



# Linking ploidy level with salinity tolerance: NADPH-dependent ‘ROS–Ca<sup>2+</sup> hub’ in the spotlight

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**Polyploidy is considered to be a driving force in plant evolution that enabled adaptation to adverse environmental conditions such as soil salinity. This phenomenon is examined by Liu *et al.* (2019) in relation to root-zone-specific ion transport, and can be explained by more efficient operation of an NADPH-dependent ‘ROS–Ca<sup>2+</sup> hub’ and desensitization of ROS-inducible cation channels in polyploid lines. Two hypotheses include that non-selective cation channels in polyploid lines are formed of chimeric tetramers, with some subunits having modified thiol groups (hence, reduced sensitivity to H<sub>2</sub>O<sub>2</sub>), or alternatively that inactivation of Ca<sup>2+</sup> channels and higher Ca<sup>2+</sup>-ATPase pump activity may reduce the level of cytosolic free Ca<sup>2+</sup> and provide a negative control over NADPH oxidase operation.**

Whole-genome duplication, or polyploidy, is considered to be a driving force in plant evolution that enabled better adaptation to some adverse environmental conditions (Adams and Wendel, 2005; Parisod *et al.*, 2010). Polyploid plants demonstrate enhanced tolerance to a range of biotic and abiotic stresses, including soil salinity (Chao *et al.*, 2013). A good example is hexaploid bread wheat (*Triticum aestivum*; genome BBAADD) that is more salt tolerant than its tetraploid wheat progenitor (*T. turgidum*) or durum wheat (*T. durum*) (Munns and James, 2003). Genome duplication improved rice resistance to salt stress (Tu *et al.*, 2014), and citrus tetraploid genotypes are more tolerant of moderate saline stress than diploids (Saleh *et al.*, 2008; Mouhaya *et al.*, 2010). The link between ploidy level and salinity tolerance seems to be reciprocal, with the recent report by Barkla *et al.* (2018) showing that salt treatment led to a significant increase in ploidy levels in the epidermal bladder cells of the halophyte *Mesembryanthemum crystallinum*.

The physiological mechanisms explaining improved salt tolerance with increasing level of ploidy remain obscure. Yang *et al.* (2014) showed superior salinity stress tolerance in a synthetic allohexaploid wheat (neo-6×) compared with its

tetraploid (*T. turgidum*; BBAA) and diploid (*Aegilops tauschii*; DD) parents, and attributed this to regulatory transition of the HKT1;5 gene from constitutive high basal expression to induced high expression upon salt stress. However, no HKT1;5 activity was measured, and the only evidence provided was a difference in the xylem Na concentration and minor variations in HKT1;5 expression in leaves at one specific timepoint (with a plethora of other reported differences in gene expression between genotypes).

Recent years have witnessed a paradigm shift towards recognition of plant tissue tolerance (e.g. a capacity of tissues to function while containing a high internal Na<sup>+</sup> and Cl<sup>-</sup> concentration; Munns *et al.*, 2016) as a key determinant of overall salinity stress tolerance. Cytosolic K<sup>+</sup> retention, i.e. an ability of root and mesophyll cells to prevent NaCl-induced K<sup>+</sup> efflux, has been shown to be an essential component of the tissue tolerance mechanism (Shabala and Pottosin, 2014; Shabala *et al.*, 2016; Wu *et al.*, 2018). Recently Chao *et al.* (2013) analyzed the elemental composition of leaves from 349 Arabidopsis accessions and 89 RILs and reported a strong correlation between the ploidy level and leaf K<sup>+</sup> content. Can this be an explanation for superior salinity tolerance in polyploids? And if so, how is this trait regulated?

