Analysis of subcellular localization of auxin carriers PIN, AUX/LAX and PGP in *Sorghum bicolor*

SuiKang Wang, ChenJia Shen, SaiNa Zhang, YanXia Xu, DeAn Jiang and YanHua Qi* State Key Laboratory of Plant Physiology and Biochemistry; College of Life Sciences; ZheJiang University; Hangzhou, China

©2011 L

Keywords: AUX/LAX, PGP, PIN, *Sorghum bicolor*, subcellular localization

Submitted: 09/02/11 Accepted: 09/06/11

DOI: 10.4161/psb.6.12.17968

*Correspondence to: YanHua Qi; Email: qyhjp@zju.edu.cn

Addendum to: Shen CH, Bai YH, Wang SK, Zhang SN, Wu YR, Chen M, et al. Expression profile of PIN, AUX/LAX and PGP auxin transporter gene families in *Sorghum bicolor* under phytohormone and abiotic stress. FEBS J 2010; 277:2954–69; PMID: 20528920; DOI:10.1111/j.1742-4658.2010.07706.x

uxin transport at least correlates **L**to the three gene families: efflux carriers PIN-formed (PIN), p-glycoprotein (PGP), and influx carrier auxin resistant 1/like aux1 (AUX/LAX) in Arabidopsis thaliana. In monocotyledon Sorghum bicolor, the biological function of these genes retains unclear. Our previous study reported that the member analysis, organ-specific expression and expression profiles of the auxin transporter PIN, PGP and AUX/LAX gene families in Sorghum bicolor under IAA, brassinosteroid, polar auxin transport inhibitors and abiotic stresses. Here we further supply the prediction of subcellular localization of SbPIN, SbLAX and SbPGP proteins and discuss the potential relationship between the subcellular localization and stress response. The predicted results showed that the most of SbPIN, SbLAX and SbPGP proteins are localized to the plasma membrane, except few localized to vacuolar membrane and endoplasmic reticulum. This data set provides novel information for investigation of auxin transporters in Sorghum bicolor.

The intercellular auxin transport depends on the polar subcellular localization of PIN-formed (PIN) auxin efflux carriers at the plasma membrane (PM) and endoplasmic reticulum (ER).¹⁻³ In Arabidopsis, PIN1–PIN4 and PIN7 localize to the PM when PIN5, 6 and 8 localize to the ER, which likely mediate the transport of auxin between the ER lumen and cytosol, and then regulate the cellular auxin homeostasis.²⁻⁶ The direction of auxin flow within tissues is mainly determined by the

asymmetric subcellular localization of PIN in each transporting cell. Since, uncovering the mechanisms controlling the subcellular dynamics of auxin transport machinery is important for plant response to all developmental signals from embryogenesis to organogenesis, vascular tissue differentiation and tropisms.7 In the other hand, the report of auxin transporter related to abiostresses was gradually revealed recently. Auxin influx transport AUX1 is essential for the lateral root proliferation component of the salt stress-induced morphogenic response;8 the promoters of the SbPIN, SbLAX and SbPGP genes contain numerous DNA elements predicted to respond to abscissic acid, drought and high salt stresses.9 To make clear if the subcellular localization of SbPIN, SbLAX and SbPGP proteins also related to abiostresses response, here we first predicted their subcellular localization by http:// wolfpsort.org/ (Table 1).10 SbPIN2, 5, 6, 7, 8 and 10 were showed the more motifs localized in PM when SbPIN1, 3, 4, 9 and 11 were showed the more motifs localized in vacuolar membrane (VM). The five SbLAXs all showed the most motifs in PM, even though there are fewer motifs in E.R or VM. The 24 SbPGPs members almost were localized in PM besides the both SbPGP5 and SbPGP11 also contained the more motifs localized in chloroplast. The above data were similar with the prediction of subcelluar localization of PIN in Arabidopsis (Table 1). Compare the prediction with the published data of subcelluar localization of six AtPINs, the prediction analysis of the AtPIN1, 3, 4 and 7 were consistent with the published data in reference 2–6. Thus, these analyses

