Seed Science Research (2013) **23**, 217–221
doi:10.1017/S0960258513000299

RESEARCH OPINION

TOUCH ME – '*Touch*' genes in the micropylar endosperm

Hiroyuki Nonogaki*
Department of Horticulture, Oregon State University, Corvallis, OR 97331, USA

(Received 5 August 2013; accepted after revision 15 August 2013; first published online 19 September 2013)

Abstract

The micropylar region of endosperm (ME) is a physical barrier to radicle emergence in seeds of many different species, including tomato (*Solanum lycopersicum*) and *Arabidopsis thaliana*. ME is thought to be weakened through cell wall-modifying proteins, and this is supported by transcriptome data showing enrichment of cell wall-associated genes in ME. Gibberellin and ethylene have been suggested to be involved in induction of these genes in ME. However, mechanisms underlying this critical event for germination still remain elusive. In addition to hormonal regulation of ME weakening, recent data from high-throughput analyses suggested that it might be important for the radicle tip to 'touch' ME (or mechanosensing), in terms of ME-specific gene induction. This emerging hypothesis can be integrated with previous hypotheses about hormonal regulation of ME-specific gene expression in seeds.

Keywords: *Arabidopsis*, micropylar endosperm (ME), tissue-specific gene expression, tomato, touch

Introduction

Seed germination is suppressed by multiple layers of molecular repression, which play an important role in seed dormancy (Nonogaki, 2008). Hormone levels in seeds are regulated by transcriptional control of hormone metabolism genes. Expression of genes encoding the rate-limiting enzymes for abscisic acid (ABA) and gibberellin (GA) biosynthesis and deactivation determines dormancy levels in seeds (Seo *et al.*, 2009). In contrast, post-transcriptional and -translational regulation, such as mRNA destabilization by microRNA (Liu *et al.*, 2007) and protein degradation or modification by the ubiquitin-26S proteasome pathway (Piskurewicz *et al.*, 2008; Lee *et al.*, 2010), is important for hormone perception and signalling, which are also important determinants of seed dormancy and germination.

Suppression of embryo emergence from seeds is also controlled at the tissue level. The testa imposes seed dormancy through its chemical properties, such as proanthocyanidins (Debeaujon *et al.*, 2000, 2007). In hard seeds, physicochemical properties of the testa, i.e. impermeability of the testa to water, inhibits seed germination (Bewley *et al.*, 2013). The endosperm provides mechanical resistance to the embryo through its rigidity or elasticity (Bewley *et al.*, 2013). An extremely rigid and thick endosperm allows little expansion of the embryo contained in a seed, while a relatively thin and elastic endosperm allows embryo expansion during imbibition but still limits radicle emergence (Liu *et al.*, 2005a, b).

In *Arabidopsis* seeds, distinct phases of seed germination are marked by testa and endosperm rupture. Testa rupture, which is not observed in dormant seeds, provides a visible sign of germination events happening in seeds, while endosperm rupture marks completion of germination (in a strict sense) (Liu *et al.*, 2005 a, b). During these two phases, elongation of the endosperm together with the enclosed embryo is observed, suggesting that embryo growth potential is generated and increased and, more importantly, for the scope of this paper, that the micropylar region of endosperm (ME) is being weakened.

Recent work on tissue-specific gene expression in *Arabidopsis* seeds (Dekkers *et al.*, 2013) presented a substantial amount of information about ME-enriched gene expression, which provided new insights into the mechanisms of embryo–endosperm interaction for regulation of ME-specific gene expression, an important research theme in seed biology. Interpretation of the new data and integration of the

*Correspondence
Email: hiro.nonogaki@oregonstate.edu

traditional and new concepts on the mechanisms of ME gene expression will be discussed in this research opinion paper.

ME weakening

It is now widely accepted that ME weakening is a prerequisite for completion of germination (Bewley *et al.*, 2013). Actual measurement of puncture forces necessary to penetrate ME provides strong evidence for reduction of mechanical resistance or weakening of ME during germination (Watkins and Cantliffe, 1983; Groot and Karssen, 1987; Chen and Bradford, 2000; Muller *et al.*, 2006; Lee *et al.*, 2012). The contention is also supported by the results of activity and localization assays and purification and characterization of cell wall enzymes and other proteins expressed in seeds (Nonogaki and Morohashi, 1996; Toorop *et al.*, 1996; Sitrit *et al.*, 1999).