## Root-zone ion transport

Liu *et al.* (2019) conducted a comprehensive study of the relationship between the ploidy level of *Ipomoea trifida* plants and root-zone-specific ion transport under saline conditions. They convincingly showed that superior tolerance of autohexaploid (6×) *I. trifida* as compared with diploid (2×) plants was conferred by reduced sensitivity of plasma membrane K<sup>+</sup>-permeable channels in the meristem root zone and increased sensitivity of Ca<sup>2+</sup>-permeable channels in the elongation and mature root zones to H<sub>2</sub>O<sub>2</sub>. This differential ROS sensitivity confers superior K<sup>+</sup> retention and Na<sup>+</sup> exclusion under

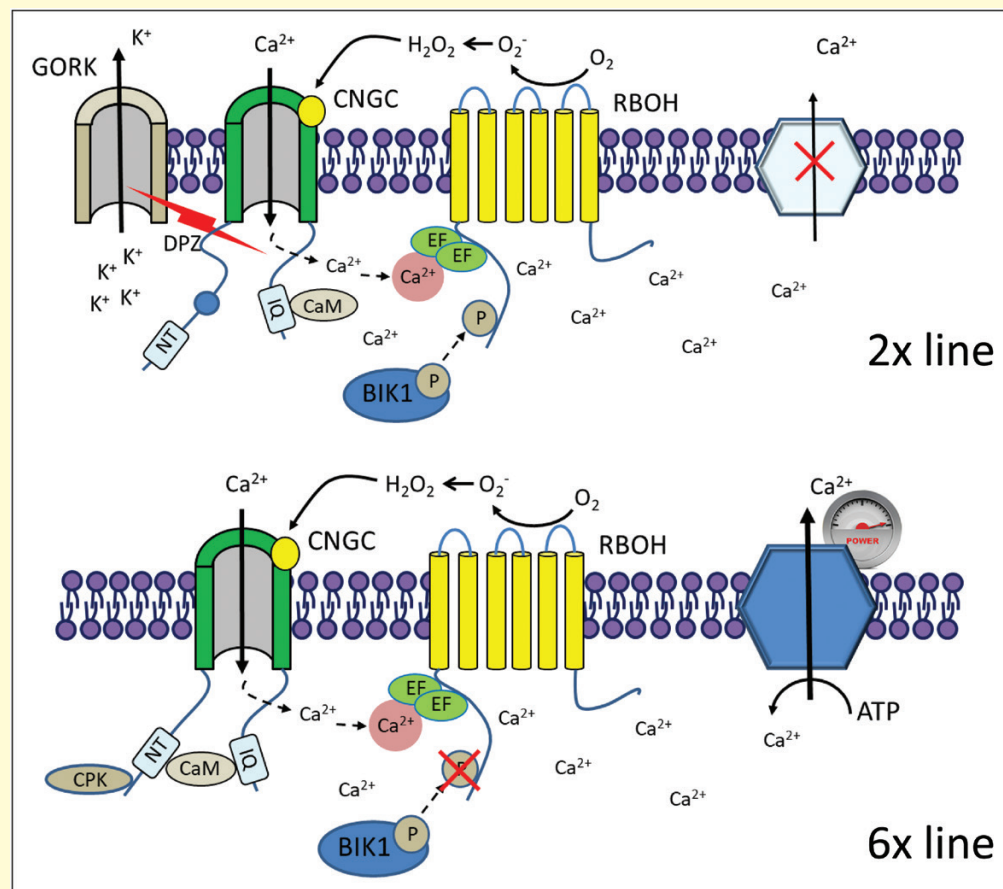
salt stress, explaining the salt-tolerant phenotype in hexaploid plants. As the reported  $\text{H}_2\text{O}_2$  levels were the same in double- and hexaploid lines, the above difference cannot be attributed to higher activity of antioxidant enzymes and suggests changes in sensitization of ROS-activated ion channels in the root epidermis.

The mechanisms of ion channel activation by ROS are poorly understood. It is generally assumed that the major

targets of ROS-induced modification of proteins are reactive cysteine residues (Alansary *et al.*, 2016). A reactive cysteine contains a thiolate group ( $\text{S}^-$ ) which reacts with  $\text{H}_2\text{O}_2$  while the thiol groups ( $\text{SH}$ ) do not react physiologically with  $\text{H}_2\text{O}_2$  unless the reaction is catalyzed (Forman *et al.*, 2004). The direct proof for this comes from experiments by Garcia-Mata *et al.* (2010), who used a heterologous expression system to show that the  $\text{K}^+$  outward-rectifying SKOR channel was activated

