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REVIEW PAPER

Variability in seeds: biological, ecological, and agricultural implications

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Abstract

Variability is observed in biology across multiple scales, ranging from populations, individuals, and cells to the molecular components within cells. This review explores the sources and roles of this variability across these scales, focusing on seeds. From a biological perspective, the role and the impact this variability has on seed behaviour and adaptation to the environment is discussed. The consequences of seed variability on agricultural production systems, which demand uniformity, are also examined. We suggest that by understanding the basis and underlying mechanisms of variability in seeds, strategies to increase seed population uniformity can be developed, leading to enhanced agricultural production across variable climatic conditions.

Key words: Dormancy, germination, noise, population, seeds, variability.

Introduction

Variability, the spread of values of some quantity across different individual samples, is present in diverse biological systems and across multiple scales ranging from populations to the molecular level (Trewavas, 2012). Throughout this article, we use 'variability' to refer to the natural differences that exist across individual biological samples, decoupled from experimental and measurement noise, and will be predominantly concerned with phenotypic variability, from transcript abundance at the cellular level to plant physiology at the organismal level.

In many instances, a species seeks to manage this natural variability to produce a constant phenotype (Waddington, 1942; Debat and David, 2001; Lempe *et al.*, 2013; Boukhibar and Barkoulas, 2016). In other instances, variability can be a positive feature in the generation of diverse phenotypes (Blake *et al.*, 2006; Fraser and Kaern, 2009).

The role of phenotypic variability at the organismal scale in evolution has been studied for centuries, but recent years have seen a large increase in the study of variability at the cellular level, exploring how genetically identical cells exhibit substantial heterogeneity in appearance and behaviour (Raser and O'Shea, 2005). Recent studies have begun to uncover the cellular mechanisms underlying the role of variability in biological systems (Elowitz *et al.*, 2002; Eldar and Elowitz, 2010), the challenges and advantages (Fraser and Kaern, 2009; Rao *et al.*, 2002) of biological variability, and even fundamental physical laws underlying cellular variability (Paulsson, 2004, 2005; Raser and O'Shea, 2005; Lestas *et al.*, 2010). These topics have been recently reviewed (Tsimring, 2014; Abley *et al.*, 2016).

Here we will focus on these principles, together with our current understanding of variability in plants and specifically within seeds. Seeds are a plant's way of moving through time and space, and securing the next generation of individuals in annual species (Finch-Savage and Leubner-Metzger, 2006). The planting of seeds in the soil also represents the starting point for the vast majority of world agriculture. Rapid and uniform crop establishment is a key determinant of crop yields (Finch-Savage and Bassel, 2015), making understanding the

variability within seeds a vital component of the global seed industry and ensuring crop security.

Variability in seeds is manifest across different scales, including the interpopulation, intrapopulation, and cellular levels. In this article, we will review classifications of biological variability and describe which apply to seeds. We consider how this variability unfolds across scales, the molecular players linked to seed variability, and describe the role of variability in seed germination and fate decisions. We also discuss the implications of this multiscale variability in biological, ecological, and agricultural contexts, and the relationships among them.

Variability and its sources

Biological cells are tumultuous environments, with interactions driven by diffusive dynamics and thermal noise (Trewavas, 2012; Paulsson, 2005). Cells and organisms exist in highly variable environments that induce variability in response to heterogeneous and time-varying conditions. Sources of variability are often described as 'intrinsic' or 'extrinsic', respectively describing processes inherent to the individual entity considered and external influences arising from the individual's environment (Swain *et al.*, 2002). We reiterate that throughout this article we use 'variability' to refer to differences in some quantity between biological individuals, unrelated to experimental uncertainty. In Fig. 1 we illustrate some of the key sources of seed variability to be discussed below.

Intrinsic and extrinsic cellular variability

Intrinsic variability refers to variability within individuals, usually at the cellular level. This intracellular variability emerges from the stochastic nature of cellular processes (notably including transcription and translation events; Swain *et al.*, 2002), which take place due to random collisions between various molecular components arising from diffusion and thermal noise.

