., Dilday, R.H., Skinner, S.L. and Collins, F.C. 1988. Association of mesocotyl and coleoptile elongation with seedling vigor in rice. *Proc. Arkansas Acad. Sci.* 42: 52-55.

Miyamoto, K., Ueda, E., Oka, M. and Ueda, J. 2011. Auxin polar transport and automorphosis in plants. *Biolog. Sci. Space* 25: 57-68.

Mori, Y., Nishimura, T. and Koshiba, T. 2005. Vigorous synthesis of indole-3-acetic acid in the apical very tip leads to a constant basipetal flow of the hormone in maize coleoptiles. *Plant Sci.* 168: 467-473.

Muday, G.K. and Murphy, A.S. 2002. An Emerging model of auxin transport regulation. *Plant Cell* 14: 293-299.

Nishimura, T., Nakano, H., Hayashi, K., Niwa, C. and Koshiba, T. 2009. Differential downward stream of auxin synthesized at the tip has a key role in gravitropic curvature via TIR1/AFBs-mediated auxin signaling pathways. *Plant Cell Physiol.* 50: 1874-1885.

Noh, B., Murphy, A.S. and Spalding, E.P. 2001. Multidrug Resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. *Plant Cell* 13: 2441-2454.

Noh, B., Bandypadhyay, A., Peet, W.A., Spalding, E.P. and Murphy, A.S. 2003. Enhanced gravi- and phototropism in plant mdr mutants mislocalizing the auxin efflux protein PIN1. *Nature* 423: 999-1002.

Oka, M., Ueda, J., Miyamoto, K., Yamamoto, R., Hoson, T. and Kamisaka, S. 1995. Effect of simulated microgravity on auxin polar transport in inflorescence axis of Arabidopsis thaliana. *Biolog. Sci. Space* 9: 331-336.

Oka, M., Ueda, J., Miyamoto, K. and Okada, K. 1998. Activities of auxin polar transport in inflorescence axes of flower mutants mislocalizing the auxin efflux protein PIN1. *Nature* 393: 999-1002.

Oka, M., Miyamoto, K., Okada, K. and Ueda, J. 1999. Auxin polar transport and flower formation in Arabidopsis thaliana transformed with indole-3-acetic acid hydrolase (iaaH) gene. *Plant Cell Physiol.* 40: 231-237.

Okada, K., Ueda, J., Komaki, M.K., Bell, C.J. and Shimura, Y. 1991. Requirement of the auxin transport system in early stage of Arabidopsis floral bud formation. *Plant Cell* 3: 677-684.

Petrašek, J. and Friml, J. 2009. Auxin transport routes in plant development. *Development* 136: 2675-2688.

Santelia, D., Henrichs, S., Vincenzetti, V., Sauer, M., Bigler, L., Klein, M., Bailly, A., Lee, Y., Friml, J., Geisler, M. and Martinoia, E. 2008. Flavonoids redirect PIN-mediated polar auxin fluxes during root gravitropic responses. *J. Biol. Chem.* 283: 31218-31226.

Skirpan, A., Guller, A.H., Gallavotti, A., Jackson, D., Cohen, J.D. and McSteen, P. 2009. BARREN INFLORESCENCE2 interaction with ZmPIN1a suggests a role in auxin transport during maize inflorescence development. *Plant Cell Physiol.* 50: 652-657.

Swarup, R., Friml, J., Marchant, A., Ljung, K., Sandberg, G., Palme, K. and Bennett, M. 2001. Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. *Genes Dev.* 15: 2648-2653.

Takahashi, K. and Kaufman, P.B. 1983. Effects of several growth regulators on the morphogenesis of mesocotyl and coleoptile of rice. *Jpn. J. Crop. Sci.* 52 (Extra 2): 178-179* (extra issue).

Takahashi, K. and Kaufman, P.B. 1992. Regulation of internodal elongation of rice seedlings by plant growth regulators. *Jpn. J. Crop. Sci.* 61:34-40*.

Thimmann, K.V. 1977. Polarity and the transport of auxin. In Hormin Action in the Whole Life of Plants. The University of Massachusetts press, Amherst, USA. 71-192.

Tatipapatanakun, B. and Murphy, A.S. 2009. Post-transcriptional regulation of auxin transport proteins: cellular trafficking, protein phosphorylation, protein maturation, ubiquitination, and membrane composition. *J. Exp. Bot.* 60: 1093-1047.

Turner, F.T., Chen, C.C. and McCanney, G.N. 1981. Morphological development of rice seedlings in water at controlled oxygen levels. *Agron. J.* 73: 566-570.

Turner, F.T., Chen, C.C. and Bollich, C.N. 1982. Coleoptile and mesocotyl lengths in semi-dwarf rice seedlings. *Crop Sci.* 22: 43-46.

Ueda, J., Miyamoto, K., Yuda, T., Hoshino, T., Fujii, S., Mukai, C., Kamiguchi, S., Aizawa, S., Yoshizaki, I., Shimazu, T. and Fukui, K. 1999. Growth and development, and auxin polar transport in higher plants under microgravity conditions in space: BRIC-AUX on STS-95 space experiment. *J. Plant Res.* 112: 487-492.

Ueda, J., Miyamoto, K., Yuda, T., Hoshino, T., Fujii, S., Mukai, C., Kamiguchi, S., Aizawa, S., Yoshizaki, I., Shimazu, T. and Fukui, K. 2000. STS-95 space experiment for plant growth and development, and auxin polar transport. *Biol. Sci. Space* 14: 47-57.

Ueda, J., Miyamoto, K., Ueda, E. and Oka, M. 2011. Auxin transport and a graviresponse in plants: Relevance to ABC transporters. *Biol. Sci. Space* 25: 69-75.

Ueda, J., Tada, T., Hoshino T., Miyamoto, K., Ueda, E. and Oka, M. 2012. Isolation of PsPINs and PsAUX1 cDNAs encoding putative auxin efflux and influx carriers and/or facilitators, respectively, from etiolated epicotyls of an agravitropic pea (*Pisum sativum*) mutant, agentropum. *Biol. Sci. Space* 26: 32-41.

Ueda, J., Toda, Y., Kato, K., Kuroda, Y., Arai, T., Hasegawa, T., Shigemori, H., Hasegawa, K., Kitagawa, J., Miyamoto, K. and Ueda, E. 2013. Identification of dehydrocostus lactone and 4-hydroxy-β-thujone as auxin polar transport inhibitors. *Acta Physiol. Plant.* 35: 2251-2258.

* In Japanese.

** In Japanese with English abstract.