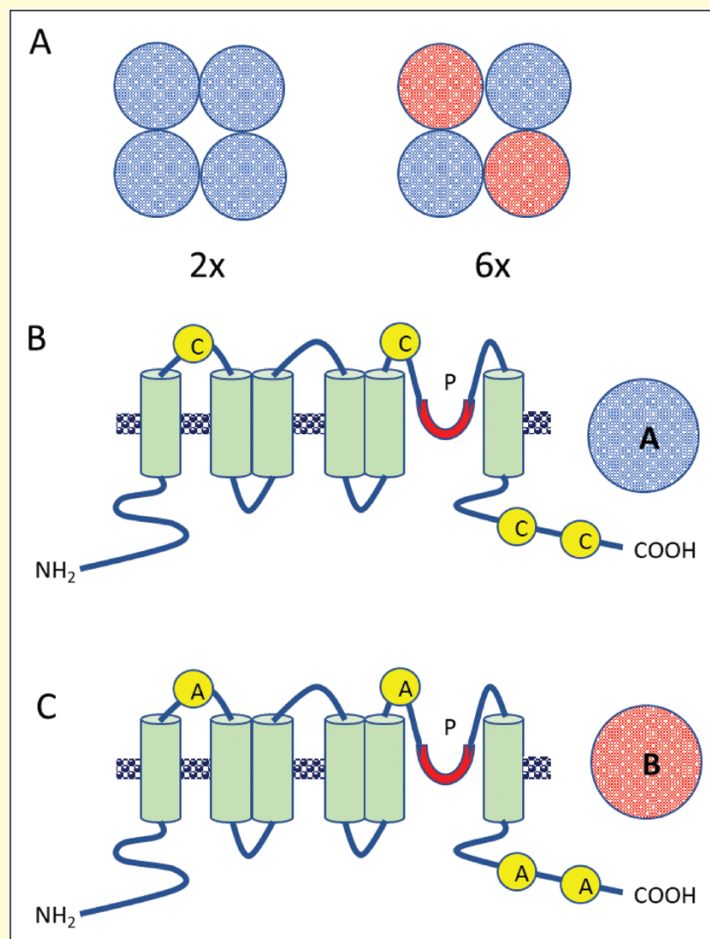
### Box 1 A tentative model for the operation of an NADPH-dependent 'ROS- $\text{Ca}^{2+}$ hub' in diploid and hexaploid lines

In the  $2\times$  line, apoplastic  $\text{H}_2\text{O}_2$  produced by NADPH oxidase stimulates  $\text{Ca}^{2+}$  uptake through non-selective cation channels (CNGC in the model) and forms a positive feedback loop, resulting in an avalanche-like increase in cytosolic free  $\text{Ca}^{2+}$ . Because of the massive  $\text{Ca}^{2+}$  influx into the cell, the plasma membrane is depolarized, triggering  $\text{K}^+$  efflux through the GORK channel. NADPH oxidase operation requires the phosphorylation of one of its terminal domains, mediated by BIK1 (Kadota *et al.*, 2014). Operation of CNGC is also dependent on binding of calmodulin (CaM) to the IQ motif in the C terminus (DeFalco *et al.*, 2016). In a hexaploid line, inactivation of  $\text{Ca}^{2+}$  channels resulting from its interaction with CaM (DeFalco *et al.*, 2016) or decreased CDPK-catalyzed phosphorylation (Zhou *et al.*, 2014) reduce NADPH oxidase activity. Higher  $\text{Ca}^{2+}$ -ATPase pump activity also reduces the level of cytosolic free  $\text{Ca}^{2+}$  and provides a negative control over NADPH oxidase operation. BIK1, the plasma-membrane-associated kinase; CPK, calcium-dependent protein kinase; NT, a putative CaM-binding motif; DPZ, depolarization; CaM, calmodulin; IQ, a conserved isoleucine-glutamine motif in the C terminus.