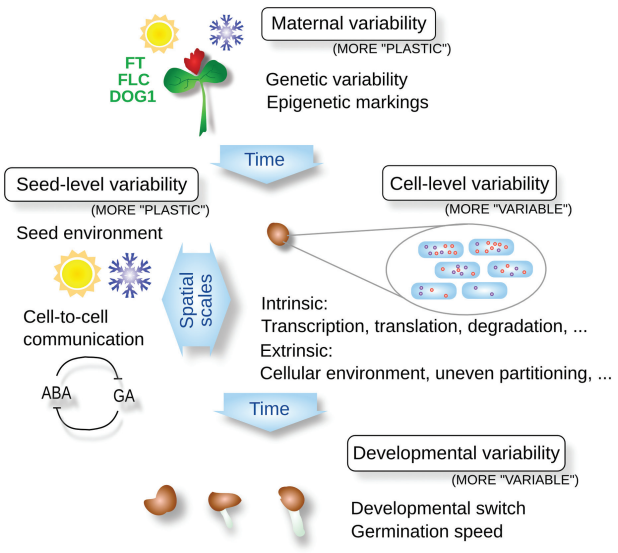

Fig. 1. Sources of seed variability. Schematic depicting the sources and scales of variability observed in seeds ranging from the maternal environment (plastic), seed level variability (plastic), cell level variability (variable), to developmental variability (variable), where the meanings of 'plastic' and 'variable' are described in the text.

It is widely accepted that the random nature of biochemical reactions, especially gene expression in cells, contributes to the stochastic behaviours exhibited by cells and organisms (Raser and O'Shea, 2005). The 'transcriptional noise' generated through gene expression within organisms is postulated to account for large degrees of variability, contributing to phenotypic differences between individuals from genetically and environmentally identical backgrounds (Swain *et al.*, 2002; Raser and O'Shea, 2005; Raj and van Oudenaarden, 2008). This inherent variability in gene expression leads to variable levels of proteins and metabolites between cells. As a result, signalling processes and responses associated with specific cellular components will be inherently variable between individual cells. Variability in organelle content between cells within a population has been linked to transcription rates, suggesting a role for cellular energy budgets in generating cell variability (Johnston *et al.*, 2012). Extrinsic variability refers to differences between individuals in a population. At the cellular level, influences such as fluctuating microenvironments, differences induced through uneven cell partitioning, and physical constraints induce cell to cell differences in behaviour (Chalancon *et al.*, 2012; Johnston, 2012).

Variability in gene expression can, in different contexts, be valuable or detrimental to cells. An active field of research aims to explore how organisms have evolved to suppress problematic variability (e.g. when a given process must be tightly controlled) and exploit beneficial variability (e.g. in cellular bet-hedging in unpredictable environments). In particular, various motifs in gene regulatory networks have been postulated as influencing genetic variability, including feed-forward loops to process environmental time variation and feedback loops to facilitate state-based control (Milo *et al.*, 2002; Swain, 2004; Chalancon *et al.*, 2012). It is important to note that a particular network structure alone is not enough to infer a particular functional role (Ingram *et al.*, 2006).

There is also evidence for cellular control acting on other variable microscopic features, including organelle content (Johnston *et al.*, 2012; Jajoo *et al.*, 2016). However, cellular control can never completely remove noise (Lestas *et al.*, 2010); there will always be an element of variability within a specific biological system. In plants, this represents a problem to the agricultural industry, which demands uniformity, as this variability remains complicated to remove.

Environmental variability and phenotypic variation

Plants, as sessile organisms, are continuously exposed to extrinsic sources of variability in the form of unavoidable changing environments. The ability to produce a consistent phenotype in the face of fluctuations is referred to as robustness (Kitano, 2004; Boukhibar and Barkoulas, 2016). This concept has been adapted from the concept of canalization, which states that members of a given population could produce the same phenotype despite the presence of environmental and genetic variation (Waddington, 1942).