Table 1. Prediction of subcellular localization of auxin carriers PIN, AUX/LAX and PGP in Sorghum bicolor

Gene name ^a	Locus identifier ^b	Accession number ^c	plasma membrane	endoplasmic reticulum	vacuolar membrane	chloroplast
AtPIN1	At01g73590	Q9C6B8	7	2	2	1
AtPIN2	At05g57090	Q9LU77	5		6	
AtPIN3	At01g70940	Q9S7Z8	7			3
AtPIN4	At02g01420	Q8RWZ6	9	2	2	
AtPIN5	At05g16530	Q9FFD0	1		12	
AtPIN6	At01g77110	Q9SQH6	6		4	2
AtPIN7	At01g23080	Q940Y5	8	2		2
AtPIN8	At05g15100	Q9LFP6	5	3	2	3
SbPIN1	Sb02g029210	C5X4P5	4		8	
SbPIN2	Sb03g029320	C5XF44	8		1	2
SbPIN3	Sb03g032850	C5XIA5	1		10	
SbPIN4	Sb03g037350	C5XMI2	3		6	1.5
SbPIN5	Sb03g043960	C5XG98	9	2	1	
SbPIN6	Sb04g028170	ND d	11			1
SbPIN7	Sb05g002150	C5Y431	8	2		3
SbPIN8	Sb07g026370	C5YI36	5		5	1
SbPIN9	Sb10g004430	C5Z4U5	5		9	
SbPIN10	Sb10g008290	C5Z7E9	6	2	2	1
SbPIN11	Sb10g026300	C5Z7A0	6		8	
SbLAX1	Sb01g026240	C5WP27	11	2		
SbLAX2	Sb01g041270	C5WR01	11	3		
SbLAX3	Sb03g040320	C5XQG2	10		3	
SbLAX4	Sb05g004250	C5Y5L4	10	3		
SbLAX5	Sb09g021990	C5YYU5	9	3.5		
SbPGP1	Sb01g039110	C5WPA9	8	3	3	
SbPGP2	Sb02g019540	C5X8A6	13			
SbPGP3	Sb03g011860	C5XI10	10		1	
SbPGP4	Sb03g023740	C5XMA7	12			
SbPGP5	Sb03g031990	C5XHH9	5	2		6
SbPGP6	Sb03g032000	C5XHI0	13			
SbPGP7	Sb03g032030	C5XHI4	11	2		
SbPGP8	Sb03g033290	C5XIE9	12	2		
SbPGP9	Sb03g047490	C5XJF5	8	2	1	1
SbPGP10	Sb04g006087	C5XX25	12		1	
SbPGP11	Sb04g006090	C5XX26	5		2	6
SbPGP12	Sb04g006100	C5XX27	3	2		3
SbPGP13	Sb04g022480	C5XU71	5	2		4
SbPGP14	Sb04g031170	C5Y0R2	11	1		
SbPGP15	Sb06g001440	C5YC52	9	2		
SbPGP16	Sb06g018860	C5Y9T7	12	2		
SbPGP17	Sb06g020350	C5YAT5	5	3		2
SbPGP18	Sb06g030350	C5Y8Z4	11			1
SbPGP19	Sb07g003510	C5YGW7	12			1
SbPGP20	Sb07g003520	C5YGW8	13			
SbPGP21	Sb07g023730	C5YMS8	5	2		
	Sb09g002940	C5YZK3	11			

Table 1. Prediction of subcellular localization of auxin carriers PIN, AUX/LAX and PGP in Sorghum bicolor (continued)

Gene name ^a	Locus identifierb	Accession number ^c	plasma membrane	endoplasmic reticulum	vacuolar membrane	chloroplast
SbPGP23	Sb09g027320	C5YUY3	14			
SbPGP24	Sb09g027330	C5YUY4	10		1	

suggested that the prediction of subcelluar localization of auxin carriers SbPIN, SbLAX and SbPGP was also useful for their experimental research. By previous report, the regulation of PIN protein polarity is needed to quickly respond and adapt plant development to internal and external stimuli.11 At the cellular level, various signals are translated into specific changes in the polar subcellular localization of PIN family thereby guiding the intercellular fluxes of auxin;11 dark treatment affects the subcellular localization of PIN1 and auxin maxima;12 various environmental and endogenous signals can modulate trafficking and polarity of PIN proteins and then change auxin distribution.¹³ Namely, the subcellular localization of PIN protein may relate to abiotic stresses responses. Future experiments of subcellular localization and abiotic stress responses need to be performed to confirm the bioinformatics prediction.