### Box 2 Suggested model explaining the desensitization of cation channels in polyploid lines by chimeric protein assembly

The model assumes that  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  fluxes across the plasma membrane are mediated by cyclic nucleotide-gated channels (CNGCs). Such CNGCs are made up of four subunits, each having one pore region and six transmembrane domains (Demidchik and Shabala, 2018). In a diploid ( $2\times$ ) line, all subunits are identical (panel A; blue) and harbour cysteine (C in the model) residues in both external and enteral loops (panel B) and, thus, can be activated by  $\text{H}_2\text{O}_2$  from either the apoplastic or the cytosolic side. In a hexaploid line ( $6\times$ ), two out of four units have cysteine replaced by the neutrally charged asparagine (A in the model; panel C). The chimeric channel is formed of two type A (blue) and two type B (red) subunits with cysteine substituted by asparagine (or with some other non-ROS-binding amino acid). Such a chimeric channel has fewer ligand ( $\text{H}_2\text{O}_2$ )-binding sites and thus reduced sensitivity to ROS. P, pore.



by  $\text{H}_2\text{O}_2$  via targeted oxidation of Cys168 at the S3  $\alpha$ -helix within the channel's voltage sensor. Thus, the difference in ROS-induced  $\text{K}^{+}$  and  $\text{Ca}^{2+}$  fluxes between  $2\times$  and  $6\times$  plants in Liu *et al.* (2019) may potentially be explained by desensitization of the appropriate transport system to  $\text{H}_2\text{O}_2$  resulting from modification of thiol groups in the sensory domain.

#### A 'ROS- $\text{Ca}^{2+}$ hub'

Another important observation by Liu *et al.* (2019) was that the magnitude of NaCl-induced  $\text{K}^{+}$  efflux in the diploid line was reduced by twofold in plants treated with DPI,

a known inhibitor of NADPH oxidase. NADPH oxidase is a plasma-membrane-bound enzyme complex from the NOX family, which faces the extracellular space (Marino *et al.*, 2012). Discovered first as part of the plant hypersensitive (HR) response to pathogens, this enzyme has recently emerged as a critical component of stress signaling mechanisms in response to a broad range of abiotic stresses, including salinity (Miller *et al.*, 2010; Ma *et al.*, 2012; Shabala *et al.*, 2015). NADPH oxidase can stabilize SOS1 transcripts (Chung *et al.*, 2008), thus assisting plants in reducing the salt load, and is involved in generating the stress-induced  $\text{Ca}^{2+}$  'signatures' that mediate rapid systemic signalling (Miller *et al.*, 2010).

The concept of a ROS–Ca<sup>2+</sup> hub was recently put forward (Demidchik and Shabala, 2018; Demidchik *et al.*, 2018) and implies that Ca<sup>2+</sup>-activated NADPH oxidases work in concert with ROS-activated Ca<sup>2+</sup>-permeable cation channels to generate and amplify stress-induced Ca<sup>2+</sup> and ROS signals (Box 1). Interestingly, an effect of DPI on K<sup>+</sup> fluxes was not observed in the 6× line (Liu *et al.*, 2019), suggesting that NADPH oxidase was already inactivated in the polyploid. This inactivation may be a result of either decreased NADPH oxidase phosphorylation by BIK1 (Kadota *et al.*, 2014; Box 1) or low activity of Rac/Rop GTPases (Baxter-Burrell *et al.*, 2002). More active Ca<sup>2+</sup>-ATPase activity in a hexaploid line or inactivation of Ca<sup>2+</sup> channels resulting from its interaction with CaM (DeFalco *et al.*, 2016) or decreased CDPK-catalyzed phosphorylation (Zhou *et al.*, 2014) may also be the reason for ROS–Ca<sup>2+</sup> hub activity ceasing (Box 1).