ME-specific genes are regulated by plant hormones. GA induces ME-specific expression of *MAN2*, an endo-β-mannanase gene (Nonogaki *et al.*, 2000), as well as germination in the GA-deficient *gib-1* tomato mutant, which does not germinate otherwise (Groot and Karssen, 1987). Other ME cell wall genes, such as *xyloglucan endotransglycosylases* (*XTHs*) and *expansins* (*EXPs*) are also dependent on GA (Chen and Bradford, 2000; Chen *et al.*, 2001). Recent studies have revealed the importance of ethylene biosynthesis and signalling in ME gene induction: ethylene promotes ME weakening and rupture in *Lepidium sativum* seeds (Linkies *et al.*, 2009). The intersection between GA and ethylene signalling in ME gene expression is not clear yet (discussed below).

ME gene expression

Dekkers *et al.* (2013) performed a comprehensive and dynamic transcriptomic analysis for the two major seed compartments – the embryo and the endosperm in *Arabidopsis thaliana*. They also divided the embryo into radicle/hypocotyl (RAD) and cotyledon (COT) regions and the endosperm into the micropylar plus charazal endosperm (MCE) and the rest of the endosperm (PE: peripheral endosperm). Furthermore, they divided the imbibition time course into 11 time points, including stages prior to and following testa rupture (TR) or endosperm rupture (ER). The high-resolution data set from this genome-wide gene expression analysis provided detailed information about temporal and spatial expression of seed germination-associated genes, which highlighted the importance of ME-specific gene expression. The data indicated that TR was marked by a large number of differentially expressed genes, compared to non-ruptured seeds, the majority of which were genes up-regulated in MCE, suggesting ME-specific activation of gene expression. Thirty out of the 104 genes up-regulated (>fivefold) in MCE were related to cell wall function (Dekkers *et al.*, 2013), which supports the idea of germination induction through ME weakening by cell wall degradation. Comparison of genes expressed 31 h after the start of imbibition showed that 200 genes were differentially expressed (>threefold), with the majority of these (95%) being up-regulated in MCE compared to PE (Dekkers *et al.*, 2013). These results suggest the significance of ME gene expression for seed germination control.

It is possible that the changes of MCE gene expression profiles before and after TR are caused by environmental factors, such as light and oxygen, because testa rupture exposes ME to these environmental factors. However, their data sets did not support this hypothesis (Dekkers *et al.*, 2013). Therefore, the large change in gene expression profiles in MCE is not caused by TR. Most likely, TR (and ER) is rather a consequence of gene expression in ME during this specific period of time of germination.

A similar transcriptomic analysis has been performed for four different tissues – EC (endosperm cap, similar to ME), LE (lateral endosperm, similar to PE), R (radicle/hypocotyl, similar to RAD), C (cotyledons, similar to COT) of germinating tomato seeds. In this study also, cell wall-associated genes were enriched in ME (Martinez-Andujar *et al.*, 2012). In this tomato seed transcriptome, ME-enriched expression of *TERF1* (*Tomato Ethylene Response Factor 1*), a transcription factor involved in ethylene signalling, and its direct targets *NP24*, *P23* and *PR5-like*, which are pathogenesis-related (PR) and wound-induced genes, was detected. It is possible that these genes are expressed as a pre-programmed defence mechanism against possible attacks by microorganisms to ME after its rupture by the radicle tip, which exposes the nutritious tissue to the outer environment. However, another possibility is that *TERF1* and the downstream targets are induced as a wounding response, in which ethylene signalling is typically involved (Johnson and Ecker, 1998). The increase in embryo growth potential probably raises pressure inside a seed, with the radicle tip pressing down on to ME, which mimics a wounding response. The latter contention invokes an idea that ME-specific gene expression may be a consequence of 'mechanosensing', in which ME senses the mechanical forces generated by the embryo (Martinez-Andujar *et al.*, 2012) (Fig. 1).

