Seed vigour and crop establishment – extending performance beyond adaptation

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Short statement: Seed vigour is critical to the yield-limiting trait of crop establishment. In this review we explore the basis of seed vigour variation and improvement in the context of crop production.

ABSTRACT

Seeds are central to crop production, human nutrition and food security. A key component of the performance of crop seeds is the complex trait of seed vigour. Crop yield and resource use efficiency depends on successful plant establishment in the field, and it is the vigour of seeds that defines their ability to rapidly, uniformly and robustly germinate and establish seedlings across diverse environmental conditions. Improving vigour to enhance the critical and yield-defining stage of crop establishment remains a primary objective of the agricultural industry and the seed/breeding companies that support it. Our knowledge of the regulation of seed germination has developed greatly in recent times, yet understanding of the basis of variation in vigour and therefore seed performance during the establishment of crops remains limited. Here we consider seed vigour at an ecophysiological, molecular and biomechanical level. We discuss how some seed characteristics that serve as adaptive responses to the natural environment are not suitable for agriculture. Past domestication has provided incremental improvements, but further actively directed change is required to produce seeds with the characteristics required both now and in the future. We discuss ways in which basic plant science could be applied to enhance seed performance in crop production.

Key words: Seed vigour, germination, pre-emergence seedling growth, seedling emergence, crop establishment, seed quality, crop production

CONTENTS:

1. INTRODUCTION

The vast majority of crops produced in world agriculture begin with the sowing of a seed to establish a new plant in the field. Successful seedling establishment is the first critical step for crop production, and determines the success or failure of the future harvest. Seed quality is an essential trait for crop production and food security, particularly during the increasing uncertainty due to climate change. In this review we describe how seed quality impacts directly yield, production efficiency and resource use efficiency to determine crop profitability and its environmental consequence. From resource-poor to industrial-scale farming, high seed quality is essential for crop production to be both sustainable and profitable and therefore widely accepted as a critically important agronomic trait.

Most crop seeds are desiccation tolerant and under the correct conditions can be stored and transported in a "dry" state with minimal loss in their ability to grow. This feature enables their distribution and sale through an international seed trade worth over $43 billion USD annually (International Seed Federation, 2015). Seeds carry the full genetic complement of the crop and are therefore the delivery system for agricultural biotechnology and crop improvement. To protect their investment in crop improvement, companies require seeds of high quality so that these benefits are not compromised when sown in the field. Moreover, farmers require seeds that ensure the reliable and successful establishment of their crops, and therefore companies must sell high quality seeds to ensure their competitive position in agricultural markets.

Seed quality includes readily measureable characteristics such as viability, seedlot purity, health and mechanical damage, but a further essential component is the more enigmatic trait of seed vigour (Perry, 1980). We explore what is required for a seed to be vigorous in an agricultural context, and show how this differs from naturally adapted seed characteristics. The influence of the environment and possible underlying mechanisms including both genetic and mechanical principles are discussed along with how these may be manipulated to enhance seed performance in practice.

What is seed vigour? Under optimal conditions, seed from different sources may result in similarly high levels of germination. However, these same seeds under the more stressful conditions experienced in the field may have vastly contrasting abilities to establish plants due to differences in their vigour (Figure 1). Although this is commonly observed, seed vigour has proven difficult to define precisely. A widely accepted definition of vigour is "the sum total of those properties of the seed that determine the potential level of activity and performance of the seed during germination and seedling emergence" (Perry, 1978, 1980). This has evolved and extended to the current International Seed Testing Association (ISTA) definition: "Seed vigour is the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments"

(ISTA, 2015). As part of this definition they point out it is not a single measurable property, but a concept associated with aspects of seed performance that include: rate and uniformity of seed germination and seedling growth; emergence ability of seeds under unfavourable environmental conditions; and performance after storage, particularly the retention of the ability to germinate. Vigour can therefore be considered as the potential performance of viable seeds in agricultural practice and this is determined by the complex interaction between genetic and environmental components (Whittington, 1973; Hodgkin and Hegarty, 1978). However, the reasons for variation in this performance are complex and remain little understood.

The effect of environment on variation in seed vigour and seed performance: In an agricultural context, seeds available for sowing come in "lots" and each is a population of seeds produced from many plants ideally in a single crop at a specific location. Each lot has its' own characteristics such that seeds sharing a common genotype can vary dramatically in their vigour depending on the maternal environment in which they developed and their subsequent harvest and handling. We discuss this further in section 3. The impact of the production environment is well known and as a result specific geographic locations having favourable climates are often selected by commercial seed companies to help ensure high quality seed production.

Earlier work on seed vigour has been largely directed towards developing the basis for testing and quantifying differences between lots, which is of great industrial relevance. Understanding how seed lots age and deteriorate, minimizing the rate and impact of this deterioration, and the negative impact of sub-optimal seed production and processing (Ellis and Roberts, 1980, 1981; Powell, 2006) has established a framework for controlling, predicting and maintaining seed performance and forms the basis of industrial seed technology and current seed vigour testing (Copeland and McDonald, 2001; Powell, 2006). Section 3 of this review summarizes this work on the behaviour of seed populations, and shows that when quantified correctly the impact of environmental conditions on seed vigour following harvest is predictable and so is the effect of differences in the initial vigour at the end of seed development. While further refinement of this understanding on the impact of environment is taking place, future advances in the enhancement of seed vigour are most likely to be achieved genetically.

The impact of seed vigour is seen during seedling establishment in the variable seedbed environment. The effect of the main seedbed variables; temperature, water availability, and soil strength on seed/seedling performance is largely predictable when appropriately quantified, for example, population based threshold models (Bradford 1995, 2005; Finch-Savage, 2004; Donohue et al., 2015; Section 4). Considering this understanding of environmental components relating to vigour we examine the less understood genetic

component in the context of producing a “robust seed”. The need to produce robust high vigour seed that resists the negative impact of variable environmental conditions during production, processing and following subsequent sowing is only enhanced by the uncertainty of climate change.

The genetic basis of seed vigour: A great deal is now known about the regulation of seed dormancy and germination and there are a number of reviews that discuss this in detail (Finch-Savage and Leubner-Metzger, 2006; Holdsworth et al., 2008a; Finkelstein et al, 2008; North et al., 2010; Weitbrecht et al., 2011; Rajjou et al., 2012; Rodriguez et al., 2015). Little research has been focused towards understanding what mechanisms determine the initial (potential) vigour of seed in an agricultural context, rather than the consequences of environmental variation. *Arabidopsis* has emerged as an excellent model for understanding the regulation of dormancy and germination. This small seeded annual has adapted to a wide range of natural environments, but has not been subjected to the selection pressures of crop domestication and therefore its’ seeds have not been challenged to perform in an agricultural context. We suggest that adaptation to the natural environment with emphasis on post-shedding dormancy to time germination to seasonal changes is very different from the agricultural necessity to germinate and progress to seedling emergence with the minimum delay following sowing (Section 5). This provides a focus for the difference since both situations require seeds to be dormant before shedding (harvest) to avoid germination on the plant leading to pre-harvest sprouting (Paulsen and Auld, 2004), but their subsequent behaviour is sharply contrasting.

After summarising why seed vigour is important below we review current understanding of seed behaviour, ageing and, its application in seed technology (pre- and post-harvest). Assuming this knowledge is fully utilised to optimize production practice, minimise negative environmental effects, and limit seed deterioration during storage, the factors that determine seed performance during crop establishment and the potential vigour of the seed are discussed. In doing this we consider what is required of a robust vigorous seed, how to achieve it, and what the underlying mechanisms may be. It is not possible here to be comprehensive in covering the literature; our aim is to provide useful illustrations of the wide range of subject areas covered. Seeds, even those of crop species, are morphologically and physiologically diverse and the review cannot be comprehensive in addressing all the specific issues. Where necessary, we therefore focus on small seeded vegetable crops where seed performance in determining timing, uniformity as well as extent of seedling emergence is particularly crucial to crop production (discussed in section 2). Nevertheless, most issues discussed are equally relevant to larger-seeded and grain crops.

2. The importance of seed vigour in agriculture:

Seed vigour and climate: Sowing time is selected on the basis of climate and seasons, but there are many crop specific drivers such as scheduling and economic incentives to fulfil production markets. Especially in temperate regions, the choice is often made to maximize the length, or optimize the environment of the growing season to increase yields, rather than the best conditions for successful crop establishment. Ongoing climate change is likely to make this choice even more precarious with the potential for more variable crop establishment. This variability has direct and negative effects on yield as we describe below. Figure 1 illustrates that on any single occasion the vigour of sugar beet seed greatly influences seedling establishment and this is magnified across sowings. Continuing with the example of sugar beet, of 254 crops studied Durrant et al. (1984) found that only in 55 was a sufficient stand achieved that did not limit yield. Similar impacts of vigour on seedling establishment from seeds of commercial quality have been observed in small seeded vegetable species (Mathews, 1980) and a wide range of other crops (reviewed by Powell, 2006). There is continuing improvement, but establishment remains variable. Even if optimum conditions for the day of sowing can be chosen the subsequent conditions that influence establishment cannot. Robust seeds with enhanced vigour mitigate these yield-limiting effects by establishing seedlings more uniformly across a wide range of environmental conditions.

Yield and profitability: The impact of variation in seed vigour on both total and marketable yield differs between species and also depends on the specific production practices and market requirements of the crop. The major impacts of variation in seed vigour manifests through a negative direct effect on seedling emergence and therefore an indirect effect on yield (Tekrony and Egli, 1991; Ellis, 1992; Finch-Savage, 1995). A set number of seeds are sown with a view to achieving a target number of seedlings (stand) in the crop that will produce a high yield per unit area. In all crops there is a clear relationship between the number of plants established per unit area and total yield (Figure 2a), therefore if seedling emergence is inadequate the amount of harvestable product is reduced (Bleasdale, 1967). No amount of effort, expense, inputs or abiotic stress resistance during later crop development will compensate for this lack of seedlings. This impact is greatest in crops that cannot compensate by tillering to fill gaps between plants. Evidence for a more direct effect of seed vigour through plant performance is more limited and is discussed further below (Ellis, 1992).

Marketable yield in horticultural crops: In many crops, the market has specific requirements for the nature of the produce if it is to be saleable and in particular to achieve a high value. Thus only a proportion of the total yield produced may meet these criteria. These more subtle effects are important because many crops including field vegetables, are not harvested and marketed in bulk at maturity (like grain crops) but marketed as individual components of plants (e.g. carrot roots, cabbage heads, onion bulbs), at different

stages during their development. The market strictly defines the characteristics required of these components (e.g. size, colour, shape). Marketable yield refers to the proportion of the crop in these defined categories and ultimately defines the difference between making a profit and not and the level of wasteage. This aspect of plant production is central in modern horticultural practice. Thus even if the numbers of emerging seedlings are adequate and total yield is high there can be more subtle crop-specific effects that limit the marketable proportion of that yield and/or its value (Tekrony and Egli, 1991; Finch-Savage, 1995).

