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Seed vigour and crop establishment – extending performance beyond adaptation

Finch-Savage W.E.¹ and Bassel G.W.²

School of Life Sciences, Warwick University, Wellesbourne Campus, Warwick CV35 9EF, UK; ²School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK

e-mail: bill.finch-savage@warwick.ac.uk; G.W.Bassel@bham.ac.uk

Corresponding author: W.E. Finch-Savage. Tel no. 024 76574968. Fax. 024 7657 4500

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Running title: Seed vigour and crop establishment

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:** *What is seed vigour?; The effect of environment on variation in seed vigour and performance; The genetic basis of seed vigour*

- 2. The importance of seed vigour in agriculture:** *Seed vigour and climate; Yield and profitability; Marketable yield in horticultural crops; Input costs and environmental impact in field crops*

- 3. Seed vigour and seed technology; a population (seed lot) characteristic:** *Seed ageing longevity and viability equations; Seed lot characteristics; Rapid ageing and vigour; Vigour, seedling normality and testing standards*

- 4. Factors limiting seed performance; stress in the agricultural seedbed environment:** *Germination and pre-emergence growth in the soil seedbed environment; Available water (water stress); Soil strength (mechanical impedance); Soil temperature and oxygen stress; Seedbed environmental effects on germination and pre-emergence seedling growth; Important seed vigour traits for predictable crop establishment; At what stage do seedlings fail to establish?*

- 5. Seed vigour as an agronomic trait; beyond natural adaptation:** *Seed strategies in the wild; Residual dormancy in crops; Domestication: Seed strategies in agriculture; Natural variation of seed vigour*

- 6. Mechanisms of seed vigour; what makes a vigorous seed in an agricultural context:** *Loss of dormancy at physiological maturity; Rate of seed germination; Rate of pre-emergence seedling growth*

- 7. Biophysics of germination and pre-emergence growth:** *Turgor; Turgor and seed vigour; Cell wall modification; Cell wall and seed vigour; Stored energy reserves and seed vigour; Cell cycle and seed vigour; Epigenetics and seed vigour; A role for cell and seed size; Spatial control of seed vigour*

- 8. Strategies to improve seed vigour:** *Role of the maternal environment; The impact of harvest time on seed vigour; Moist seed treatment before drying (continued seed development?); Moist seed treatment after drying (imbibition, hardening, priming: continued physiological advancement); Pre-germinated seed treatments; Conventional breeding; Genetic manipulation*

- 9. Conclusion and perspective:**

1 1. INTRODUCTION

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

11

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

22

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

30

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

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

50

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

60

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

74

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

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

85

86 ***The genetic basis of seed vigour:*** A great deal is now known about the regulation of seed
87 dormancy and germination and there are a number of reviews that discuss this in detail
88 (Finch-Savage and Leubner-Metzger, 2006; Holdsworth et al., 2008a; Finkelstein et al,
89 2008; North et al., 2010; Weitbrecht et al., 2011; Rajjou et al., 2012; Rodriguez et al.,
90 2015). Little research has been focused towards understanding what mechanisms
91 determine the initial (potential) vigour of seed in an agricultural context, rather than the
92 consequences of environmental variation. *Arabidopsis* has emerged as an excellent model
93 for understanding the regulation of dormancy and germination. This small seeded annual
94 has adapted to a wide range of natural environments, but has not been subjected to the
95 selection pressures of crop domestication and therefore its’ seeds have not been
96 challenged to perform in an agricultural context. We suggest that adaptation to the natural
97 environment with emphasis on post-shedding dormancy to time germination to seasonal
98 changes is very different from the agricultural necessity to germinate and progress to
99 seedling emergence with the minimum delay following sowing (Section 5). This provides a
100 focus for the difference since both situations require seeds to be dormant before shedding
101 (harvest) to avoid germination on the plant leading to pre-harvest sprouting (Paulsen and
102 Auld, 2004), but their subsequent behaviour is sharply contrasting.