The combination of extrinsic and intrinsic variability across scales produces phenotypic variation. This variability/variation can be broken down into four different types: (i) invariant, where phenotypes remain consistent across a range of environmental conditions; (ii) plastic, where phenotypes are consistently altered by the environment; (iii) variable, where a range of phenotypes are observed across constant environmental conditions; and (iv) variable and plastic, which is a combination of these two (reviewed in Abley *et al.*, 2016).

Variability observed in seed populations can be placed in the fourth category (Fig. 1). Environmental conditions in the mother plant can influence the germination of seeds made subsequently, accounting for the plastic component, while seeds from the same population also show variability in their germination behaviour under constant conditions. These two aspects of variability will be considered separately, along with their underlying mechanisms. We will also consider the three scales of variability acting within seed populations mentioned previously.

The plastic component: seed variability induced by maternal environment

Extensive variability in both germination rate and dormancy between different seed populations has been previously reported in a wide variety of species (Bradford and Trewavas, 1994; Kanno *et al.*, 2010). In the context of agriculture, variability between seed lots represents a key obstacle to the uniform establishment of crops, and harmonizing seed behaviour remains a key objective of this industry (Finch-Savage and Bassel, 2015).

Seeds have been shown to exhibit higher levels of germination in association with reduced dormancy when developed in specific parental conditions, including high temperature, drought, and short-day periods (Fenner, 1991). These maternal effects act as instructive signals for the next generation to time their germination in accordance with the season in which they are produced.

Recent research has begun to uncover the mechanisms underlying this interpopulation variability. It has been shown that the composition of the seed coat can directly influence germination and dormancy of seeds (Debeaujon, 2000), while maternal conditions have been shown to effect levels of flavonoid production within the seed coat of developing seeds (MacGregor *et al.*, 2015). During low temperatures, the levels of phenylpropanoid gene expression increased, leading to higher flavonoid concentrations and increased seed dormancy.

The influence of the maternal environment on seed behaviour was shown to be mediated by *FLOWERING LOCUS C* (*FLC*) in a temperature-dependent manner (Chiang *et al.*, 2009). A similar role for *FLOWERING LOCUS T* (*FT*) within seeds linking the maternal environment to seed behaviour has been shown (Chen *et al.*, 2014). These observations could suggest that *FLC* and *FT* act as part of the mechanism that mediates the plastic component of seed behaviour.

The common influence of these genes which mediate the environmental contribution towards flowering time also impacts germination timing, suggesting the conservation of genetic mechanisms mediating plant developmental transitions in response to the environment (Chiang *et al.*, 2009; Chen *et al.*, 2014). This hypothesis was supported by a genome-wide co-expression network which also identified flowering time regulators as mediators of this developmental phase transition (Bassel *et al.*, 2011).

The quantitative expression of *DELAY OF GERMINATION 1* (*DOG1*) transcripts in seeds has also been correlated with maternal temperatures during development (Chiang *et al.*, 2011; Footitt *et al.*, 2011, 2015). This connection may provide a quantitative molecular link between maternal temperatures and future seed behaviour.

Another potential mechanism by which the maternal environment may be passed onto progeny is through epigenetics. Epigenetic effects are thought to play a role in the regulation of key temperature-responsive genes within seeds including *DOG1* and *FLC* (Angel *et al.*, 2011; Footitt *et al.*, 2015). There are suggestions that epigenetics can be influenced by environmental factors which may provide a link between the environment and future gene expression within seeds.

The variable component: variability between individuals from the same population

Variability exists at the intrapopulation level, whereby individuals from within the same population exhibit differences in behaviour or responses. Aspects of this variability underpin an evolutionary strategy termed bet-hedging, whereby a range of phenotypes in the next generation, while potentially reducing mean population fitness, increases the chances of generational survival in an uncertain or changing environment (Slatkin, 1974). This reduces the risk of a population becoming extinct across variable conditions, and is advantageous to ensure the success of the future generation when large numbers of progeny are generated. In this sense, noise is valuable in a variable environment as it can serve an adaptive purpose towards the staggering of seedling establishment from a population. The cost of this approach is the suboptimal performance of populations under a given set of conditions, as a mitigating approach to loss under diverse conditions is being applied.