Small-seeded vegetable crops often have unpredictable timing of seedling emergence, which can disrupt planned schedules of production (Gray and Finch-Savage, 1994). Sub-optimal uniformity at emergence can impact directly resulting in poor uniformity in plant size at harvest. This limits the potential for mechanized harvesting, or in the case of manual harvesting this limits the proportion of the crop that is economic to harvest with a single pass of field workers, for example in lettuce (Wurr and Fellows, 1983). Alternatively, the proportion of the crop in high value size grades for example in carrot production, can be limited due to poorly timed or widely spread seedling emergence (Finch-Savage, 1987; Benjamin 1990). Figure 2b shows that as stand and therefore plant density increases the size of individual plants is reduced. In order to achieve the desired marketable size of plant produce, the stand achieved must be precise. If seedling emergence is spread more in time the size of seedlings has a greater spread (Figure 2c) and fewer plants in the population achieve the desired size to achieve a greater marketable yield.

Co-ordinating plant development through tightly controlled germination timing and seedling establishment is therefore crucial to the ability to maximize both marketable yield potential and profit. This explains why farmers choose to spend more on the purchase of high quality seed to ensure economic returns. A single high quality tomato seed, for example, can cost a grower up to 1 USD.

Input costs and environmental impact in field crops: For bulk harvested field crops such as cereals, it is the total harvested weight per unit area at full reproductive maturity that is important. In these bulk-harvested crops, the differences in emergence time and uniformity within the plant stand that affect marketable yield of many vegetable crops tend to diminish later in crop development through plant competition. Therefore in these crops, if the plant stand is adequate, or compensated for by tillering, there is no significant impact of seedling establishment on total yield (Tekrony and Egli, 1991; Finch-Savage 1995). Germination timing is therefore less important in bulk field crops.

Seed vigour, through its impact on seedling emergence, contributes directly to the economic success of all commercial crops. Vigour also has important indirect effects on crop production. The inputs such as fertilizers, irrigation, pesticides are the same whether

200 the stand is adequate or not and so adequate stands are essential for production to be
201 resource efficient. The timing and uniformity of field crop seedling emergence also alters their
202 competitive advantage with weeds. This has an immediate impact upon the efficacy of
203 herbicide applications, weeding strategies and other aspects of crop production that determine
204 cost effectiveness and impact on the environment. In other production practices, such as
205 establishment of vegetable transplants and ornamentals in the glasshouse, poor seed vigour
206 can have a direct financial penalty through wasted glasshouse space, planting materials,
207 increased labour costs, and reduced product quality resulting from non-uniformity.
208
209 **3. Seed vigour and seed technology: A population (seed lot) characteristic**
210 During seed development seeds on the mother plant progressively gain the ability to
211 germinate and the capacity to produce a seedling (Bewley et al., 2013; Figure 3a). Seed
212 vigour then progressively increases to physiological maturity (PM) the point of maximum
213 seed quality (Dornboss, 1995a; Still and Bradford, 1998; Bewley et al., 2013). Seed vigour
214 can continue to increase after severing the connection with the mother plant and so PM can
215 occur after mass maturity (MM, maximum seed dry weight) and usually before harvest
216 maturity (HM, seeds first have to dry on the mother plant). The precise time of maximum
217 vigour will differ between species (e.g. TeKrony and Egley, 1997). Seed vigour begins to
218 decline from PM as seeds age before and after harvest and ultimately the seed loses
219 viability during storage. Vigour not only influences field performance, but also storage
220 potential. The progress of individual seeds throughout this process differs in time and
221 therefore vigour, like other seed characteristics, is a property best examined on a
222 population level. The vigour of seed lots has a mean and distribution that should be
223 evaluated in order to determine the probability of the response of seeds to the environment.
224 We report how this approach has supported innovations in seed technology, and will
225 underpin future progress in the genetic enhancement of seed performance.
226
227 In their classic work, Ellis and Roberts (1980) discuss the difficulty faced in working with
228 vigour and in providing a clear definition and concluded that it is a “vague qualitative
229 concept” and therefore cannot be measured. This is recognized in the current ISTA
230 definition quoted above. However, Ellis and Roberts (1980) point out that there are facets
231 of seed quality that can be considered in quantitative terms by developing a “unified view of
232 seed deterioration”. This is possible because most crop seeds can survive drying to low
233 moisture contents, which extends their longevity in a predictable way. Seed quality in these
234 so-called orthodox seeds greatly influences deterioration and therefore longevity, which can
235 be quantified in absolute terms using viability equations (described below; Roberts, 1972,
236 1973; Ellis and Roberts, 1981; Pritchard and Dickie 2003).
237
238 ***Seed ageing, longevity and viability equations:*** Weathering on the plant before harvest
239 influences ageing to alter their vigour before harvest (reviewed by Powell, 2006). When

orthodox seeds are subsequently stored in constant conditions of temperature and seed moisture content, they age and viability decreases over time in the form of a cumulative normal distribution of negative slope (survival curve; Figure 3a). When transformed to probits this deterioration has a linear relationship over time (Figure 3b) that is the basis of viability equations. With this approach the longevity of any seed lot, within a given storage environment, can be predicted from the intercept on the Y axis and the slope of the survival curve. The slope is the same for all seed lots within a given storage environment and is therefore defined by the storage conditions. Whereas, the intercept is the initial theoretical probit percent viability (viability constant K_i; Figure 3b), which is a function of genotype and pre-storage factors. Thus the value of K_i may represent an absolute measure of seed vigour (Ellis and Roberts, 1980). However, although a valuable concept, It should be pointed out that questions have been raised about its' applicability as an overall measure of vigour (Hampton and Coolbear, 1990).

Seed lot characteristics: How fast a seed germinates is an easy and recognizable indication of the concept of vigour. Germination rate is often incorrectly used in the literature as the proportion of seeds that germinate, but it is the reciprocal of germination time (rate) and provides a useful measure to compare seed lots or individuals within seed lots. Germination rate is predictably influenced by temperature and water potential (Bradford 1995; Finch-Savage, 2004; Section 4) and so must be compared under the same accurately controlled germination environments. Often rate is quantified by the time for half the viable seeds to germinate (T50), but this only accurately represents the whole seed population if the times to germination of individuals in the population are normally distributed. To avoid this often-incorrect assumption mean germination time can be calculated (MGT = , days = sum(*Dn*)/Sum *n*, where *n* = number of seeds which germinate on day *D*, and *D* is the number of days counted from the beginning of the germination test; Heydecker, 1966) and this has been found to have a consistent relationship with percentage viability in a seed lot as it deteriorates (Figure 3c,d), independent of the storage conditions (Ellis and Roberts, 1980). This relationship holds true for different species, but the slope differs.

The distribution of germination times in a seed lot is also correlated with percentage germination in many species, including carrot (Gray, 1984; Finch-Savage and McQuistan, 1988a). Other seed lot characteristics such as seedling length, variation in seedling length, their subsequent seedling emergence and seedling weight can also be correlated with percentage germination and speed of germination (e.g. carrot, leek, onion, cauliflower; Finch-Savage, 1986; Finch-Savage and McQuistan, 1988a). Time to germination and seedling size measurements are often confounded in reported experiments. A late germinating seed will always have a smaller seedling at a given time from sowing than a seedling from a fast germinator. Therefore it is important in the work highlighted here that

seedling measurements were made independently following germination. Nevertheless, the importance of MGT or an indicative timed single count as an indicator of rate and final seedling emergence has been demonstrated in a wide range of crop species (Mathews et al. 2012)

Germination time is determined by both genetics and production environment (Figure 3e). The genetic component can be determined by quantitative genetic analyses of genotypes produced under the same environmental conditions (Bettey et al., 2000) and this can provide a basis for improvement of seed performance in practice (Section 9).

Rapid ageing and vigour: In the absence of deterioration, a link exists between the theoretical initial percentage viability (K_i) and the full "potential" speed of germination and other seed lot characteristics under optimum production conditions. Unfortunately it is not practical to measure percentage viability sufficiently accurately in standard germination tests. For example, in a standard germination test of 400 seeds (e.g. 8 replicates x 50 seeds) it is not possible to statistically determine seed lot differences less than 7-8% in seeds of commercial quality (Ellis and Roberts, 1980). As we show in Figure 1 such statistically non-significant differences in relatively high percentage viability seed lots may mask significant differences in other seed quality components that result in a difference in field performance potential.

A more accurate determination of this performance potential is possible if seeds are first subjected to rapid ageing under controlled conditions. There are two accepted methods for this: accelerated- ageing (TeKrony, 1993), and controlled-deterioration (Powell, 1995). Both methods elevate seed moisture content and temperature for a fixed period of time to accelerate progress down the viability curve before germination testing (Figure 3b). Differences between seed lots are enhanced after the treatment and can be seen at a single point in time. This now forms the basis of ISTA validated tests used in commercial seed testing for specified species (Powell, 2006; ISTA, 2015). For research purposes it is also possible to do several successive measurements in time to construct a survival curve and estimate K_i as described above (Ellis and Roberts, 1980). Thus ageing is a key characteristic that is both a cause of differences in vigour and a basis for vigour testing. Other validated ISTA vigour tests, the electrical conductivity test, and the radicle emergence test are also related to physiological changes that occur during ageing (ISTA, 2015). These tests are based on a large body of physiological evidence linking seed vigour difference to ageing both before and after seed harvest (reviewed by Powell, 2006). Other types of vigour tests have been described (ISTA, 1995; AOSA, 1983), but these have not undergone the extensive comparative testing used in the ISTA validation procedure.

Vigour, seedling normality and testing standards: The central importance of seed quality to agriculture has led to the creation of associations dedicated to the maintenance of standards in seed lot quality assessment. The Association of Official Seed Analysts (AOSA) operates in North America and the International Seed Testing Association (ISTA) has member laboratories throughout the world. The latter have a vision of 'uniformity in seed quality evaluation worldwide' and provide a framework within which quality may be evaluated and compared. To do this they have developed tests and methodologies that are accepted internationally (ISTA, 2015). To be of high quality seed lots the need to be genetically pure, free from physical damage and disease, and have high viability so that almost all seeds complete germination and produce normal seedlings. The science of seed technology has evolved to develop a range of processes, techniques, and testing that are common throughout the seed industry, including procedures for harvesting and handling that minimize seed deterioration (Dornbos 1995b; Copeland and Mc Donald, 2001).

Survival curves are theoretically the same for high and low vigour seeds of the same lot (same genetics and production). They therefore take the same amount of time to pass through the defective stages that precede death and lead to abnormal development following germination (Figure 3a); they are just in different places on the curve (Figure 3b,c). However, because they are further down the curve low vigour seed appear to deteriorate more quickly as it takes less time to see measurable differences in germination rate and the number of abnormal seedlings. In seed testing practice a non-dormant viable seed may germinate and either produce a seedling that can be normal or abnormal. However, a seed that produces an abnormal seedling is not included in the germination percentage in the test result. This is because they are less likely to establish a plant under field conditions. Therefore in commerce, percentage germination and viability may not be the same. There are accepted criteria for assessing seedling normality, which provides a better estimate of field performance. These criteria appear in the rules for seed testing from both ISTA and AOSA and details of their most recently updated rules are available on their web sites (www.seedtest.org; www.aosaseed.com respectively). Deterioration in storage is one particular stress, but seeds are subjected to many stresses once sown in the seedbed (Section 4). Higher seed vigour results in greater resistance to all these growth-limiting stresses (Figure 3f).