Interestingly, the *Arabidopsis* seed transcriptome mentioned above demonstrated a more than eightfold increase of expression of *TOUCH3* and *TOUCH4* in MCE after TR (Dekkers *et al.*, 2013), which are known to be expressed rapidly in response to touch or thigmotropism (Braam, 2005). This result provides evidence to support the idea of mechano- or

https://doi.org/10.1017/S0960258513000299 Published online by Cambridge University Press

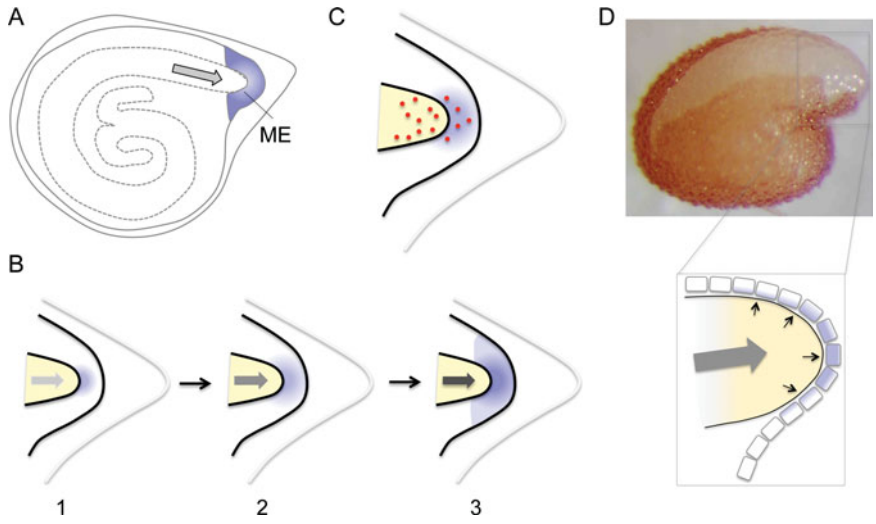

Figure 1. (colour online) Schematic representation of possible mechanisms of embryo–endosperm interaction for gene induction in the micropylar endosperm. (A) Schematic representation of tomato seed with the micropylar endosperm (ME) highlighted by shading and embryo growth potential by a grey arrow. (B) Three progressive stages (1–3) of tomato ME illustrating the mechano- or touch-sensing hypothesis. In this hypothesis, embryo growth potential (grey arrow) increases during imbibition (indicated by increased intensities), which causes the radicle tip to press ('touch') on to ME. This pressure is perceived by ME as a wounding response, which triggers ethylene signalling and ME gene induction (diffuse shading). (C) An alternative hypothesis for ME gene induction, in which gibberellins or insoluble secondary messengers (dots) are transferred from the embryo to ME. See text for details. (D) Photograph of *Arabidopsis* seeds with testa rupture and endosperm elongation (top). The scheme (bottom) shows that mechano- or touch-sensing (shading) described for tomato seeds may also be occurring in the single-cell layer ME in *Arabidopsis* seeds. Small black arrows indicate pressure.

touch-sensing as a possible mechanism for ME-specific gene induction. Their finding was further strengthened by the comparison of the set of MCE TR up-regulated genes in *Arabidopsis* seeds with genes up-regulated upon touch in the aerial part of *Arabidopsis* plants (Lee *et al.*, 2005), which showed a 30% overlap between the two sets of gene expression data. The overlap was even more striking when GO (gene ontology) was considered for analysis; touch-induced signalling resulted in relatively higher abundance of genes associated with cell wall, calcium-binding, disease resistance, kinase and transcription factor (Lee *et al.*, 2005), which matched well with the GO classes of the MCE TR genes (Dekkers *et al.*, 2013). Thus, both the *Arabidopsis* and tomato seed research suggest mechano- or touch-sensing as a possible mechanism of ME gene induction.