While this bet-hedging strategy is advantageous in an ecological context for the survival of species in response to unpredictable environments, it is disadvantageous in an agricultural context as it results in non-uniform and suboptimal crop establishment, leading to reductions in yield and increased difficulties in crop and pest management (Finch-Savage and Bassel, 2015). Agricultural environments are more predictable and less challenging than those faced in uncontrolled environments. We expect that yield gains are possible if plants are engineered to avoid generating the variability in their seed populations, a trait historically needed to deal with uncertainty, which is reduced in agriculture. The cost associated with bet-hedging, namely the suboptimal cellular performance of growth-limited individuals, represents an additional limiting factor to agricultural productivity which can be altered and balanced with stress tolerance (Achard *et al.*, 2006).

Variable germination in a seed lot can be observed in seeds harvested from mother plants which are grown under constant environmental conditions (Philippi, 1993). The mechanisms underlying the emergence of this seed to seed variability within populations has been explored previously at a whole-plant level, and linked to maternally derived spatial features. The position of a seed within a plant can impact its subsequent germination performance, and has been proposed to be linked to competition in the availability of nutrients from the mother plant (Susko and Lovett-Doust, 2000). This has been demonstrated in grasses, where lower positioned seeds showed enhanced germination traits compared with those in the distal upper parts of the plant (González-Rabanal *et al.*, 1994), and in the weedy *Brassica* species *Alliaria petiolata* (Susko and Lovett-Doust, 2000). This has also been observed in seeds from the *Umbelliferacea* family which show differences between primary and secondary inflorescences (Thomas *et al.*, 1977). This last example raises a second source of temporal variability whereby asynchronous flowering and pollination leads to non-uniform seed development. In both instances, when seeds from a plant are bulk harvested and treated as a single population, differences arising from the position on the mother plant where the seed developed, and the time at which seed development was initiated, will contribute towards the heterogeneity of the seed population. These whole-plant phenomena have been described previously using traditional plant physiology approaches (Gutterman, 1980), and their impacts on seed variability are minimized by conventional production practices within the seed industry.

In some species, multiple seed morphologies, or seed heteromorphisms, can be produced by the same mother plant (Silvertown, 1984). This occurs principally in the *Chenopodiaceae* and *Asteraceae* families (Imbert, 2002). These multiple morphologies can lead to contrasting germination behaviours within a seed lot, and variability within seed lots. This phenomenon is largely present in non-cultivated weedy species, and shows limited prevalence in crops.

In the context of interpopulation variability, we propose there to be at least two distinct developmental stages in seeds which are acted upon following seed development. The first is the rate at which the developmental fate switch that initiates the germination programme is flipped (Bassel, 2016), and the second is the rate at which the germination programme is executed after the flipping of the switch (Finch-Savage and Bassel, 2015).

The developmental fate switch and modelling the effects of seed variability

Seeds shed from their mother plant are typically in a dormant state, where they retain desiccation tolerance and storability. The combination of developmental time, through prolonged dry storage or after-ripening, and inputs from the environment leads to the flipping of a developmental fate switch which leads to the irreversible decision to commence germination. This results in the loss of sustained desiccation tolerance (reviewed in Bassel, 2016). Variability of dormancy levels is present within seed populations as part of the ecologically advantageous bet-hedging strategy which seeds employ. In the context of agriculture, this dormancy is typically reduced through a controlled period of after-ripening (Holdsworth *et al.*, 2008). When after-ripening treatments are not saturating, residual dormancy levels are retained within these seed populations, and the time at which individuals flip their fate switches is still not necessarily synchronous (Donohue *et al.*, 2015). As a result, the germination profile of the population can be observed as variable to this population-level variability.