4. Factors limiting seed performance: stress in the agricultural seedbed environment

Unlike experiments generally performed in labs on Petri dishes, seeds in the field are encased in a soil matrix where they experience a variety of different stresses discussed below. In order to understand and improve seed vigour and establishment it is necessary to understand the field-based limiting factors in the environment that are similar for seeds of all species. We have shown above that not only the percentage seedling emergence, but the speed and uniformity of emergence are important in many crops especially small

seeded vegetable crops. Thus seed vigour and the seedbed environment are particularly crucial for seeds of these crops, especially those with epigeal germination (Figure 4). For brevity, after describing the nature of stress in the seedbed, we then focus on understanding and modelling its' impact on such crops.

Germination and pre-emergence growth in the soil seedbed environment: The soil seedbed is a complex environment in which seeds and seedlings are exposed to multiple stresses (Braunack and Dexter, 1989; Hadas, 2004; Whalley and Finch-Savage, 2006, 2010). The literature provides no clear description of the soil conditions that lead to either good or poor crop emergence; this is because seedlings are not at all sensitive to soil type or condition *per se*, but are extremely sensitive to the physical stresses that a soil imposes during germination and seedling expansion (Whalley and Finch-Savage, 2006). These soil physical stresses (available water, mechanical impedance, oxygen and temperature) interact with each other and vary with water content, but neither the seed nor seedling is sensitive directly to water content. In order to understand what is required of a robust seed/seedling it is important to understand the nature of the physical stresses and their interaction that must be overcome when seedbeds are either drying or wetting. In the seedbed, temperature influences timing, but water stress and mechanical impedance have been identified as the two stresses most likely to limit germination and emergence respectively (Whalley and Finch-Savage, 2006, 2010)**.**

Available water (water stress): The seed and seedlings are not sensitive to the water content of soil *per se*, but the availability of water measured as water potential (MPa); the sum of matric potential (adhesion of water to soil structure) and osmotic potential (influence of solutes). It is this potential that is referred to in hydrothermal time models for seed germination. In saline soils, the osmotic potential can be of sufficient magnitude to affect water uptake by seeds, but in most cases it is the matric potential that will determine the availability of water to seeds in the soil. Soil water retention characteristics differ between soil types and thus for a given matric potential, water content can differ greatly between different soil types. It is often said that good seed to soil contact is important in facilitating water uptake by seeds. In myxospermic seeds the mucilage produced has hydrogel properties that may hold water around the seed or enhance water uptake to mediate germination, especially during imbibition under water or salt stress (Western, 2012). However, there is evidence suggesting that seeds can also uptake water effectively in the vapour phase (Wuest et al., 1999).

Soil Strength (mechanical impedance): Soil strength is very unlikely to affect the germination of seeds (Whalley and Finch-Savage, 2006). However, increasing soil strength (also measured as MPa) has a considerable negative impact on the rate of elongation of

roots (Jin et al., 2013) and in particular shoots of pre-emergent seedlings (Whalley et al, 1999). In the seedbed the strength of soil tends to be due to the capillary pressure of water in the pores holding the soil particles together. The effective stress model of soil strength, allows for the interaction between soil strength and water stress to be understood. Root and shoot elongation both tend to decrease as a linear function of water stress and as a nonlinear function of soil strength (Whalley and Finch-Savage, 2010). Thus, not only does soil strength increase rapidly as the soil dries, but the expansive growth of a seedling is far more sensitive to changes in soil strength than it is to changes in water stress (Weaich et al., 1992). Clay soils tend to have a higher degree of saturation (thus greater capillary pressure) at a given matric potential than sandy soils and so their effective stress is higher and they tend to be stronger (Whalley and Finch-Savage, 2006).

Soil structure is a term that is used to describe the arrangement of soil particles and pores (Braunack and Dexter, 1989). Crop establishment differs according to the structure of the seedbed such as the distribution of aggregate sizes. Tillage alters particle size and this is influenced by soil moisture content. However, it is difficult to make recommendations since there are species-specific optimal aggregate distributions, which depend on environmental conditions (Braunack and Dexter, 1989). Differences in soil structure also affect the rate at which physical stresses change with water content. For example, a well-structured soil will provide a seedbed that is relatively weak when dry but relatively strong when wet. In an ideal situation the soil structure will help to minimize water loss by evaporation and it will remain a mechanically weak growth environment (Whalley and Finch-Savage, 2006, 2010). The presence or otherwise of an impeding soil crust following heavy rainfall or irrigation is also an important issue.

Soil temperature and oxygen stress: Temperature is one of the key variables in germination and seedling growth models (see below). Solar radiation largely determines seedbed temperature, but for a given solar radiation soil water content and therefore evaporative cooling influences soil temperature. Thus dry seedbeds warm up quickly in early spring, but dry surfaces also cool down quicker and are prone to frost at night. Soil moisture and temperature also greatly influence the activity of soil microbes, which in turn largely determines oxygen supply in the seedbed. Thus oxygen stress has greatest impact in hot wet conditions. Oxygen sensitivity differs between species; in general monocot species and/or high starch content seeds are less sensitive to oxygen than those of dicot species and/or high lipid content seeds.

Seedbed environmental effects on germination and pre-emergence seedling growth: Although we focus our discussion on crop species with small seeds the key points are similar for larger and/or monocot seeds. Firstly it should be pointed out that the initial uptake of water can cause imbibitional damage particularly in grain legumes (Powell et al.,

1984) resulting from loss of membrane integrity (Powell, 1985). There are a number of factors that influence the extent of damage, for example, when the testa is not intact or seeds have low vigour through ageing (reviewed by Powell, 2006). Once imbibed, our own work on carrot, onion and *B. oleracea* illustrates how seeds germinate and seedlings grow in the soil and mimimise the stress they encounter (Finch-Savage and Phelps, 1993; Finch-Savage et al., 1998, 2001; Rowse and Finch-Savage, 2003; Whalley et al., 1999; Finch-savage et al. 2010). Soil moisture fluctuates rapidly in the surface layers of the soil where seeds are sown and seeds have adapted to this situation so that completion of germination will only occur when there is likely to be adequate moisture in the soil for subsequent growth (Finch-Savage and Phelps, 1993; Finch-Savage et al., 1998). For example, the germination process and pre-emergence seedling growth can proceed at water potentials below that which will prevent the completion of germination (Ross and Hegarty, 1979). Thus the completion of germination is a critical moisture-sensitive stage that controls rate of progress from sowing to seedling emergence from the soil (Figure 4).

Once the seed has germinated, it is essential to have rapid downward growth to maintain root contact with receding moisture in the seedbed during a subsequent dry period. Both the root and initial hypocotyl contribute to this initial downward growth. The hypocotyl subsequently forms a crook and grows upward (Figure 4). Contact with moisture tends to be maintained because the seedbed dries from the surface at a rate determined, in large part, by temperature. The root also grows down from the surface at a rate determined by temperature (Finch-Savage et al., 2001). This contact with moisture limits water stress within the seedling for upward shoot growth (post-crook extension of the hypocotyl) through the soil, but continued drying of the surface increases soil strength and impedance to this growth (Whalley et al., 1999).

The influence of the seedbed environment on this pattern of germination and seedling growth can be described and modeled using the following population-based threshold modelling approach (Finch-Savage, 2004). A negative relationship exists between increasing stress from a component of the seedbed environment and the progress towards germination completion (temperature, water potential, oxygen) or pre-emergence seedling growth (additionally soil strength) such that progress reduces to zero at a threshold value (base). The models assume progress is proportional to the component value above the base and ceases below the base when the level of stress prevents progress. The development and applications of such models (thermal-, hydro-, and hydrothermal time) for seed germination in the laboratory have been reviewed (Bradford, 1990,1995; Finch-Savage, 2004) and the principles extended to other environmental factors (Bradford, 2002; 2005; Donohue et al., 2015) and to pre-emergence seedling growth (Finch-Savage et al., 2001). The threshold, base water potential is a key unifying parameter relating germination performance to seedbed stress that is likely determined by the physical restraint to

germination of surrounding tissues and cell wall extensibility (Welbaum et al., 1998; Section 7); and has potential as a measure of seed vigour (Still and Bradford, 1998). Furthermore, developmental threshold models may have much wider application to understanding phenology and fitness in variable and changing environments (Donohue et al., 2015).

The population-based threshold modeling approach has been shown to have predictive ability in the field for crops (Finch-Savage and Phelps, 1993; Finch-Savage et al., 1998). Pre-emergence seedling growth models have also been extended to include soil strength and therefore mechanical impedance (Whalley et al., 1999) and combinations of these component models with suitable models of the seedbed environment (Hadas, 2004) can be used to simulate the impact of seedbed environment on the progress from sowing to seedling emergence from the soil (Finch-Savage, 2004). Thus although detailed knowledge is limited the impact of stress in the seedbed environment on germination and pre-emergence seedling growth can be predicted; but crucially climate, weather and therefore the timing and extent of stress cannot, nor can the variability that is inherent in the seedbed environment. Robust seeds are therefore required to withstand variation in seedbed stresses.

Important seed vigour traits for predictable crop establishment: As a result of the above work three key seed vigour traits have been identified as necessary to establish well across a wide range of seedbed conditions (Finch-Savage et al., 2010). The seed must: 1, germinate rapidly; 2, have rapid initial downward growth; and 3, have high potential for upward shoot growth in soil of increasing impedance (Figure 4). All these features reduce the time between sowing and seedling emergence when the seedbed can be deteriorating. This suggests that a strategy of stress avoidance, through rapid germination when adequate moisture is present and subsequent rapid pre-emergence seedling growth, has an advantage in agriculture that may differ from the natural situation. Rapid germination and subsequent growth in impeded soils are therefore key phenotypes of vigorous seeds that are known to differ with genetic background for example in *Brassica* species (Hodgkin and Hegarty, 1978; King et al., 1986; Bettey et al., 2000; Finch-Savage et al., 2010).

At what stage do seedlings fail to establish? It is important to consider at what stage seeds/seedlings die and fail to establish, but there are few detailed studies that have addressed this question. Finch-Savage et al. (1998) conducted a detailed investigation of carrot seedling emergence in 15 different seedbed environments that exposed the seeds to different levels of stress (Figure 5). Surprisingly in every environment seed germination in the soil (measured by exhuming seeds) eventually reached the same high level as that achieved in unstressed laboratory conditions. Under more stressful conditions the seeds took much longer to germinate, but did not die while waiting for conditions that would allow completion of germination. However, throughout that time the seedbed was deteriorating

increasing the stress experienced during the post germination, pre-emergence seedling growth phase. Consequently seedling emergence declined in more stressful conditions and rarely achieved the level of emergence measured in unstressed laboratory conditions. Thus seeds/seedlings are often lost post-germination. A further consideration is the impact of soil-borne fungi on pre-emergence mortality. For example, low vigour or physiologically aged grain legume seeds have increased leakage of solutes that attract fungi and the presence of dead tissue provides a food base for infection (Powell et al. 1984).