Contrary to animal systems, plant genomes do not encode any Ca<sup>2+</sup>-selective ion channels (Demidchik *et al.*, 2018), with Ca<sup>2+</sup> transport across the plasma membrane mediated by non-selective cation channels (NSCCs). While the genetic origin of NSCCs remains unknown, two major classes – CNGCs (cyclic nucleotide-gated channels) and GLRs (glutamate receptors) – are known in Arabidopsis (with 20 members in each class; Maser *et al.*, 2001). NSCCs can be activated by ROS (Demidchik *et al.*, 2018). GLRs are believed to be tetramers consisting of different subunits (Price *et al.*, 2013). CNGCs can also form chimeric channels (Zhong *et al.*, 2003), and plants harbouring the ATCNGC11/12 gene showed a phenotype with constitutively activated (ROS-burst-related) defence responses to pathogens (Yoshioka *et al.*, 2006). Keeping this in mind, one may hypothesize that polyploid lines may encode chimeric NSCCs with altered ligand-gated properties and reduced sensitivity to H<sub>2</sub>O<sub>2</sub> (Box 2). It was shown that replacement of the positively charged lysine (Lys1110) with the neutrally charged asparagine (K1110N) or the negatively charged amino acid glutamic acid (K1110E) in the mammalian TRPM2 channel generated mutants that failed to induce an increase in free cytosolic calcium concentration in response to H<sub>2</sub>O<sub>2</sub> (Kim *et al.*, 2013). It remains to be shown if the similar substitution of one or several amino acids in chimeric NSCCs may desensitize them, thus altering ROS–Ca<sup>2+</sup> hub operation kinetics and affecting plant salt stress signaling and ionic homeostasis, explaining salt-tolerant phenotype in polyploid lines.

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## References

Adams KL, Wendel JF. 2005. Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology* **8**, 135–141.

Alansary D, Schmidt B, Dörr K, Bogeski I, Rieger H, Kless A, Niemeyer BA. 2016. Thiol dependent intramolecular locking of Orail channels. *Scientific Reports* **6**, 33347.

Barkla BJ, Rhodes T, Tran KT, Wijesinghege C, Larkin JC, Dassanayake M. 2018. Making epidermal bladder cells bigger: developmental- and salinity-induced endopolyploidy in a model halophyte. *Plant Physiology* **177**, 615–632.

Baxter-Burrell A, Yang Z, Springer PS, Bailey-Serres J. 2002. RopGAP4-dependent Rop GTPase rheostat control of Arabidopsis oxygen deprivation tolerance. *Science* **296**, 2026–2028.

Chao DY, Dilkes B, Luo H, Douglas A, Yakubova E, Lahner B, Salt DE. 2013. Polyploids exhibit higher potassium uptake and salinity tolerance in Arabidopsis. *Science* **341**, 658–659.

Chung JS, Zhu JK, Bressan RA, Hasegawa PM, Shi H. 2008. Reactive oxygen species mediate Na<sup>+</sup>-induced SOS1 mRNA stability in Arabidopsis. *The Plant Journal* **53**, 554–565.

DeFalco TA, Marshall CB, Munro K, Kang HG, Moeder W, Ikura M, Snedden WA, Yoshioka K. 2016. Multiple calmodulin-binding sites positively and negatively regulate Arabidopsis CYCLIC NUCLEOTIDE-GATED CHANNEL12. *The Plant Cell* **28**, 1738–1751.

Demidchik V, Shabala S, Isayenkov S, Cuin TA, Pottosin I. 2018. Calcium transport across plant membranes: mechanisms and functions. *New Phytologist* **220**, 49–69.

Demidchik V, Shabala S. 2018. Mechanisms of cytosolic calcium elevation in plants: the role of ion channels, calcium extrusion systems and NADPH oxidase-mediated 'ROS–Ca<sup>2+</sup> Hub'. *Functional Plant Biology* **45**, 9–27.

Forman HJ, Fukuto JM, Torres M. 2004. Redox signaling: thiol chemistry defines which reactive oxygen and nitrogen species can act as second messengers. *American Journal of Physiology. Cell Physiology* **287**, C246–C256.

Garcia-Mata C, Wang J, Gajdanowicz P, Gonzalez W, Hills A, Donald N, Riedelsberger J, Amtmann A, Dreyer I, Blatt MR. 2010. A minimal cysteine motif required to activate the SKOR K<sup>+</sup> channel of Arabidopsis by the reactive oxygen species H<sub>2</sub>O<sub>2</sub>. *The Journal of Biological Chemistry* **285**, 29286–29294.

Kadota Y, Sklenar J, Derbyshire P, *et al.* 2014. Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. *Molecular Cell* **54**, 43–55.

Kim TK, Nam JH, Ahn WG, *et al.* 2013. Lys1110 of TRPM2 is critical for channel activation. *The Biochemical Journal* **455**, 319–327.