Threshold-based models for the developmental fate switch in seeds have been proposed previously (Bradford and Trewavas, 1994; Bradford, 2002; Trewavas, 2012; Donohue *et al.*, 2015; Bassel, 2016). These models are effective towards modelling rates of developmental transitions in the face of environmental variability (Donohue *et al.*, 2015). Accumulated inputs over time lead to fate switching once a sensitivity threshold has been exceeded (Angel *et al.*, 2015). These sensitivity thresholds can be modulated by external and internal factors, such as hormones, making for a dynamic system, and providing the ability for individuals in the population to be different from one another (Trewavas, 2012). The inclusion of both noise and positive feedbacks enables biological systems to have dose-dependent outputs in response to environmental signals according to the length of time they are exposed to them (Trewavas, 2012).

Elegant studies have combined theoretical and experimental approaches to characterize the quantitative influences of factors including temperature and water potential on germination, leading to the concept of 'thermal time' and its variations (Allen *et al.*, 2007). These studies have shown that the variation in thermal time—a product of the time and excess temperature spent above a given germination threshold—to germination in a population of seeds is well modelled by a normal distribution, corresponding to the often-observed sigmoidal behaviour with time in the germinating proportion of seeds in a population (Batlla and Benech-Arnold, 2015). Extensions of the thermal time model have been developed to account for variability in, and influence of, other environmental conditions (Alvarado and Bradford, 2002), which have been linked with experimental evidence to describe and predict successfully the effects of seed variability in ecological and agricultural contexts (Finch-Savage, 2004). The width of the underlying distributions (i.e. the magnitude of response variation between seeds), and the microscopic features governing this width, are of key interest in the study, and control, of seed variability.

The use of population-based threshold models describing oxygen consumption in single seeds provided a link between variability in respiration and germination (Bello and Bradford, 2016). This study was able to link variability in organelle activity with that in the developmental output of germination; reflecting a similar link found between organelle content and developmental behaviour in other systems (Johnston *et al.*, 2012).

Other threshold models focusing on the hormonal regulation of germination have been presented. The hormone

balance theory proposes that the relative levels of two endogenous plant hormones, abscisic acid (ABA) and gibberellic acid (GA), determine whether a seed progresses through germination, or remains dormant. The levels of these hormones antagonize one another in order either to promote germination in the case of a high GA state, or to maintain dormancy in the case of a high ABA state (Karssen and Lacka, 1986). Here, it is not the absolute levels of hormones present that is important but rather the relative ratios between the two (Toh *et al.*, 2008), representing a relative hormone abundance threshold switch in seeds (Bradford and Trewavas, 1994).

While the position in which a seed develops within a plant, or within a fruit, can influence its germination behaviour, this is not the sole source of interpopulation variation. Intrinsic factors independent of seed position have also been shown to amplify interindividual differences. Up to 3-fold differences in ABA content have been measured on single genetically identical seeds from within the same silique in *Arabidopsis thaliana*, even in two seeds adjacent to one another (Kanno *et al.*, 2010). These striking observations demonstrate that hormone abundance and thresholding are highly variable within individuals in the same position within the mother plant. It is not known how this intrinsic variability is generated within genetically and environmentally identical individuals (though several candidate mechanisms, described above, could be responsible), but it does indicate the presence of hormone synthesis mechanisms which are sensitive to intrinsic variability.

A role for *DOG1* expression as a molecular threshold governing developmental fate decisions has also been proposed (Footitt *et al.*, 2015), yet the mechanisms leading to the enhanced expression of this gene, and its down-regulation, remain elusive.

Rate at which the germination programme is executed

Following the flipping of the developmental fate switch, it has been proposed that a series of sequential steps is followed by seeds on their way to the completion of germination. Evidence for these sequential steps has been previously reported as peaked patterns of gene expression across the germination process (Bassel *et al.*, 2011; Dekkers *et al.*, 2013).