To support the development of seedlings prior to autotrophic growth, protein and energy reserves are deposited during seed development. These reserves are mobilized during germination and seedling establishment and support photosynthesis-independent growth. As plant growth is a mechanically-driven process, the impact of physical stresses in the seedbed can be dependent of the pattern of this reserve dependent post-germination, pre-emergence seedling development (Figure 4). Species that have hypogeal germination, such as cereal grains, leave seed reserves below the soil surface when they emerge. In this case after the primary root emerges the coleoptile is pushed upward by elongation of the mesocotyl. Extension of the coleoptile then takes it above the soil surface. In contrast, seeds with epigeal germination such as in many small seeded dicots including *Arabidopsis* and other *Brassicaceae*, the principle seed reserve storage organ (cotyledons) have to emerge through the soil and form the initial photosynthetic unit. Soil impedance has a greater impact on seedling emergence in the latter. Further negative impact results from reduced photosynthetic competency when emergence is delayed (Tamet et al., 1996). Irrespective of the pattern of pre-emergence seedling growth, the majority of viable seeds are most likely to fail in the post-germination pre-emergence seedling growth phase.

5. Seed vigour as an agronomic trait: beyond natural adaptation

Seed strategies in the wild: Seeds used for the establishment of crops are harvested, stored in a dry state generally by seed producers, and then sown at times selected by growers and farmers. These seeds should complete germination rapidly upon sowing (Figure 6). In an ecological context, seeds are shed from the mother plant and remain on/in the soil with the function of germinating at a time and place best suited to establishing a new plant. In the majority of cases species adapted to natural conditions shed seeds that are dormant (Baskin and Baskin, 1998, 2004; Finch-Savage and Leubner-Metzger, 2006). The depth of this dormancy at shedding is not fixed; it is determined by genotype and maternal environment and is altered further by environmental conditions following shedding (Footitt et al., 2011, 2013). In this way dormancy mechanisms are adapted in different species to result in germination completion at different times of year and under different environmental conditions.

Within species, seeds are likely to germinate at a similar time of year but are unlikely to germinate uniformly and may often germinate across years to spread the risk of failure (Cohen, 2006). Such bet-hedging strategies where there is a probabilistic diversification of phenotypes expressed by a single genotype acts to buffer against unpredictable environmental conditions (Seger and Brockmann, 1987). Even within a given year, germination is spread in time and this less extreme adaptive bet hedging may be a response to variable environments on a shorter time scale. It is interesting in this context to note that seed populations have a characteristic sigmoid cumulative germination curve, and although this can be approximated to a normal distribution to aid analysis, in most cases it is positively skewed (Bewley and Black, 1994). This is consistent with bet hedging in the case of temporal environmental variation where geometric-mean fitness is more sensitive to variance than is the arithmetic-mean fitness (Seger and Brockmann, 1987). There are examples of germination behaviour in seeds that are considered to be both dormant or non-dormant that is interpreted as adaptive bet-hedging (Tielborger et al., 2012 and Watt et al., 2011 respectively).

Bet-hedging is observed in diverse biological contexts including seed and bud dormancy in plants (Nilsson et al., 1996; Springthorpe and Penfield, 2015), and is an effective adaptive mechanism to increase the likelihood of survival of one's offspring. This strategy may be more important in the future as shifts in germination phenology resulting from global climatic changes will directly influence population dynamics and productivity of all agrosystems (Walck et al., 2011). As a consequence of global warming, seeds will have to cope with climatic changes that include higher temperatures and lower water availability. In the context of agriculture, bet-hedging leads to reduced germination synchronicity and therefore decreased seed lot vigour, even in the absence of significant dormancy (Figure 6). In contrast to this adaptive strategy observed in undomesticated seed populations, the potential for rapid and synchronous germination following sowing into a suitable seedbed (long dashed line; Figure 6a) is agronomically ideal. Unfortunately as we discuss above seedbed conditions are not predictable and so seeds must also be robust to cope with this.

A group of species that have naturally "very fast germination", e.g. germinating in under 24 h, have been described (reviewed by Parsons, 2012). These species tend to inhabit high-stress environments, and are likely to be pioneer species or highly invasive weeds. They can rapidly exploit favourable conditions for germination, which can have a significant selective advantage. Fast germination allows the root to grow into the soil surface as it dries. This is similar to the requirements of vigorous seeds in an agricultural context (sections 4,6) to overcome seedbed conditions created by the farmer (section 4) who will try to sow into moisture below the surface having selected sowing times during or entering drier periods, for example, to allow access of machinery onto the soil. In nature this is a high- risk strategy and so species with the fast germinating seeds have often co-evolved

dimorphic seeds; a proportion of fast germinators and others more adapted to creating a persistent seed bank (Parsons, 2012).

In extreme cases very fast germinating species have significant physical adaptations such as curved or spiral embryos (Parsons, 2012). These embryos have rapid cell expansion on water uptake causing them to uncoil and rupture the seed coat (Wallace et al., 1968). However, more generally, they tend to have fully differentiated non-dormant embryos, small to very small seeds and little endosperm with soft thin seed coats. They therefore have high embryo to seed ratio, and furthermore tend to exude mucilage. Small embryos in significant amounts of endosperm represent a more ancestral state where the embryo takes a long time to grow and therefore germinate (Forbis et al., 2002). In contrast, Parsons et al. (2014) show that species with very fast germination have evolved independently many times and are mainly restricted to advanced clades. This suggests that it is a derived trait that evolved as an adaptation to either arid, saline or floodplain habitats. Parsons et al. (2014) further suggest that very fast germination is associated with substantial changes in seed morphology, including soft thin seed coats and increased nutrient storage in the embryo relative to non-embryonic tissues. This suggests interesting parallels to changes resulting from selection during domestication of crop species with the requirement of rapid germination following sowing. In contrast as we point out above, crop seeds are larger than their wild relatives and this occurs even in species where seeds are not the harvested yield component (Fenner, 1991).

Residual seed dormancy in crops: Dormancy exists on a scale that forms a continuum with germination (Finch-Savage and Leubner-Metzger, 2006; Finch-Savage and Footitt, 2012). The depth of dormancy on this scale alters the requirements for germination and thus anything that alters the conditions that enable germination are altering (inducing/relieving) dormancy. As dormancy is progressively relieved, for example by low temperature or afterripening (Yamauchi et al., 2004; Holdsworth et al., 2008a), seeds become able to germinate in a greater range of conditions, but seeds remain dormant outside of those specific conditions. This residual dormancy imposes limitations to the ability to complete germination. Residual dormancy that reduces the speed, but not percentage germination, can be determined in the so-called germination-resistance test, which essentially has repeated germination counts used to estimate MGT (Gordon, 1971). For example, during afterripening maximum percentage germination may be reached, but further treatment may still increase the speed of germination as residual dormancy is relieved.

In an agricultural context dormancy throughout development is essential to prevent the pre-harvest sprouting that can result in very severe economic loss in many crops, in particular grain crops (Clarke et al., 2005). The timing of exit from dormancy in these crops is

637 therefore crucial, remains an area of active research, but remains to be resolved as a
638 practical problem (reviewed in Benech-Arnold, 2004; Rodriguez et al., 2015). Ideally crops
639 should then be completely non-dormant at sowing as any residual dormancy at sowing
640 directly affects the potential performance of seeds; which could then be considered as less
641 vigorous according to the accepted definition of vigour we quote. In many cases it will be
642 difficult to distinguish whether a seed has residual dormancy or less capacity for vigour. It is
643 therefore instructive to consider reduced performance resulting from residual dormancy that
644 may be relieved by treatment (e.g. after-ripening, low temperature) and the true genetic
645 "potential vigour". Thus in this case the desirable agricultural characteristic of being non-
646 dormant can be separated from the germination component of seed vigour *per se*. i.e.
647 cellular mechanisms underlying vigour that are not involved in dormancy. However,
648 dormancy and vigour cannot be entirely separated since there will also be repressive
649 mechanisms that separately both delay germination by enhancing dormancy and inhibit
650 post-germination growth rate. A completely non-dormant seed has been defined as having
651 the capacity to germinate over the widest range of normal physical environmental factors
652 possible for that genotype (Baskin and Baskin, 1998, 2004). When this is the case the
653 genotype, not the environment, determines seed vigour in terms of the range of
654 germination permissive conditions and the speed at which germination completion can take
655 place in those conditions.
656
657 ***Domestication: Seed strategies in agriculture:*** The comparatively controlled
658 environment including uniform sowing, weed control to limit competition, as created by field
659 agriculture, removes the need for the bet hedging strategies discussed above as rapid and
660 uniform germination of seed lots does not compromise the success of individuals. The
661 process of crop domestication selected for useful traits in crop wild relatives. The collection
662 and resowing of wild seeds rapidly selected for those that emerged first and led to the
663 growth of the largest plants. These individuals were likely selected for both their greater
664 yield and rapid timing to the next generation of planting. Selection would therefore favour
665 genotypes that were less dormant, and at the same time showed reduced bet hedging and
666 consistently faster germination across generations. A recent meta-analysis of germination
667 characteristics of 243 species shows that on average crop seeds germinate faster, their
668 range of threshold temperatures and water potential threshold values is wider and some
669 crops have higher optimum and maximum temperatures indicating that domestication has
670 enabled them to grow in a wide range of environments where agriculture has developed
671 (Durr et al., 2015). This consistent rapid germinating phenotype will have served as a basis
672 for selection in terms of seed behaviour. However, it has been argued that the selection
673 pressure against dormancy during domestication in some cases may have gone too far
674 resulting in the pre-harvest sprouting reported above (Rodriguez et al., 2015). Pre-harvest
675 sprouting is highly important in terms of seed quality as a component of crop yield, but not
676 directly for vigour of seeds as a propagule.

Natural variation of seed vigour: The undomesticated species *Arabidopsis* has been very useful for quantitative genetics of many seed behaviour traits including seed dormancy (Clerkx et al., 2004; Bentsink et al., 2006; Joosen et al., 2012; 2013). However, the lack of agronomic selection in this species means bet-hedging characteristics persist (Penfield and Springthorpe, 2015). It could therefore be argued that, although excellent for developing understanding, *Arabidopsis* may not serve as the most useful model to uncover QTLs for seed vigour in agriculture. The lack of performance-based selection in this species means that genetic loci controlling successful seedling establishment in an ecological context will dominate in *Arabidopsis*. Alleles for vigour (consistent seedling establishment in an agricultural context) may not be available in wild populations as the vigour required in agriculture presents an adaptive disadvantage by removing the bet hedging strategy leading to synchronous germination. Conversely a domesticated crop species will have undergone some selection based on these traits as outlined above in the seedbed environments of agricultural production practice.