Reconsideration of embryo–endosperm interaction in ME gene induction

The traditional hypothesis about the mechanisms of ME gene expression is that genes expressed in ME are induced by GA, which is secreted from the embryo (Groot and Karssen, 1987). This hypothesis is supported by the fact that ME-specific gene expression is induced in *gib-1* tomato seeds by exogenous GA (Chen and Bradford, 2000; Nonogaki *et al.*, 2000; Chen *et al.*, 2001). MAN expression is induced in isolated tomato ME by co-incubating it with the embryonic axes, which can be replaced by exogenous GA (Martinez-Andujar *et al.*, 2012). This result suggests that the embryo has the capacity to secrete GA, which also supports the traditional view. However, it is not explained why such a diffusible signal like GA does not stimulate gene expression in the rest of the endosperm (LE or PE). Isolated (embryo-less) tomato LE responds to GA and induces MAN in this tissue (Martinez-Andujar *et al.*, 2012). Therefore, it is unlikely that GA receptors localize exclusively in ME. Non-diffusible secondary messengers, such as peptide ligands, could be produced by the embryo and transferred to ME (Fig. 1). However, such factors have not been identified and, if any, the modes of transport and perception of these signals between the radicle tip and ME need to be explained.

In the traditional hypothesis, GA was assumed to affect both the embryo (growth potential increase) and endosperm (ME gene expression) in parallel (Groot and Karssen, 1987). However, it is possible that GA is primarily responsible for changes in the embryo and affects ME gene expression indirectly or secondarily. The latter contention is well integrated with an alternative new hypothesis of mechano- or touch-sensing into a comprehensive scheme, in which the embryonic (growth potential increase) and endospermic (ME gene expression) events are placed

https://doi.org/10.1017/S0960258513000299 Published online by Cambridge University Press

sequentially (GA biosynthesis/signalling in the embryo → embryo growth → touch ME → ME gene expression → ME weakening → germination). The traditional view of GA dependency of ME gene expression and the new hypothesis of mechano- or touch-sensing are not mutually exclusive, because GA is still necessary for the embryo to 'touch' ME and cause ME-specific gene expression in the new hypothesis also. The new hypothesis also explains why ethylene signalling, which is typical of wounding, touch or thigmotropism, is important for ME gene expression. Probably, it is a logical next step to test this emerging hypothesis for comprehensive understanding of the biological significance of ME events during seed germination, while other possibilities, including direct stimulation of ME gene expression by GA or secondary messengers from the embryo should not be excluded.

Perspectives

This opinion paper focused only on the 'touch ME' mechanism. However, there were more discoveries from the high-throughput transcriptomes mentioned in this paper. For example, the *Arabidopsis* transcriptome (Dekkers *et al.*, 2013) identified *INFLORESCENCE DEFICIENT IN ABSCISSION (IDA)-LIKE1* (*IDL1*) as one of the highest differentially expressed genes in MCE (>20-fold). This gene encodes a peptide ligand, which interacts with receptor kinases (RLKs). This peptide–RLK interaction is important for cell separation during lateral root emergence (Kumpf *et al.*, 2013), which requires penetration of cell layers covering the lateral root primordia and somewhat mimics endosperm rupture by the radicle in seeds (Belin and Lopez-Molina, 2010). Cell separation may be an important final determinant of ME rupture (Linkies *et al.*, 2009). The discovery of *IDL* induction in ME after TR has provided a good target of research to elucidate the mechanisms of cell separation in ME, an uncharacterized, but possibly the most important, last step of seed germination. Furthermore, *IDL1* identification in ME also opened a whole new area of research – peptide–RLK signalling in ME, which could also mediate communication between ME and the radicle tip. IDA and IDL share sequence and functional similarities with CLAVATA3 (CLV3), one of the best-characterized signalling peptides in plants (Kondo *et al.*, 2006), a modified form of which (MCLV3) suppresses plant stem cell fate ((Ito *et al.*, 2006). *CLV3* was originally identified as *EMBRYO SURRONDING REGION* (*ESR*), which is specifically expressed in ME of developing maize seeds (Opsahl-Ferstad *et al.*, 1997). It is conceivable that peptide–RLK signalling is involved in the biology of ME. Thus, recent data have opened possibilities of new research development in ME and seed germination biology.