The rate at which the germination programme is executed, or the speed at which these progressive peaks are executed, may also represent a means by which variability leading to the completion of germination between individual seeds is regulated. These sequential steps may represent checkpoints at which this developmental transition can be arrested under stress conditions (Nambara *et al.*, 2000; Lopez-Molina *et al.*, 2001). While these checkpoints may serve a key role in the ecological context of seed survival, they can also present an obstacle to rapid and uniform crop establishment (Finch-Savage and Bassel, 2015). Understanding what these checkpoints are and how they are regulated will probably provide a means to enhance uniformity in seed populations for agricultural purposes. This could include the identification of the genetic regulators of these checkpoints, for example *ABI5* in a post-germination checkpoint (Lopez-Molina *et al.*, 2001), as well as markers to identify the developmental stage at which seeds are present.

The final output of the signalling promoting the germination process is the expression of genes encoding cell wall-modifying enzymes, which promotes the expansion of the cells of the embryo (Bassel, 2016), and degrades the cell walls of the surrounding endosperm which limit embryo growth (Bewley, 1997). The cell wall-degrading enzyme endo-β-mannanase has been proposed to participate in the weakening of the micropylar endosperm of germinating tomato seeds, facilitating the exit of the embryo (Nonogaki *et al.*, 2000). It has also been shown that endo-β-mannanase activity can vary up to 1000-fold in germinating tomato endosperm caps (Still and Bradford, 1997). This single seed variability did not, however, show any relationship to the germination rate, but does highlight the presence of underlying molecular heterogeneity within individual seeds. This variation in biochemical activity within seeds prior to germination suggests variability in the quantitative levels of expression in individuals following the flipping of the developmental fate switch. This may be due to the intensity at which the germination programme is executed within individuals and the timing of the flipping of the fate switch within individuals of tomato seed populations. This pronounced variability between individuals also highlights that measurements made using pooled samples diminishes the ability to resolve variability in populations, averaging over individual quantities (see below).

Variability in seed sensitivity

The combination of both hormone levels and cellular sensitivity results in an induced hormone response. Increases in either hormone abundance or sensitivity can therefore have a similar effect on the output of a signalling pathway (Bradford and Trewavas, 1994).

The presence of sensitivity thresholds to hormones in seeds has been observed by performing titrations using the germination-promoting hormone GA with dormant seeds (Ni and Bradford, 1993). Here, subsets of the seed population are responsive to varying hormone concentrations, resulting in their germination. The sustained observation of these seeds indicates that the population which is sensitive and germinates is fixed within the population, so that after the addition of the hormone, the percentage of germination plateaus. This saturation indicates that a subsection of the population has a greater GA sensitivity, which is fixed across developmental time. A Poisson distribution of sensitivity to hormone thresholds is observed, indicating that stochastic mechanisms underly single seed sensitivities (Trewavas, 2012).

One potential sensitivity component vital in initiation of the switch is the GA receptor GID1A (Ueguchi-Tanaka *et al.*, 2005). It has recently been suggested that increased levels of GID1A transcript and protein accumulate following the dormancy-breaking process of after-ripening (Hauvermale *et al.*, 2015). This increased abundance of the GA receptor may create a greater capacity for GA response and the breaking of dormancy, and may explain the threshold responses in seed populations to GA titration. This receptor-mediated mechanism of increasing the sensitivity to GA had been previously proposed

prior to the identification of this protein (Rodbard, 1973; Hilhorst, 1990*a*, *b*). Similar mechanisms for ABA sensitivity at the level of PYRABACTIN RESISTANCE1 (PYR1) receptor abundance may also be present (Mosquna *et al.*, 2011).