Crucially as discussed above a degree of seed dormancy must be retained in crops during domestication to prevent germination on the mother plant (vivipary; pre-harvest sprouting; Rodriguez et al., 2015). Thus a key selection pressure during domestication is likely to be the rapid switch from dormant to non-dormancy at the end of seed development and the elaboration of such a mechanism. A role for seed desiccation, a signal involved in this switching mechanism has been proposed previously (Kermode and Bewley, 1985). This may serve as an adaptive mechanism to enable seeds that have not fully completed development to gain competence to germinate.

6. Mechanisms of seed vigour: what makes a seed vigourous in an agricultural context?

Seed vigour is a quantitative trait influenced by the interaction between genetics and the environment. Despite the central role of vigour in the success of crops, it has seldom been a priority in commercial breeding programs that largely concentrate directly on other plant yield components and disease resistance. We have shown above that when quantified correctly the behaviour of seeds following PM on the mother plant and the impact of storage environment is predictable (section 3). The impact of environmental factors including temperature, water potential, soil impedance, on seed germination and pre-emergence seedling growth is also predictable (section 4). Once this is understood and seed technology becomes fully developed to minimize the negative influences on production and handling, the key to further vigour improvement is likely to be through targeted genetic enhancement.

716 The mechanisms that control seed vigour remain poorly understood and this lack of
717 understanding at a mechanistic level represents a key gap in our ability to enhance seed
718 vigour. We consider below 4 key stages, the first discussed briefly above is the rapid
719 transition from dormancy to non-dormancy at physiological maturity. Figure 4 illustrates that
720 the establishment of seedlings involves three further key stages including rapid seed
721 germination, the downward growth of the root and upward growth of the hypocotyl. The
722 genetic factors controlling these 4 stages of plant development represent points where
723 vigour may be controlled and are likely to be quantitative traits (dormancy: Bentsink et al.,
724 2010; germination and seedling growth: Finch-Savage et al., 2010). It is beyond the scope
725 of this review to discuss these in depth, and so will be discussed in a broad sense below:
726
727 ***Loss of dormancy at physiological maturity***: The maternal environment is key to
728 controlling dormancy levels in seeds. In *Arabidopsis* this has been shown to be largely
729 mediated by the DOG1 locus (Bentsink et al., 2006), and discussed further below. A
730 progressive loss of dormancy occurs with prolonged storage in the dry state through the
731 poorly understood process of dry after-ripening (Holdsworth et al., 2008a; Leymarie et al.,
732 2012). This process and other mechanisms underlying the decision of a seed to complete
733 germination are being uncovered and have been extensively reviewed elsewhere (reviewed
734 by Finch-Savage and Leubner-Metzger, 2006; Holdsworth et al., 2008a; Finkelstein et al.,
735 2008; North et al., 2010, Weitbrecht et al., 2011; Rodriguez et al., 2015). In a vigorous seed
736 this transition from dormant to non-dormant must be rapid, because particularly in the case
737 of cereal grains moderate to high levels of dormancy are required for protection against
738 pre-harvest sprouting (Rodriguez et al., 2015).
739
740 ***Rate of seed germination:*** Less is known about the mechanisms that control the rate at
741 which the germination program is executed in the absence of dormancy. On a conceptual
742 level, vigour may be thought of as the rate and intensity at which a developmental program
743 driving the seed to seedling transition is executed (Figure 6b).
744
745 Firstly, the composition and translation of stored mRNAs remaining after seed development
746 may play a role in seed vigour (Gallandt and Rajjou, 2015). During the final stages of seed
747 development, changes in mRNA dynamics result in the accumulation of so-called ‘long-
748 lived’ or ‘stored' mRNA that are stored in the dry seed (Nakabayshi et al., 2005; Bazin et
749 al., 2011). Their mobilization during imbibition is important for the germination process
750 since *Arabidopsis* mutants in mRNA degradation showed severe germination and seedling
751 establishment defects (Goeres et al., 2007). The presence of stored transcripts may as well
752 provide a link between the maternal environment in which seeds developed and their
753 subsequent vigour following imbibition. Rajjou et al. (2004) report from work using inhibitors
754 that germination can reach completion in the absence of transcription, but not translation.
755 Therefore they suggest the key level of regulation is translation/post-translation. However,

although transcription may not be absolutely required to complete germination, rate and uniformity of germination were considerably affected by inhibiting transcription suggesting new transcripts must be synthesised during imbibition to enhance germination vigour (Holdsworth et al., 2008b). The active recruitment of transcripts to polysomes also represents a potential control point by which stored transcripts may influence the vigour of seeds (Basbouss-Serhal et al., 2015).

A link has been made between genome integrity and seed quality so that early imbibitional repair has been suggested as an essential component of seed vigour required to repair germination-limiting damage accumulated during seed production and storage (Elder and Osborne, 1993; Powell and Mathews, 2012; Waterworth et al., 2015). At a practical level Mathews and Khajeh Hosseini (2007) suggest the extent of previous deterioration and the time taken to repair it can determine the length of the lag period and therefore rate of germination in maize. Furthermore, ageing and repair are suggested as the overall physiological basis explaining the principles behind germination and vigour tests that predict subsequent seed performance (Mathews et al, 2012). Specifically Waterworth et al. (2015) argue that DNA repair represents an important limitation to seed vigour with potential for the development of markers for predicting or improvement of seed vigour.

A wide range of other early events in the germination process have been outlined (Weitbrecht et al., 2011) that must be completed and the progression through germination is thought to be controlled by a series of repressors and checkpoints (Nonogaki et al., 2006; Catusse et al., 2008). The presence of these sequential steps progressing through the germination process has been demonstrated by gene expression profiling of germinating *Arabidopsis* seeds (Bassel et al., 2011; Dekkers et al., 2013). Progressive waves of co-ordinated gene expression are observed during the germination program, which are observed as peaks of co-expressed genes over a time course of germination. Dynamic shifts in the translation of expressed transcripts have also been observed (Galland et al., 2014).

First it is necessary to identify the key checkpoints and where within seeds they are executing their function. Important to this is the proteolytic degradation of repressor proteins, which act to block events leading to the completion of germination. Central to the repression of seed germination are the DELLA proteins (Lee et al., 2002, Bassel et al., 2004), which are degraded in response to the perception of the hormone GA (Harberd et al., 2009). The growth repression activity of DELLA is therefore relieved upon GA binding its' receptor GID1 and the F-box protein SLEEPY. Removal of DELLA proteins in seeds leads to a de-repression of cell wall remodeling gene expression and in turn growth of the embryo (Stamm et al., 2012; Cao et al., 2006).

Another proteolytic checkpoint in seedling establishment is mediated by ABA-INSENSITIVE5 (ABI5) in *Arabidopsis*, which acts to promote ABA-mediated growth arrest during a late stage of seed germination (Lopez-Molina et al. 2003). The stability of the ABI5 protein is regulated by both ABI5 BINDING PROTEIN (AFP) (Lopez-Molina et al., 2003) and KEEP ON GOING (KEG) (Liu and Stone, 2010) as a mechanism to control ABA response at this stage of development.

A role for microRNAs in the targeted removal of repressive transcripts has been demonstrated as a mechanism involved in the control of seed germination (Martin et al., 2010). The targeted removal of transcripts, which act to repress germination represents another level of targeted removal of repressors and another possible mechanism by which the sequential steps of germination are regulated. A role for miR159 targeting *MYB33* and *MYB101* (Reyes and Chua, 2007) and miR160 targeting *ARF10* (Liu et al., 2007) represent examples of how seed behaviour is influenced by miRNAs.

The control of germination by phytochrome is repressed by the bHLH transcription factor PHYTOCHROME INTERACTING FACTOR 3-LIKE 5 (PIL5) (Oh et al., 2006). This appears to be a very late checkpoint, and may serve as a final cue before the commitment to complete germination. PIL5 is also proteolytically degraded in response to germination-stimulating light conditions by currently unknown mechanisms (Oh et al., 2006).

In the context of seedling establishment and vigour, a rapidly establishing vigorous seed will pass through these sequential steps more rapidly than a less vigorous seed (Figure 6c,d). As a result, it will take longer for the non-vigorous seed to reach the end of the program and to complete germination. Identifying factors that control the overall progression through the sequential series of checkpoints, rather than targeting the individual checkpoints themselves may more effectively enhance the speed of germination. A route to this may be found in understanding the mechanism underlying adaptive bet hedging that results in a spread of germination times between seeds.

Rate of pre-emergence seedling growth: Following germination rapid downward growth of the root is required to maintain contact with moisture in the seedbed. Following the commitment to commence germination the quiescent meristems of both the root and shoot are activated and will generate all post-embryonic plant growth. During root meristem activation, both endoreduplication (Sliwinska et al., 2009) and cell divisions (Masubelele et al., 2005) begin within the germinating radicle in *Arabidopsis*. Very little is known about the molecular components mediating the re-activation of the root meristem during germination, though it seems probable that an induction of the genetic factors that mediate cellular patterning in the mature root will be involved (Petricka et al., 2012). Activation of the shoot

apical meristem does not impact upon the definition of vigour and seedling establishment as defined herein.

In many species the growth of the hypocotyl represents the final stage of seed reserve dependent growth resulting in emergence through the soil and the start of autotrophic development. This upwards growth, like the germination of the embryo, is driven through cell expansion events in the absence of cell division, for example in carrot, but in other species by both cell division and expansion, for example onions (Whalley et al., 1999). The growth of hypocotyls has been studied extensively, however the relationships between these mechanisms and seed vigour remain unexplored. We consider below how this growth occurs and how this is regulated.

7. Biophysics of germination and pre-emergence growth

Plant growth is a mechanically driven process that is manifest by the opposing forces of intercellular turgor pressure, and the constraint of the surrounding cell wall. The ability to grow fast and strong results from the capacity to generate greater mechanical forces. The ability to unconditionally generate this force across a wide range of stress conditions defines the vigour of a seed. In many regards vigour can be considered a mechanically driven crop trait and we explore this concept below by reviewing the factors involved individually (principally cell wall modification and turgor) and then immediately for each how they relate directly to seed vigour.

Turgor: Turgor is the internal pressure generated by the cell contents on cell walls, which is the driving force of expansion. The generation of turgor by a germinating embryo cell depends on the local availability of water within the heterogeneous soil matrix (see section 4 above). Depending on how tightly bound the water is by the soil and the overall abundance of water, will determine the capacity of the seed to generate cellular turgor promoting cell expansion. It has not yet been demonstrated what the solute is in germinating embryos that generates cellular turgor pressure.

Turgor and seed vigour: The relationship between turgor and seed vigour is poorly understood. This is partially limited by the inability to measure cellular turgor using a pressure probe, as in our experience the cellular contents of embryonic cells tend to plug the tip of the probe. Following germination, gradients in water potential form the driving force for the movement of water for cell enlargement within soybean hypocotyls and water potentials are much lower in the elongating region (hypocotyl crook) than towards the root (Cavalieri and Boyer, 1982). However, osmotic potential differed in parallel so that turgor pressure was uniform along the hypocotyl. When seedlings were grown under water stress, water and osmotic potentials both decreased thus maintaining turgor (Cavalieri and Boyer,

1982). Mechanisms to alter turgor in seeds and seedlings may also present an avenue to enhance the generation of mechanical force and increase seed vigour.