Acknowledgements

I am grateful to Henk Hilhorst, Editor-in-Chief, for providing an opportunity for me to submit this opinion paper to *Seed Science Research*.

Financial support

This work was supported by US–Israel Binational Science Foundation grant number 2009173 to H.N.

Conflicts of interest

None.

References

Belin, C. and Lopez-Molina, L. (2010) Endosperm rupture as a model for lateral root emergence in Arabidopsis? *Plant Signaling Behavior* **5**, 564–566.

Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M. and Nonogaki, H. (2013) *Seeds: Physiology of development, germination and dormancy*. New York, Springer.

Braam, I. (2005) In touch: plant responses to mechanical stimuli. *New Phytologist* **165**, 373–389.

Chen, F. and Bradford, K.J. (2000) Expression of an expansin is associated with endosperm weakening during tomato seed germination. *Plant Physiology* **124**, 1265–1274.

Chen, F., Dahal, P. and Bradford, K.J. (2001) Two tomato expansin genes show divergent expression and localization in embryos during seed development and germination. *Plant Physiology* **127**, 928–936.

Debeaujon, I., Leon-Kloosterziel, K.M. and Koornneef, M. (2000) Influence of the testa on seed dormancy, germination, and longevity in arabidopsis. *Plant Physiology* **122**, 403–414.

Debeaujon, I., Lepiniec, L., Pourcel, L. and Routaboul, J.M. (2007) *Seed coat development and dormancy*. pp. 25–49 *in* Bradford, K.J.; Nonogaki, H. (Eds) *Seed development, dormancy and germination*. Oxford, Blackwell Publishing.

Dekkers, B.J., Pearce, S., Van Bolderen-Veldkamp, R.P., Marshall, A., Widera, P., Gilbert, J., Drost, H.G., Bassel, G.W., Muller, K., King, J.R., Wood, A.T., Grosse, I., Quint, M., Krasnogor, N., Leubner-Metzger, G., Holdsworth, M.J. and Bentsink, L. (2013) Transcriptional dynamics of two seed compartments with opposing roles in Arabidopsis seed germination. *Plant Physiology* **163**, 205–215.

Groot, S.P.C. and Karssen, C.M. (1987) Gibberellins regulate seed germination in tomato by endosperm weakening: A study with gibberellin-deficient mutants. *Planta* **171**, 525–531.

Ito, Y., Nakanomyo, I., Motose, H., Iwamoto, K., Sawa, S., Dohmae, N. and Fukuda, H. (2006) Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science* **313**, 842–845.

Johnson, P.R. and Ecker, J.R. (1998) The ethylene gas signal transduction pathway: A molecular perspective. *Annual Review of Genetics* **32**, 227–254.

Kondo, T., Sawa, S., Kinoshita, A., Mizuno, S., Kakimoto, T., Fukuda, H. and Sakagami, Y. (2006) A plant peptide encoded by *CLV3* identified by in situ MALDI-TOF MS analysis. *Science* **313**, 845–848.

Kumpf, R.P., Shi, C.L., Larrieu, A., Sto, I.M., Butenko, M.A., Peret, B., Riiser, E.S., Bennett, M.J. and Aalen, R.B. (2013) Floral organ abscission peptide IDA and its HAE/HSL2 receptors control cell separation during lateral root emergence. *Proceedings of the National Academy of Sciences USA* **110**, 5235–5240.

Lee, D., Polisensky, D.H. and Braam, J. (2005) Genome-wide identification of touch- and darkness-regulated arabidopsis genes: A focus on calmodulin-like and *XTH* genes. *New Phytologist* **165**, 429–444.

Lee, K.J., Dekkers, B.J., Steinbrecher, T., Walsh, C.T., Bacic, A., Bentsink, L., Leubner-Metzger, G. and Knox, J.P. (2012) Distinct cell wall architectures in seed endosperms in representatives of the Brassicaceae and Solanaceae. *Plant Physiology* **160**, 1551–1566.