103

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

118

119 **2. The importance of seed vigour in agriculture:**

120 **Seed vigour and climate:** Sowing time is selected on the basis of climate and seasons,
121 but there are many crop specific drivers such as scheduling and economic incentives to
122 fulfil production markets. Especially in temperate regions, the choice is often made to
123 maximize the length, or optimize the environment of the growing season to increase yields,
124 rather than the best conditions for successful crop establishment. Ongoing climate change
125 is likely to make this choice even more precarious with the potential for more variable crop
126 establishment. This variability has direct and negative effects on yield as we describe
127 below. Figure 1 illustrates that on any single occasion the vigour of sugar beet seed greatly
128 influences seedling establishment and this is magnified across sowings. Continuing with
129 the example of sugar beet, of 254 crops studied Durrant et al. (1984) found that only in 55
130 was a sufficient stand achieved that did not limit yield. Similar impacts of vigour on seedling
131 establishment from seeds of commercial quality have been observed in small seeded
132 vegetable species (Mathews, 1980) and a wide range of other crops (reviewed by Powell,
133 2006). There is continuing improvement, but establishment remains variable. Even if
134 optimum conditions for the day of sowing can be chosen the subsequent conditions that
135 influence establishment cannot. Robust seeds with enhanced vigour mitigate these yield-
136 limiting effects by establishing seedlings more uniformly across a wide range of
137 environmental conditions.

138

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

153

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

160 stages during their development. The market strictly defines the characteristics required of
161 these components (e.g. size, colour, shape). Marketable yield refers to the proportion of the
162 crop in these defined categories and ultimately defines the difference between making a
163 profit and not and the level of wastage. This aspect of plant production is central in
164 modern horticultural practice. Thus even if the numbers of emerging seedlings are
165 adequate and total yield is high there can be more subtle crop-specific effects that limit the
166 marketable proportion of that yield and/or its value (Tekrony and Egli, 1991; Finch-Savage,
167 1995).

168

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

182

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

188

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

196 Germination timing is therefore less important in bulk field crops.

197 Seed vigour, through its impact on seedling emergence, contributes directly to the
198 economic success of all commercial crops. Vigour also has important indirect effects on
199 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

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

253

254 **Seed lot characteristics:** How fast a seed germinates is an easy and recognizable
255 indication of the concept of vigour. Germination rate is often incorrectly used in the
256 literature as the proportion of seeds that germinate, but it is the reciprocal of germination
257 time (rate) and provides a useful measure to compare seed lots or individuals within seed
258 lots. Germination rate is predictably influenced by temperature and water potential
259 (Bradford 1995; Finch-Savage, 2004; Section 4) and so must be compared under the same
260 accurately controlled germination environments. Often rate is quantified by the time for half
261 the viable seeds to germinate (T_{50}), but this only accurately represents the whole seed
262 population if the times to germination of individuals in the population are normally
263 distributed. To avoid this often-incorrect assumption mean germination time can be
264 calculated ($MGT = \sum(Dn)/\sum n$, where n = number of seeds which germinate
265 on day D , and D is the number of days counted from the beginning of the germination test;
266 Heydecker, 1966) and this has been found to have a consistent relationship with
267 percentage viability in a seed lot as it deteriorates (Figure 3c,d), independent of the storage
268 conditions (Ellis and Roberts, 1980). This relationship holds true for different species, but
269 the slope differs.

270

271 The distribution of germination times in a seed lot is also correlated with percentage
272 germination in many species, including carrot (Gray, 1984; Finch-Savage and McQuistan,
273 1988a). Other seed lot characteristics such as seedling length, variation in seedling length,
274 their subsequent seedling emergence and seedling weight can also be correlated with
275 percentage germination and speed of germination (e.g. carrot, leek, onion, cauliflower;
276 Finch-Savage, 1986; Finch-Savage and McQuistan, 1988a). Time to germination and
277 seedling size measurements are often confounded in reported experiments. A late
278 germinating seed will always have a smaller seedling at a given time from sowing than a
279 seedling from a fast germinator. Therefore it is important in the work highlighted here that

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

284

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

289

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

300

301 A more accurate determination of this performance potential is possible if seeds are first
302 subjected to rapid ageing under controlled conditions. There are two accepted methods for
303 this: accelerated- ageing (TeKrony, 1993), and controlled-deterioration (Powell, 1995). Both
304 methods elevate seed moisture content and temperature for a fixed period of time to
305 accelerate progress down the viability curve before germination testing (Figure 3b).