Variability within individuals

Variability is also present within individual seeds at a cellular level. As multicellular structures, embryos within seeds represent interacting communities of cells. As discussed above, gene expression determines the dynamic behaviour of the genetic regulatory networks determining cell behaviour and fate decisions. Variability in gene expression, organelle content, and other subcellular quantities can induce larger scale downstream variability between cells. For example, cell to cell variability in organelle content and gene expression can influence cell fate choice (Johnston *et al.*, 2012; Katajisto *et al.*, 2015). Non-uniform behaviour within these cellular populations at a gene expression level has been observed previously in germinating Arabidopsis endosperms with respect to the promoter of the lipid-mobilizing gene *PHOSPHOENOLPYRUVATE CARBOXYKINASE1* (Penfield *et al.*, 2004), and proposed to act at the level of both GA and ABA responses (Bradford and Trewavas, 1994). Here it has been proposed that the average expression levels of all cells collectively decide when the hormone-mediated developmental fate switch flips in seeds. According to this model, cellular interactions produce a tissue-scale output through intracellular signalling. The mechanisms governing these interactions between heterogeneous cells, and their amalgamation to a macroscopic signal, within the embryo is an active topic of current research.

Agricultural and economic impact of variability in seed populations

Most plant agriculture begins with the planting of a seed, and the quality of that which is planted represents a key determinant of future success. The ‘vigour’ of seeds describes the sum total of their properties that enable seedlings to establish rapidly and uniformly across a wide variety of environmental conditions (reviewed by Finch-Savage and Bassel, 2015). Producing seeds with relatively constant and predictable levels of seed vigour is central to ensuring food security and streamlining agricultural growing practices, while the sale of high quality and high vigour seeds underpins the modern seed industry. This vigour concept also highlights the relationship between variability in seeds and yield potential.

In an ideal scenario, a farmer would sow a lot of seeds which would germinate at 100% and rapidly. This outcome, combined with appropriate seed spacing, will result in the suppression of weeds and uniform crop development, and allow harvest with a single pass of the field. Such a harvest would be of high quality with the harvestable commodity being highly uniform and at full maturity.

The ability to synchronize germination in agricultural seed lots, however, remains a persistent challenge. The bet-hedging mechanisms which serve to enhance plant survival in an ecological context negatively impact the uniform establishment of crops (Finch-Savage and Bassel, 2015). This in turn results in gaps within the field which can be filled by weed competitors and increase crop production costs, and chemical inputs which have negative impacts on the environment, which also negatively impacts profitability in crop production. The ability to harvest crops in a single pass is compromised while the marketable yield for high value crops is reduced when commodities are not brought to market while prices are favourable, a timing which is typically set at the time of planting.

Understanding the mechanisms of variability and its impacts on seed behaviour will become increasingly important with climate change. Here, phenotypic changes in seed populations will impact the reliability and predictability with which crops are established and maintained (Walck *et al.*, 2011). As a result, in the future it will become increasingly difficult to produce high quality seeds and for food producers to plan crop planting and harvesting schedules as variability within populations increases as a result of environmental fluctuation. Large economic costs will probably follow as farmers have to account for reduced yields and profits which will contribute to the long-standing issue of future food security.

Strategies to reduce variability in seed populations

Due to the persistent issue of seed lot variability, the agricultural industry continues to invest in approaches to minimize these yield- and profit-limiting effects.

The extent to which the ability to enhance variability is genetically encoded remains poorly understood. While it should be possible to identify genes which promote variability and alter these traits using conventional breeding approaches, it remains complicated in practice to pursue this line of research.

One method of reducing the variability within a given population of seeds is to perform the process of ‘seed priming’ (Paparella *et al.*, 2015). The premise of priming is to imbibe non-dormant seeds and impose a block upon these (typically using a stress) such that their germination is not completed. Holding the population under these conditions for a period of time enables the individual seeds to progress through their sequential stages of germination, and for the population to be collectively arrested at a late stage through this progression. Following the re-imbibition of these seeds, the germination of the population is rapid and more uniform in absolute terms.

A common way of imposing this block to germination is through osmopriming, where seeds are imbibed under water-limiting conditions which do not enable the completion of germination (expansion of the embryo). Imposing this water stress enables seeds to progress through the germination process without it being completed. The mechanisms underlying this process remain unclear, though they are likely to involve previously characterized stress response pathways. Other priming

methods used by the seed industry to reduce variability in seed lots have recently been reviewed (Paparella *et al.*, 2015).