Liu Y, Yu Y, Sun J, Cao Q, Tang Z, Liu M, Xu T, Ma D, Li Z, Sun J. 2019. Root-zone-specific sensitivity of K<sup>+</sup> and Ca<sup>2+</sup>-permeable channels to H<sub>2</sub>O<sub>2</sub> determines ion homeostasis in salinized diploid and hexaploid *Ipomoea trifida*. *Journal of Experimental Botany* **70**, 1389–1405.

Ma L, Zhang H, Sun L, Jiao Y, Zhang G, Miao C, Hao F. 2012. NADPH oxidase AtrbohD and AtrbohF function in ROS-dependent regulation of Na<sup>+</sup>/K<sup>+</sup> homeostasis in Arabidopsis under salt stress. *Journal of Experimental Botany* **63**, 305–317.

Marino D, Dunand C, Puppo A, Pauly N. 2012. A burst of plant NADPH oxidases. *Trends in Plant Science* **17**, 9–15.

Mäser P, Thomine S, Schroeder JI, *et al.* 2001. Phylogenetic relationships within cation transporter families of Arabidopsis. *Plant Physiology* **126**, 1646–1667.

Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell & Environment* **33**, 453–467.

Mouhaya W, Allario T, Brumos J, Andres F, Froelicher Y, Luro F, Talon M, Ollitrault P, Morillon R. 2010. Sensitivity to high salinity in tetraploid citrus seedlings increases with water availability and correlates with expression of candidate genes. *Functional Plant Biology* **37**, 674–685.

Munns R, James RA. 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant and Soil* **253**, 201–218.

Munns R, James RA, Gilliam M, Flowers TJ, Colmer TD. 2016. Tissue tolerance: an essential but elusive trait for salt-tolerant crops. *Functional Plant Biology* **43**, 1103–1113.

Parisod C, Holderegger R, Brochmann C. 2010. Evolutionary consequences of autopolyploidy. *New Phytologist* **186**, 5–17.

- Price MB, Kong D, Okumoto S.** 2013. Inter-subunit interactions between glutamate-like receptors in Arabidopsis. *Plant Signaling & Behavior* **8**, e27034.
- Saleh B, Allario T, Dambier D, Ollitrault P, Morillon R.** 2008. Tetraploid citrus rootstocks are more tolerant to salt stress than diploid. *Comptes Rendus Biologies* **331**, 703–710.
- Shabala S, Pottosin I.** 2014. Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. *Physiologia Plantarum* **151**, 257–279.
- Shabala S, Wu H, Bose J.** 2015. Salt stress sensing and early signalling events in plant roots: current knowledge and hypothesis. *Plant Science* **241**, 109–119.
- Shabala S, Bose J, Fuglsang AT, Pottosin I.** 2016. On a quest for stress tolerance genes: membrane transporters in sensing and adapting to hostile soils. *Journal of Experimental Botany* **67**, 1015–1031.
- Tu Y, Jiang A, Gan L, et al.** 2014. Genome duplication improves rice root resistance to salt stress. *Rice* **7**, 15.
- Wu HH, Zhang XC, Giraldo JP, Shabala S.** 2018. It is not all about sodium: revealing tissue specificity and signalling roles of potassium in plant responses to salt stress. *Plant and Soil* **431**, 1–17.
- Yang CW, Zhao L, Zhang HK, Yang ZZ, Wang H, Wen SS, Zhang CY, Rustgi S, von Wettstein D, Liu B.** 2014. Evolution of physiological responses to salt stress in hexaploid wheat. *Proceedings of the National Academy of Sciences, USA* **111**, 11882–11887.
- Yoshioka K, Moeder W, Kang HG, Kachroo P, Masmoudi K, Berkowitz G, Klessig DF.** 2006. The chimeric Arabidopsis CYCLIC NUCLEOTIDE-GATED ION CHANNEL11/12 activates multiple pathogen resistance responses. *The Plant Cell* **18**, 747–763.
- Zhong H, Lai J, Yau K-W.** 2003. Selective heteromeric assembly of cyclic nucleotide-gated channels. *Proceedings of the National Academy of Sciences, USA* **100**, 5509–5513.
- Zhou L, Lan W, Jiang Y, Fang W, Luan S.** 2014. A calcium-dependent protein kinase interacts with and activates a calcium channel to regulate pollen tube growth. *Molecular Plant* **7**, 369–376.