Acknowledgments

This research is supported by the National Natural Science Foundation of China

(grant no. 31071392, 30971703 and 3117462), the Genetically Modified Organisms Breeding Major Projects (2009ZX08009-1238) and the Natural Science Foundation of Zhejiang province, China (grant no. Y3080111).

References

- Wisniewska J, Xu J, Seifertová D, Brewer PB, Ruzicka K, Blilou I, et al. Polar PIN localization directs auxin flow in plants. Science 2006; 312:883; PMID:16601151; DOI:10.1126/science.1121356.
- Petrásek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertová D, et al. PIN proteins perform a ratelimiting function in cellular auxin efflux. Science 2006; 312:914-8; PMID:16601150; DOI:10.1126/ science.1123542.
- Mravec J, Skůpa P, Bailly A, Hoyerová K, Krecek P, Bielach A, et al. Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. Nature 2009; 459:1136-40; PMtD;19596555; DOI:10.1038/nature08066.
- Wabnik K, Kleine-Vehn J, Govaerts W, Friml J. Prototype cell-to-cell auxin transport mechanism by intracellular auxin compartmentalization. Trends Plant Sci 2011; 16:468-75; PMID:21665516; DOI:10.1016/j.tplants.2011.05.002.
- Yang H, Murphy AS. Functional expression and characterization of Arabidopsis ABCB, AUX 1 and PIN auxin transporters in *Schizosaccharomyces pombe*. Plant J 2009; 59:179-91; PMID:19309458; DOI:10.1111/j.1365-313X.2009.03856.x.

- Utsuno K, Shikanai T, Yamda Y, Hashimoto T. AGR, an Agravitropic locus of *Arabidopsis thaliana*, encodes a novel membrane protein family member. Plant Cell Physiol 1998; 39:1111-8; PMID:9871369.
- Forestan C, Varotto S. PIN1 auxin efflux carriers localization studies in *Zea mays*. Plant Signal Behav 2010; 5:436-9; PMID:20383059; DOI:10.4161/ psb.5.4.11339.
- Zolla G, Heimer YM, Barak S. Mild salinity stimulates a stress-induced morphogenic response in *Arabidopsis thaliana* roots. J Exp Bot 2010; 61:211-24; PMID:19783843; DOI:10.1093/jxb/erp290.
- Shen C, Bai Y, Wang S, Zhang S, Wu Y, Chen M, et al. Expression profile of PIN, AUX/LAX and PGP auxin transporter gene families in Sorghum bicolor under phytohormone and abiotic stress. FEBS J 2010; 277:2954-69; PMID:20528920; DOI:10.1111/ j.1742-4658.2010.07706.x.
- Horton P, Park KJ, Obayashi T, Fujita N, Harada H, Adams-Collier CJ, Nakai K. WoLF PSORT: protein localization predictor. Nucleic Acids Res 2007; 35:585-7; PMID:17517783; DOI:10.1093/ nar/gkm259.
- Grunewald W, Friml J. The march of the PINs: developmental plasticity by dynamic polar targeting in plant cells. EMBO J 2010; 29:2700-14; PMID:20717140; DOI:10.1038/emboj.2010.181.
- Yoshida S, Mandel T, Kuhlemeier C. Stem cell activation by light guides plant organogenesis. Genes Dev 2011; 25:1439-50; PMID:21724835; DOI:10.1101/gad.631211.
- Friml J. Subcellular trafficking of PIN auxin efflux carriers in auxin transport. Eur J Cell Biol 2010; 89:231-5; PMID:19944476; DOI:10.1016/j. ejcb.2009.11.003.