Cell wall modification: The other half to the facilitation of plant growth is the weakening of the mechanical properties of the cell wall (Cosgrove, 2005). This has been demonstrated to occur through genetically encoded enzymes that are secreted from within cells to the cell wall. This provides a link between cellular signaling and the control of plant growth though changing the biophysical properties of the cell wall. Different classes of growth promoting gene expression, which modify different components of the complex wall include expansin, xyloglucan endotransglucosylase (XTH) (Rose et al., 2002) and some classes of pectin modifying enzymes including pectin methylesterase (PME) (Peaucelle et al., 2015). A role for PME inhibitor gene activity in the promotion of *Arabidopsis* seed germination has been previously shown (Muller et al., 2013) demonstrating a role for cell wall modification in the control of germination. Furthermore, PME activity has been demonstrated to regulate a bipolar mechanical asymmetry during post-germinative hypocotyl growth (Peaucelle et al., 2015), supporting a role for pectin modification in the control of seedling growth. A role for ABA in the inhibition of cell wall loosening in *Brassica napus* has also been previously reported (Schopfer and Plachy, 1985), indicating hormonal control of these biomechanical changes. All signals controlling the germination process ultimately function through the regulation of the cell wall remodelling enzymes (CWREs), which drive embryonic growth. These CWREs represent the downstream targets of all upstream regulatory processes and are the workhorses of the seed to seedling developmental transition. However, it remains poorly understood how signaling pathways directly impact upon CWREs on a mechanistic level and how these impact upon the physical properties of the embryo. These represent key gaps in our understanding of seed germination and vigour.

Cell wall and seed vigour: Previous studies have demonstrated that the physical properties of the cell wall control both seed germination and seedling establishment under stress conditions in both *Arabidopsis* and tobacco (Li et al., 2011; Lü et al., 2013). *Arabidopsis* plants ectopically expressing the cell wall loosening protein expansin (Lü et al., 2013) have dramatically increased germination and seedling establishment under osmotic stress. These observations suggest that a greater degree of cell wall loosening has the capacity to confer vigour upon seedlings that have decreased turgor under osmotic stress. The increased loosening of the wall may facilitate growth under osmotically limiting conditions given the reduction in cellular turgor due to limited water availability. These observations suggest that expansins represent downstream targets of seed quality and vigour, and their high level induction can confer resistance to osmotic stress at the germination and seedling establishment stages. Following seedling establishment, these plants show a greater sensitivity of osmotic stress in terms of root growth and leaf

production (Lü et al., 2013). Thus altering plant mechanical properties has different effects at different stages of development.

Seed germination and seedling establishment occur within the soil seedbed matrix, which is a very different environment from the agar plate and filter paper-based assays that are widely used in laboratory experiments (Section 4). Mechanical impediment by increased soil strength through seedbed deterioration and at its worst, a soil crust, must be physically overcome by greater force generated by the seedling. Stress experienced by plant organs has been shown to result in decreased extensibility of the cell wall. The application of compaction stress to growing pea roots by placing bricks on top of soil led to increased stiffening of the cell wall in this organ (Croser et al., 2000). An increase of 36% in hypocotyl cross-sectional area occurred in snap beans after physical impedance (Taylor and Tan Broeck, 1988). In carrot and onion redistribution of seed reserves to thicken both roots and hypocotyls was observed when grown under increased mechanical resistance (Whalley et al., 1999). This was accompanied by reduced growth rates that were enhanced by subsequent removal of the mechanical impedance. Unpublished work in our laboratory indicates that seeds give off similar quantities of CO_2 per unit length of growth when rate of growth is progressively reduced by mechanical impedance (Finch-Savage and Whalley unpublished). Thus seed reserves are not used up faster by enhanced respiration during growth under stress. However, more seed reserves would be used per unit length during the thickening of the hypocotyl as observed by Whalley et al. (1999). Consequently this would reduce the soil depth from which the seedling can emerge. At greater sowing depths larger seeds can therefore appear to enhance vigour (see below).

Stored energy reserves and seed vigour: Seeds store carbon reserves in the form of oil bodies to support pre-autotrophic growth during germination and seedling establishment. It is the mobilization of such stored oil reserves that drives these developmental transitions, and mutants that are impaired in oil mobilization are impaired in their germination (Kelly et al., 2011). Such mutants include the *COMATOSE* (*CTS*) locus (Foottit et al., 2002), which is required for the import of a range of biologically important molecules into the peroxisome, including very-long chain fatty acids associated with breakdown of seed-storage lipids. Germination of this mutant is restored with the exogenous application of sucrose. Further support for the role of lipid breakdown in driving seedling growth comes from the *PHOSPHOENOLPYRUVATE CARBOXYKINASE1* (*PCK1*) mutant, which is compromised in lipid breakdown. Mutant *pck1* seedlings have reduced hypocotyl length that can also be reverted with the external application of sucrose (Penfield et al., 2004). How the metabolic by-products of lipid catabolism feed back onto gene expression has not been clearly established and may represent a link between these metabolic events and the sequential steps of the germination process. The rate at which lipid reserves may impact upon the rate at which seedlings can establish, with more rapid energy availability being a means to

enhance the rate of germination. A second gluconeogenic pathway that uses pyruvate, a breakdown product of storage protein catabolism, has been described (Eastmond et al., 2015). This provides an alternative energy source from stored seed reserves that may be used to drive seedling growth.

Cell cycle and seed vigour: The cell cycle and endoreduplication have been strongly correlated with plant cell expansion. Following the induction of germination there is a discrete activation of the cell cycle from G1 concurrent with the cell expansion events that drive germination and seedling establishment (Vázquez-Ramos and de la Paz Sánchez, 2003; Sliwinska et al., 2009). A role for the cell cycle regulatory protein KRP5 in the control of endoreduplication and promotion of cell expansion has been demonstrated in the embryonic axis in *Arabidopsis* (Wen et al., 2013). The *krp5* mutant shows impaired germination and represents a molecular link between the cell cycle and the regulation of seed germination. The TCP14 and TCP15 transcription factors have also been shown to promote germination downstream of GA and cell divisions within the radicle meristem (Resentini et al., 2015).

Epigenetics and vigour: Epigenetics may also play a role in the vigour of seeds given the environmental inheritance of vigour depending on the maternal condition. A role for epigenetic marks in the control of *DOG1* expression in response to the environment has been established (Footitt et al., 2015). Whether similar mechanisms persist in the control of gene expression programs controlling vigour beyond its control of residual dormancy through *DOG1* expression remains to be established.

A role for cell and seed size in vigour: Seed companies have selected larger seeds for sale as they are thought to show greater vigour characteristics than their smaller counterparts. A reason for this is that larger seeds lead to larger seedlings, which fulfil a criteria for being more vigorous. Larger seeds as well contain more reserves that may account for enhanced growth as discussed above.

Enhanced vigour in larger seeds may also be due to mechanical advantages. According to computational 3D mechanical models (Bassel et al., 2014), bigger cells have a greater capacity to grow and generate force in response to growth-promoting gene expression. Given that the number of cells in mature plant embryos of a given species is relatively fixed, this means that larger seeds have larger cells, and vice versa. This raises the possibility that bigger seeds also perform better than their smaller counterparts under stress conditions for mechanical reasons.

Seed size can differ greatly within a species dependent on the seed production environment (Fenner, 1991) and may significantly influence the success of seedling

993 emergence and therefore arguably seed vigour. Seeds of domesticated plants are normally
994 much larger than those of their wild counterparts and this likely results from selection pressure
995 for yield (Doganlar et al., 2000). However, this increase in seed size with domestication also
996 occurred in species where seed is not the primary component of yield and so may have also
997 occurred by passive selection for improved germination and seedling vigour. In some crops,
998 in particular small seeded vegetable crops, there is a direct relationship between plant size
999 and its variation at harvest and that at seedling emergence as this is dependent of seed
1000 reserves (Benjamin, 1990, Bettey et al., 2000). For this reason larger seeds are favoured
1001 since they tend to produce larger and in some cases more uniform seedlings especially from
1002 deeper sowings (Fenner, 1991).
1003
1004 There is also a positive influence of seed weight on the ability of *B. oleracea* hypocotyls to
1005 grow through strong soil (Finch-Savage et al., 2010). Furthermore, in a study of seeds from
1006 nine vegetable crops Taylor and Ten Broeck, (1988) demonstrated that the seedling
1007 emergence force generated was greater for larger seeded crops and for larger seeds within
1008 crops. However, in general small-seeded crops exerted greater force per stored energy than
1009 large-seeded crops. On the negative side there is anecdotal evidence that the hypocotyls of
1010 seeds with large cotyledons, for example in legumes with epigeal germination can become
1011 damaged during emergence in strong soils and so lose vigour.
1012
1013 Although in general larger seed size is a benefit for seedling establishment, there appears no
1014 consistent link with germination characteristics. For example, in *B. oleracea* there is no
1015 correlation between seed size and mean germination time or final percentage germination
1016 (Finch-Savage et al., 2010), but under hypoxic conditions germination was negatively
1017 correlated with seed weight (Finch-Savage et al., 2005). This is likely due to the reduced
1018 penetration of oxygen into larger seeds. There are contradictory reports where small seeds
1019 completed germination faster than larger seeds (Fenner, 1991); in tomato inheritance of
1020 time to germination completion was closely related to seed size, with smaller seeds
1021 germinating earlier (Whittington and Fierlinger, 1972).
1022
1023 ***Spatial control of seed vigour:*** While there is a growing amount of information on the
1024 regulatory factors controlling germination at various stages, there is almost no information
1025 on the spatial and temporal dynamics of where these factors act. We therefore do not know
1026 the cellular sites in seeds where vigour may be manipulated. Techniques that quantify the
1027 dynamic changes in embryo cell shape, gene and protein abundance (Bassel et al., 2014;
1028 Montenegro-Johnson et al., 2015; Barbier de Reuille et al., 2015) may help uncover which
1029 cells drive vigour within crop embryos.
1030
1031 The cells of the embryonic hypocotyl in most crop species are substantially larger than
1032 those in the radicle. Given that cells which are larger have a greater capacity to grow and

generate force from an equivalent amount of gene expression relative to smaller cells, this suggests that greater forces driving seedling establishment come from the hypocotyl rather than the radicle. This presents the hypocotyl as the prime cellular subdomain for the manipulation of seed vigour, as manipulation of these larger cells will have a greater potential to impact on embryo and seedling growth force than within the small celled radicle.

8. Strategies to improve seed vigour

The limited knowledge of the underlying mechanisms that drive seed vigour makes developing approaches to enhancing this crop trait challenging. Here we discuss approaches that have been taken, and may be taken in the future to approach this problem.