306 Differences between seed lots are enhanced after the treatment and can be seen at a
307 single point in time. This now forms the basis of ISTA validated tests used in commercial
308 seed testing for specified species (Powell, 2006; ISTA, 2015). For research purposes it is
309 also possible to do several successive measurements in time to construct a survival curve
310 and estimate K_i as described above (Ellis and Roberts, 1980). Thus ageing is a key
311 characteristic that is both a cause of differences in vigour and a basis for vigour testing.

312 Other validated ISTA vigour tests, the electrical conductivity test, and the radicle
313 emergence test are also related to physiological changes that occur during ageing (ISTA,
314 2015). These tests are based on a large body of physiological evidence linking seed vigour
315 difference to ageing both before and after seed harvest (reviewed by Powell, 2006). Other
316 types of vigour tests have been described (ISTA, 1995; AOSA, 1983), but these have not
317 undergone the extensive comparative testing used in the ISTA validation procedure.

318

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

332
333 Survival curves are theoretically the same for high and low vigour seeds of the same lot
334 (same genetics and production). They therefore take the same amount of time to pass
335 through the defective stages that precede death and lead to abnormal development
336 following germination (Figure 3a); they are just in different places on the curve (Figure
337 3b,c). However, because they are further down the curve low vigour seed appear to
338 deteriorate more quickly as it takes less time to see measurable differences in germination
339 rate and the number of abnormal seedlings. In seed testing practice a non-dormant viable
340 seed may germinate and either produce a seedling that can be normal or abnormal.
341 However, a seed that produces an abnormal seedling is not included in the germination
342 percentage in the test result. This is because they are less likely to establish a plant under
343 field conditions. Therefore in commerce, percentage germination and viability may not be
344 the same. There are accepted criteria for assessing seedling normality, which provides a
345 better estimate of field performance. These criteria appear in the rules for seed testing from
346 both ISTA and AOSA and details of their most recently updated rules are available on their
347 web sites (www.seedtest.org; www.aosaseed.com respectively). Deterioration in storage is
348 one particular stress, but seeds are subjected to many stresses once sown in the seedbed
349 (Section 4). Higher seed vigour results in greater resistance to all these growth-limiting
350 stresses (Figure 3f).

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

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

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

363

364 **Germination and pre-emergence growth in the soil seedbed environment:** The soil
365 seedbed is a complex environment in which seeds and seedlings are exposed to multiple
366 stresses (Braunack and Dexter, 1989; Hadas, 2004; Whalley and Finch-Savage, 2006,
367 2010). The literature provides no clear description of the soil conditions that lead to either
368 good or poor crop emergence; this is because seedlings are not at all sensitive to soil type
369 or condition *per se*, but are extremely sensitive to the physical stresses that a soil imposes
370 during germination and seedling expansion (Whalley and Finch-Savage, 2006). These soil
371 physical stresses (available water, mechanical impedance, oxygen and temperature)
372 interact with each other and vary with water content, but neither the seed nor seedling is
373 sensitive directly to water content. In order to understand what is required of a robust
374 seed/seedling it is important to understand the nature of the physical stresses and their
375 interaction that must be overcome when seedbeds are either drying or wetting. In the
376 seedbed, temperature influences timing, but water stress and mechanical impedance have
377 been identified as the two stresses most likely to limit germination and emergence
378 respectively (Whalley and Finch-Savage, 2006, 2010).

379

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

394

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

398 roots (Jin et al., 2013) and in particular shoots of pre-emergent seedlings (Whalley et al,
399 1999). In the seedbed the strength of soil tends to be due to the capillary pressure of water
400 in the pores holding the soil particles together. The effective stress model of soil strength,
401 allows for the interaction between soil strength and water stress to be understood. Root
402 and shoot elongation both tend to decrease as a linear function of water stress and as a
403 nonlinear function of soil strength (Whalley and Finch-Savage, 2010). Thus, not only does
404 soil strength increase rapidly as the soil dries, but the expansive growth of a seedling is far
405 more sensitive to changes in soil strength than it is to changes in water stress (Weaich et
406 al., 1992). Clay soils tend to have a higher degree of saturation (thus greater capillary
407 pressure) at a given matric potential than sandy soils and so their effective stress is higher
408 and they tend to be stronger (Whalley and Finch-Savage, 2006).