It is worth noting that priming does not necessarily reduce variability within the seed population when time to germination is taken in relative terms. It does, however, reduce the absolute time to the completion of germination which is of agricultural importance. By reducing the time for crop establishment, vigour is enhanced as the variability in absolute terms is reduced within the seed population.

While priming can reduce the variable component, the careful selection of regions where seeds are produced can help minimize the plastic component. As a result, companies that produce seeds carefully choose growing locations throughout the world which have environmental conditions well suited to producing high quality seeds when subsequently planted for agricultural purposes. With increasing demands on the agricultural food production systems, priming methods and understanding the associated molecular mechanisms will become increasingly important to produce high yielding crops reliably across variable environmental conditions.

Another approach to reduce variability in seed populations is through the rational design of novel signal integration mechanisms. By understanding the network topologies and motifs which generate intrinsic variation in seeds, novel edges can be introduced to mitigate their noise-generating effects (Mangan and Alon, 2003). An alternative approach could be to generate parallel pathways to engineer redundancy into the system, limiting the impacts of noisy inputs (Trewavas, 2012).

Perspectives in understanding variability in seeds

A key obstacle to understanding variability in seeds is the destructive nature of sampling methods. Current approaches to measuring gene expression, protein abundance, or hormone abundance involve killing the sample, making it impossible to know whether the individual was close to the completion of germination or not. It is also not possible to follow the dynamics of these events over developmental time within individuals.

This highlights the need for non-destructive approaches to follow gene expression, protein abundance, and hormone concentrations in real time in order to investigate seed variability. Luciferase-based systems have been developed in other plant systems to monitor gene expression in real time (Millar *et al.*, 1995), while FRET (fluorescence resonance energy transfer)-based sensors have been developed to measure hormone concentrations in real time, including ABA (Jones *et al.*, 2014). Key obstacles to the adaptation of these approaches in seeds include the permeability and opacity of the seed coat, and the optical heterogeneity of cells within the seed due to refractive cell walls and oil bodies (Bassel *et al.*, 2014).

There is also a need to perform single seed analyses in order to address variability within populations. As mentioned above, there exists extensive variability at the individual level, and reporting only the results of sampling sets of individuals or whole populations loses information about this variability. This approach fails to capture key variation within and between populations. Variability between individuals encodes valuable information that can be used to distinguish the biological mechanisms underlying the generation of variability (and a wealth of other biological processes) (Altschuler and Wu, 2010; Johnston, 2014). For example, the shape of distributions of gene expression levels in individual cells can be used to support or refute noise-generating transcriptional mechanisms (Shahrezaei and Swain, 2008). The traditional reporting of 'mean ± standard error' statistics loses much of the information on structured variability in populations (Motulsky, 2015), and the technical challenge of decoupling experimental error (Grün *et al.*, 2014) has led to a dearth of this important information in the literature, representing a key gap in our understanding.

Finally, addressing variability at the single-cell level within individuals will enable spatial and temporal scales to be bridged, and to understand how collective cellular behaviour gives rise to organ-level responses. Recently developed techniques enable single-cell analyses to be performed using whole-mount 3D imaging, and provide an avenue to address this. The ability to do this in living tissues represents an obstacle yet to be surmounted in seeds.

Conclusion

Developing our understanding of seed variability is important from both a scientific and agronomic viewpoint, and we hope this review highlights this issue and its importance. Understanding variability within seeds across different scales, and the role of these mechanisms in modern food production systems, provides a path forward to enhancing crop synchronicity and yield across variable climatic conditions. This knowledge and its implementation into practical technologies will be increasingly important in the future with increasing climate change in terms of temperature and elevated CO_2, both of which influence the timing of seed set and the quality of seeds during their development (Madan *et al.*, 2012). Single-seed, and single-cell, measurements, facilitated by emerging technology, will allow unprecedented progress in describing and elucidating the mechanisms responsible for this variability.

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