Role of the maternal environment: The environment in which a seed develops impacts its behaviour in subsequent generations. As we describe above seed producers carefully select production areas for their beneficial climates, however a mechanistic understanding between the maternal environment and seed quality remains to be clearly demonstrated. It has been shown that flavonoid production in the mature seed coat is influenced by maternal temperatures (MacGregor et al., 2014) and a role for the flowering time regulator *FT* is linked to this process (Chen et al., 2014). A role for seed coat pigments in the control of dormancy and germination has also been demonstrated (Debeaujon et al., 2000) providing a potential link between the maternal environment and seed quality. High permeability of the seed coat was selected for during legume domestication possibly due to seed quality properties (Sun et al., 2015). In soybean, seed coat permeability was shown to be controlled by a single transmembrane protein GmHs1-1 (Sun et al., 2015). The mechanisms by which seed coat properties influence seed behaviour remain unknown.

A positive correlation between the quantitative expression of the *Arabidopsis* dormancy QTL DOG1 and depth of seed dormancy has been previously demonstrated (Chiang et al., 2011; Kendall et al. 2011). The expression level of this gene is also strongly influenced by the maternal environment strengthening this correlation. The mechanistic mode of action for DOG1 remains elusive, and the link between an increased abundance of this gene or protein and the maternal control of seed dormancy remains unknown (Dekkers and Bentsink, 2015).

The impact of harvest time on seed vigour: In many species, including *Brassicaceae* and *Umbelliferae*, seed development is not uniform within the inflourescence and so a seedlot harvested at any single time from the mother plant can contain seeds that have different developmental stages and even seeds that have begun to deteriorate (e.g. Still and Bradford, 1998; Still, 1999; Copeland and McDonald, 2001; Bewley et al., 2013). Seed companies employ strategies to limit these problems, but they have not been eradicated.

Thus individual seeds will vary in vigour and other seed characteristics resulting in heterogeneity. In wild species, seeds would be shed at different times as they mature, but may still vary since they would have experienced different environments during development. This is natural bet hedging adaptive behaviour. In many crops domestication has selected characteristics that avoid seeds being shed at different times to maximize yield. Therefore a dilemma exists in some species when to harvest plants for seeds destined to sow the next generation, rather than yield. Where plants fully senesce before harvest, the effect of different developmental stages is minimized, but this impact varies between species.

We have discussed above that seeds deteriorate both on and off the plant following physiological maturity (Figure 3). For this reason in commerce seeds may be harvested early before full HM and dried rapidly under controlled conditions to maintain initial quality by reducing pre-HM deterioration (Bewley et al., 2013). There is clearly a balance to be drawn since it is well documented that following disconnection from the mother plant there is a post MM developmental programme that enhances seed vigour, which is terminated by continued loss of moisture from the seed (summarized by Bewley et al., 2013). Once dried, seed performance can also be enhanced, by holding seeds in the lag phase of subsequent seed imbibition (priming) either by limiting water availability (Heydecker and Coolbear, 1977) or cell wall extension (ABA: Finch-Savage and McQuistan, 1988b, 1991) to prevent germination completion. We discuss this below, however, there may be other opportunities to manipulate seed performance. Seeds could be harvested before HM, (i.e. still moist), but then held under appropriate conditions to avoid drying, potentially enhancing seed lot uniformity by allowing less developed seeds to complete their developmental program. Generally this may not be practical on a crop scale due to physical harvest damage and difficulty in extraction from the surrounding fruit structures. Nevertheless, a range of techniques to improve seeds physiologically are being developed (Halmer, 2004). We discuss below strategies to alter seed performance both before and after seed drying as a means of overcoming adaptive variation not lost during domestication. Although we do not describe them here there is also a number of physical techniques under the heading of conditioning or processing (e.g. mechanical size and density grading) that are routinely carried out by seed companies following harvest to refine the quality of bulk seed lots (Halmer, 2004).

Moist seed treatment before drying (continued seed development?): *Prunus avium* (cherry) seed performance differs between individuals seeds, harvest occasions, areas of production and mother trees due to differences in the extent of seed development (Finch-Savage et al., 2002). *P. avium* is a wild species that has domesticated cultivars selected for horticultural qualities in the harvested fruit. The seed is deeply dormant (Suszka, 1962) and so represents an extreme of commercial crop seed, but illustrates how variation in

seed performance is generated and can be overcome by understanding the source of variation and adopting natural treatments. *P. avium* seeds mature at different times on the mother tree, but for practical reasons seeds are harvested on a single occasion and so many remain immature. The most mature seeds respond to relatively simple dormancy breaking treatments; the least mature require complex treatments lasting up to 26 weeks that have repeated warm and cold periods to mimic repeated winter and summer seasons (Suszka et al., 1996).

Finch-Savage et al. (2002) show that if freshly harvested seeds are cleaned (fruit removed), prevented from drying by storing in moist sand which allows aeration, and held at 15^{o}C (neutral, neither dormancy inducing or relieving) for up to 8 weeks the performance of individuals in and between the seed lots progressively becomes more homogeneous and responds to simple treatments to break dormancy. Thus seed development appears to continue and all seeds acquire the characteristics of fully mature seeds. However, further detailed work is required to confirm there is no other explanation for the seed improvement. The benefit is retained upon drying. The extent to which this is possible with optimization in any other species is not known, but there is little logic in allowing seeds to dry and potentially age before physiological treatment (e.g. priming).

Moist seed treatment after drying (imbibition, hardening, priming: continued physiological advancement?): Several methods have been developed to improve seed performance physiologically by manipulating imbibition (Halmer, 2004). These include short-term imbibition allowing repair as a result of ageing (Walters, 1998; Powell et al., 2000); the process of seed hardening, which involved repeatedly wetting and drying seed (Hegarty, 1978), and several approaches under the banner of seed priming (Heydecker and Coolbear, 1977).

Seed priming techniques limit the availability of water to the seed so there is sufficient to progress metabolism, but insufficient for radicle extension and completion of germination; seeds therefore remain desiccation tolerant. Water can be limited by placing seeds on absorbent paper soaked in an osmoticum of appropriate strength (osmotic priming; Heydecker et al., 1975); or in aerated osmoticum (Bujalski and Nienow, 1991); or seeds separated from the osmoticum by dialysis membrane (membrane priming; Rowse et al., 2001). Water can also be limited by mixing it with various solids to create an appropriate matrix potential (solid matrix priming; Taylor et al., 1988). Water may be applied in limited quantity in a rotating drum (drum priming; Rowse and McKee, 1999) or by other means (Halmer, 2004). Alternatively, germination completion may be inhibited by placing seeds in an ABA solution, followed by washing to remove the inhibitor (ABA priming; Finch-Savage and McQuistan, 1988b, 1991). In all cases seeds are then dried and stored or sown.

Seeds after all these treatments are metabolically advanced leading to a shortened lag phase on re-imbibition and thus more rapid and uniform germination. The mechanism is not fully understood, but may result partly from repair (Powell et al., 2000) and interestingly priming can result in an increase in cell wall elasticity (Karssen et al., 1989) suggesting that cell wall remodifying gene expression is induced under this treatment. It has been questioned whether priming this improves vigour *per se*, or whether is merely advances the stage of germination of the seed. Conceptually it seems plausible that this protracted process takes both fast and slow germinating seeds to an advanced checkpoint in the germination process such that their subsequent germination requires only minor additional advances before germination is completed (Figure 6).

Priming treatments are typically carried out upon dry “mature” seed usually of high-value vegetable and flower species. It is interesting to note that seeds of species that benefit most from these treatments tend to be those with indeterminate floral development and thus heterogeneous seed development, often containing under-developed seeds such as those in the *Umbelliferae* and many ornamental species. In commerce, poor quality aged seeds do not respond as well to seed priming as do higher quality fresh seeds, and thus it is not generally economic to use the technique to reverse the deterioration of ageing, rather overcome the problems of heterogeneous development.

Most experimental results present laboratory germination and there are relatively few fully replicated field experiments, which are needed to properly evaluate the efficacy of seed-enhancing treatments. In one such experiment, the seedling emergence performance of primed and unprimed carrot seeds were compared in 37 seedbed environments (Finch-Savage, 1990). Priming seeds reduced the variation in percentage seedling emergence (Figure 7; primed 75%, SD 11; untreated 65%, SD 15) and so by the definitions quoted above can be considered to have improved vigour. However, the data was analysed on thermal time scales to determine predictability of timing across environments. Thermal time to emergence in non-stressed conditions was subtracted in all environments to indicate the relative effect of the seedbed. Although the results showed mean seedling emergence was earlier from primed (210 ^{o}Cd) than untreated seeds (244 ^{o}Cd) the timing of seedling emergence across environments (standard deviation (SD) of the means; 81.8 and 63.2 respectively) was not significantly different and so the result was no more predictable. Much of the variation in both cases across environments was due to the timing of water availability in the seedbed, and not germination *per se.*

Priming treatments can also be used to overcome specific dormancy issues. Examples of this include advancing the stage of germination in seeds to a point beyond when they are sensitive to thermoinhibition, a particularly important issue in lettuce, by exposing them to

light beyond the point of sensitivity, and by enabling immature embryos to complete their development, which happens in seeds such as celery (Halmer, 2004).

Another issue in seed quality is that of imbibitional damage (Powell, 2006). This occurs when the water potential of embryo cells is very low and upon imbibition in pure water, cells lyse due to the rapid generation of high pressure, and is typically associated with planting in low temperature soils (Bennett and Waters, 1987). Controlled hydration rates can help alleviate this problem (Taylor et al., 1992). For example, seed priming at low osmotic potential can minimize the effects of imbibitional damage by giving cells the opportunity to adjust their osmolytes accordingly.

In order to develop novel approaches to prime seeds, we must first understand the molecular nature of the sequential steps of germination, and how arrest at various stages relates to the overall developmental program. Once this is understood, novel opportunities to manipulate this system will emerge by targeting checkpoints or the processes that control the overall rate of progression through these checkpoints. In the context of priming, arresting the germination program at the latest possible checkpoint would be advantageous in order to have the most rapid completion of germination following subsequent imbibition.

Pre-germinated seed treatments: A further opportunity to enhance seed performance exists where seeds are allowed to progress beyond emergence of the radicle to sow moist (Fluid Drilling; Gray, 1981), or to dry before desiccation tolerance is lost (Finch-Savage, 1988; Finch-Savage and McKee, 1988); or when desiccation tolerance is re-introduced (Finch-Savage, 1989; Bruggink and van der Toorn, 1995, 1997). Although the basis of such commercial treatments and products has been developed, to date practical issues have limited use in commerce. The key advantage in this approach is that seeds can be selected from a seed lot as germinated (100% viable) or the first to germinate (most vigorous). Indeed there is evidence that first germinators from seed lots of different quality have similar high vigour (Finch-Savage, 1986; Finch-Savage and McQuistan, 1988a).

The concept of synthetic seeds allowing the direct genetic manipulation of the embryo through somatic embryogenesis offers potential for the production of superior performing seeds (Redenbaugh 1993), but the technology is not currently sufficiently developed or economic.