Lee, K.P., Piskurewicz, U., Turečková, V., Strnad, M. and Lopez-Molina, L. (2010) A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in arabidopsis dormant seeds. *Proceedings of the National Academy of Sciences USA* **107**, 19108–19113.

Linkies, A., Muller, K., Morris, K., Tureckova, V., Wenk, M., Cadman, C.S.C., Corbineau, F., Strnad, M., Lynn, J.R., Finch-Savage, W.E. and Leubner-Metzger, G. (2009) Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using *Lepidium sativum* and *Arabidopsis thaliana*. *The Plant Cell* **21**, 3803–3822.

Liu, P.P., Koizuka, N., Homrichhausen, T.M., Hewitt, J.R., Martin, R.C. and Nonogaki, H. (2005a) Large-scale screening of Arabidopsis enhancer-trap lines for seed germination-associated genes. *The Plant Journal* **41**, 936–944.

Liu, P.P., Koizuka, N., Martin, R.C. and Nonogaki, H. (2005b) The *Bme3* (*Blue Micropylar End 3*) Gata zinc finger transcription factor is a positive regulator of Arabidopsis seed germination. *The Plant Journal* **44**, 960–971.

Liu, P.-P., Montgomery, T.A., Fahlgren, N., Kasschau, K.D., Nonogaki, H. and Carrington, J.C. (2007) Repression of *AUXIN RESPONSE FACTOR10* by microRNA160 is critical for seed germination and post-germination stages. *The Plant Journal* **52**, 133–146.

Martinez-Andujar, C., Pluskota, W.E., Bassel, G.W., Asahina, M., Pupel, P., Nguyen, T.T., Takeda-Kamiya, N., Toubiana, D., Bai, B., Gorecki, R.J., Fait, A., Yamaguchi, S. and Nonogaki, H. (2012) Mechanisms of hormonal regulation of endosperm cap-specific gene expression in tomato seeds. *The Plant Journal* **71**, 575–586.

Muller, K., Tintelnot, S. and Leubner-Metzger, G. (2006) Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and endosperm rupture of cress and *Arabidopsis thaliana*. *Plant and Cell Physiology* **47**, 864–877.

Nonogaki, H. (2008) Repression of transcription factors by microRNA during seed germination and postgermination: Another level of molecular repression in seeds? *Plant Signaling and Behavior* **3**, 65–67.

Nonogaki, H. and Morohashi, Y. (1996) An endo-β-mannanase develops exclusively in the micropylar endosperm of tomato seeds prior to radicle emergence. *Plant Physiology* **110**, 555–559.

Nonogaki, H., Gee, O.H. and Bradford, K.J. (2000) A germination-specific endo-β-mannanase gene is expressed in the micropylar endosperm cap of tomato seeds. *Plant Physiology* **123**, 1235–1246.

Opsahl-Ferstad, H.-G., Deunff, E.L., Dumas, C. and Rogowsky, P.M. (1997) *ZmESR*, a novel endosperm-specific gene expressed in a restricted region around the maize embryo. *The Plant Journal* **12**, 235–246.

Piskurewicz, U., Jikumaru, Y., Kinoshita, N., Nambara, E., Kamiya, Y. and Lopez-Molina, L. (2008) The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. *The Plant Cell* **20**, 2729–2745.

Seo, M., Nambara, E., Choi, G. and Yamaguchi, S. (2009) Interaction of light and hormone signals in germinating seeds. *Plant Molecular Biology* **69**, 463–472.

Sitrit, Y., Hadfield, K.A., Bennett, A.B., Bradford, K.J. and Downie, A.B. (1999) Expression of a polygalacturonase associated with tomato seed germination. *Plant Physiology* **121**, 419–428.

Toorop, P.E., Bewley, J.D. and Hilhorst, H.W.M. (1996) Endo-β-mannanase isoforms are present in the endosperm and embryo of tomato seeds, but are not essentially linked to the completion of germination. *Planta* **200**, 153–158.

Watkins, J.T. and Cantliffe, D.J. (1983) Mechanical resistance of the seed coat and endosperm during germination of *Capsicum annuum* at low temperature. *Plant Physiology* **72**, 146–150.