Conventional breeding: In the pursuit of vigour we seek to push plants to perform in ways they would not naturally, and to adopt a more agronomic behaviour than that they have adapted to. A diversity of genetic backgrounds may have the capacity to be vigorous, and it becomes a question of unlocking this genetic potential and realizing the full capacity of

plant performance in the field. One approach is that of Quantitative Trait Loci (QTL) analysis we discuss further below (section 9)

Altering the timing of dormancy loss following physiological maturity and harvest is an active area to reduce the problems of residual dormancy while preventing pre-harvest sprouting in grain crops. It is suggested that studies linking genetics with our physiological understanding appear to be most promising, for example genes controlling sensitivity to ABA and GA may be radically altered by desiccation marking the end of seed development (Benech-Arnold, 2004) as has been shown previously in tomato (Kermode and Bewley, 1985).

There are examples of specific potential genetic improvements through breeding that affect seed performance, for example hard-seediness in beans, or mechanical damage in navy beans (Copeland and McDonald, 2001). Other modification to seed coats that alter the rate of water uptake, and minimize the negative impact of imbibitional damage on seed vigour may be possible (Powell, 2006). Furthermore, the advantage of hybrid vigour in crops where hybrid production is practiced like maize can extend to the quality of the seeds produced (Copeland and McDonald, 2001). The extent of quality improvement is influenced by the method of crossing, but the enhanced quality can lead to improved stands under adverse conditions following sowing.

Increasing understanding of the regulation of germination and pre-emergence seedling growth may facilitate more targeted selections and screens in breeding programs for specific attributes and vigorous phenotypes. This is particularly true for our understanding of the cellular basis of vigour and where within embryos this trait is conferred.

Genetic manipulation: Transgenic approaches may also be taken to improve seed vigour. Targeted alteration of a positive ABA signalling factor, or negatively acting GA signalling factor results in the more rapid germination of seeds and enables germination to occur within stress conditions. While these manipulations conform to the definition of increased seed vigour, there is also a loss in stress tolerance within these seedlings (Achard et al., 2006). This appears to be a common trend when targeting loci that are central to fundamental signalling pathways in plants. “Green revolution” alleles in wheat DELLA proteins were capable of enhancing yield, while a cost to seed vigour at deeper planting depths has recently been reported (Amram et al. 2015).

Systems-based approaches to understanding the interactions between these key loci and their downstream targets may provide a means for the rational manipulation of the system in an advantageous fashion. More sophisticated and subtle approaches to manipulating multiple genes using designer genetic circuits in a synthetic biology context may make

1271 these key loci suitable targets for the enhancement of seed performance. Alternatively,
1272 mutant screens designed to identify vigour-enhancing mutations may provide novel alleles
1273 to enhance this trait, though such a screen would be logistically complicated.
1274
1275 The identification of central signalling loci from genetic screens may represent mutations in
1276 genes, which have too dramatic an effect on the germination system. These drastic
1277 mutations are contrasted by natural variant alleles, which were selected for or arose over
1278 the course of crop domestication. QTLs for developmental traits in plants rarely correspond
1279 to the same genes as those identified using mutant screens as they are more likely
1280 involved in a complex series of subtle interactions with multiple key loci involved in
1281 controlling a system. These fine-tuning, naturally occurring alleles therefore represent more
1282 adaptive options to enhance a crop trait such as seed vigour.
1283
1284 **9. Conclusion and perspective**
1285 We have shown above that seed vigour is a complex trait that is determined during different
1286 stages of mother plant and seed development to seed imbibition and greatly influenced by
1287 the prevailing environment. Furthermore its affects act from seed imbibition through to
1288 seedling emergence and depend on the prevailing environment. We summarise these
1289 factors in Figure 8.
1290
1291 When the trait is understood and then studied and analysed systematically it may be both
1292 feasible and practical to improve the complex trait of seed vigour to create robust seeds
1293 that enhance seed performance and crop establishment. A key approach to future
1294 improvement of such complex traits is to use marker assisted introgression and other
1295 methods of exploiting novel genes/alleles identified from natural variation using, for
1296 example QTL analysis. Amongst others, examples of such QTL analyses can be found in
1297 *Arabidopsis* (Clerkx et al., 2004; Joosen et al., 2012, 2013), tomato (Foolad et al., 2007;
1298 Kazmi et al., 2011; Khan et al., 2012) and lettuce (Argyris et al., 2011). Rodriguez et al.
1299 have also recently reviewed this approach to understanding natural variation in dormancy
1300 as an approach to eliminating pre-harvest sprouting in rice and wheat. In *B. oleracea*, QTL
1301 that influence seed vigour have been identified (Bettey et al., 2000; Finch-Savage et al.,
1302 2010) and these loci have been fine mapped. Furthermore, initial studies indicate that
1303 beneficial alleles at these loci can be introduced to enhance *B. oleracea* seed performance
1304 in commercial lines (Finch-Savage et al., 2013) indicating the future potential of this
1305 approach.
1306
1307 The issue of seed vigour is of central importance to agriculture and the seed industry, yet is
1308 still poorly understood and generally overlooked in academic research. With the rapidly
1309 growing human population and rapid changes in climate, the significance of seed vigour is
1310 increasing with time. We hope that with this review we were able to highlight what is known

about seed vigour, its impact on crop production, and the various approaches that could be taken to improve it. Further research and novel approaches into understanding this enigmatic and complex trait are needed and will help to ensure a more reliable food supply into the future.

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Figure legends:

Figure 1: Differences in field performance are caused by differences in seed vigour. Percentage germination of nine commercial sugar beet seeds lots in standardised laboratory germination tests (dark grey columns), and percentage seedling emergence (light grey columns) following sowing under commercial field conditions. The germination tests carried out under near optimal conditions cannot statistically distinguish between the seed lots, but difference in vigour result in very different field performance (seedling emergence) under field conditions (Finch-Savage, unpublished).

Figure 2: Crop establishment and crop yield. Schematics of the influence of plant density on total yield (a) and plant size (b); and (c) the distribution of plant sizes resulting from uniform (solid line) and non-uniform (dotted line) seedling emergence. The vertical lines indicate the range of sizes acceptable for sale; thus uniform seedling emergence greatly increases the marketable yield (value) of the crop. Copyright 2004, from Finch-Savage, 2004. Reproduced by permission of Taylor and Francis Group, LLC, a division of Informa plc.

Figure 3: **A schematic illustration of the establishment and loss of seed vigour in a seed population (seed lot).** The illustration shows progressive changes in the development and loss of seed vigour within a population of seeds and how this affects their performance in practice. These changes and the influence of environment are predictable when quantified correctly. Throughout, 4 seed lots are considered (A – D) that have different ages and consequently different vigour. (a) seeds gain the ability to germinate and produce normal seedlings progressively during seed development. Subsequently seed vigour increases through mass maturity (MM; maximum seed weight) to physiological maturity (PM; maximum seed quality) after which seeds progressively deteriorate, first on the plant and then independently following harvest maturity (HM; sufficiently dry to harvest). This represents soybean redrawn from Dornbos 1995a from an original in Miles 1985. Copyright 1995, from Dornbos, 1995a. Reproduced by permission of Taylor and Francis Group, LLC. (b) a linear relationship can describe seed deterioration in storage; the origin on the Y axis (K_i) is the theoretical initial viability. (c,d) germination time increases predictably as viability declines. (e) the vigour (time to 50% germination; T50) of B. oleracea seeds differs at 7 production environments, but seeds that have genetically higher vigour are less affected by increasingly harsher production environments (G x E plot: gene = individual value of seed lots; environment = mean across seed lots; Finch-Savage unpublished). (f) seed lots with reduced vigour are less able to resist stress during germination.

Figure 4: Influence of the seed bed environment on seed germination and seedling emergence. The illustration shows the progression of an epigeal carrot (Daucus carota)

seed from sowing to seedling emergence. Hypogeal bean (Vicia faba) and wheat (Triticum aestivum) seedlings are shown at emergence for comparison. Successful crop establishment can be considered as a balance between seedbed deterioration and rate of seedling development; both are determined by the prevailing environment, but the latter is greatly influenced by vigour. The seedbed begins to deteriorate following sowing at a rate dependent on soil type, soil structure and weather. As a consequence the strength of the soil that opposes seedling growth progressively increases. Temperature and water availability drive the rate of progress through seed imbibition, germination completion and growth to emergence, and therefore how long the seedbed has to deteriorate before seedling emergence. If seedbed moisture is sufficient, seeds will complete germination (Trait 1). Once the radicle has emerged, extension growth occurs at a rate determined by temperature as does the rate of seedbed drying from the surface downwards. Thus rapid downward growth (Trait 2) facilitates the seedling root tip maintaining contact with receding moisture. However, continued drying increases soil strength through which the hypocotyl has to extend before it emerges and the seedling can become autotrophic. Enhanced vigour in Traits 1-3 will tip the balance in favour of successful crop establishment. Figure redrawn from Finch-Savage et al. 2010, Copyright 2010, with Permission from Elsevier.

Figure 5: Seeds eventually germinate, but seedlings are lost during the post germination phase. Percentage carrot seed germination in the field recorded by exhuming seeds at intervals (dark gray columns) and percentage seedling emergence in adjacent field plots (light gray columns). Data was recorded on 5 different occasions each with three seedbed treatments; a, no irrigation; b, pre-emergence irrigation; c, pre-sowing and pre-emergence irrigation to provide 15 seed-bed environments. Horizontal dotted line is germination and dashed line represents seedling emergence recorded under non-stressed laboratory conditions. Redrawn from Finch-Savage et al. (1998).

Figure 6: Schematic of domestication of vigorous seed populations for agriculture. **(a)** Even in the absence of deterioration demonstrated in Figure 3d seed populations with different genetic backgrounds and production environments have germination curves with different synchronicity. The dotted line indicates where the agronomic trait of seed quality has been selected and a corresponding undomesticated seed lot (short dashed line) where a bet-hedging strategy is still being employed even with minimal or no dormancy. The dashed and dotted line represents an ideal with no residual dormancy giving the potential for rapid and synchronous germination and seedling emergence when seeds are sown into favourable seedbeds. (**b)** These indicate rates of the execution of the germination program in individual seeds that are either vigorous or non-vigorous. The y-axis indicates developmental time starting at the origin that is the seed state and the upper limit representing an established seedling. **(c-d)** The sequential steps underlying the germination program (see section 6) and the rate at which these are executed **(c)** non-

vigorous and **(d)** vigorous individual seed. A total of four steps were selected for schematic purposes. The increased rate at which these sequential steps are passed are illustrated as the program reaching completion faster in (**b**). This must happen uniformly in the population of seeds to provide a steep germination curve **(a).**

Figure 7. Osmotic seed priming reduced variation in percentage seedling emergence across seedbed environments. The frequency of percentage seedling emergence in decile categories from a comparison of osmotically primed and unprimed seed in 37 seedbed environments. Data recalculated from Finch-Savage, 1990.

Figure 8. A summary of factors influencing seed vigour and seed performance in progressing from fertilisation on the mother plant to emergence of the seedling through the soil following sowing. PM is physiological maturity.