409

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

422

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

433

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

438 1984) resulting from loss of membrane integrity (Powell, 1985). There are a number of
439 factors that influence the extent of damage, for example, when the testa is not intact or
440 seeds have low vigour through ageing (reviewed by Powell, 2006). Once imbibed, our own
441 work on carrot, onion and *B. oleracea* illustrates how seeds germinate and seedlings grow
442 in the soil and minimise the stress they encounter (Finch-Savage and Phelps, 1993; Finch-
443 Savage et al., 1998, 2001; Rowse and Finch-Savage, 2003; Whalley et al., 1999; Finch-
444 savage et al. 2010). Soil moisture fluctuates rapidly in the surface layers of the soil where
445 seeds are sown and seeds have adapted to this situation so that completion of germination
446 will only occur when there is likely to be adequate moisture in the soil for subsequent
447 growth (Finch-Savage and Phelps, 1993; Finch-Savage et al., 1998). For example, the
448 germination process and pre-emergence seedling growth can proceed at water potentials
449 below that which will prevent the completion of germination (Ross and Hegarty, 1979).
450 Thus the completion of germination is a critical moisture-sensitive stage that controls rate of
451 progress from sowing to seedling emergence from the soil (Figure 4).

452

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

463

464 The influence of the seedbed environment on this pattern of germination and seedling
465 growth can be described and modeled using the following population-based threshold
466 modelling approach (Finch-Savage, 2004). A negative relationship exists between
467 increasing stress from a component of the seedbed environment and the progress towards
468 germination completion (temperature, water potential, oxygen) or pre-emergence seedling
469 growth (additionally soil strength) such that progress reduces to zero at a threshold value
470 (base). The models assume progress is proportional to the component value above the
471 base and ceases below the base when the level of stress prevents progress. The
472 development and applications of such models (thermal-, hydro-, and hydrothermal time) for
473 seed germination in the laboratory have been reviewed (Bradford, 1990,1995; Finch-
474 Savage, 2004) and the principles extended to other environmental factors (Bradford, 2002;
475 2005; Donohue et al., 2015) and to pre-emergence seedling growth (Finch-Savage et al.,
476 2001). The threshold, base water potential is a key unifying parameter relating germination
477 performance to seedbed stress that is likely determined by the physical restraint to

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

482

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

495

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

508

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

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

525

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

542

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

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

556

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

572

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

586

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

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

599

600 In extreme cases very fast germinating species have significant physical adaptations such
601 as curved or spiral embryos (Parsons, 2012). These embryos have rapid cell expansion on
602 water uptake causing them to uncoil and rupture the seed coat (Wallace et al., 1968).

603 However, more generally, they tend to have fully differentiated non-dormant embryos, small
604 to very small seeds and little endosperm with soft thin seed coats. They therefore have high
605 embryo to seed ratio, and furthermore tend to exude mucilage. Small embryos in significant
606 amounts of endosperm represent a more ancestral state where the embryo takes a long
607 time to grow and therefore germinate (Forbis et al., 2002). In contrast, Parsons et al. (2014)
608 show that species with very fast germination have evolved independently many times and
609 are mainly restricted to advanced clades. This suggests that it is a derived trait that evolved
610 as an adaptation to either arid, saline or floodplain habitats. Parsons et al. (2014) further
611 suggest that very fast germination is associated with substantial changes in seed
612 morphology, including soft thin seed coats and increased nutrient storage in the embryo
613 relative to non-embryonic tissues. This suggests interesting parallels to changes resulting
614 from selection during domestication of crop species with the requirement of rapid
615 germination following sowing. In contrast as we point out above, crop seeds are larger than
616 their wild relatives and this occurs even in species where seeds are not the harvested yield
617 component (Fenner, 1991).

618

619 ***Residual seed dormancy in crops:*** Dormancy exists on a scale that forms a continuum
620 with germination (Finch-Savage and Leubner-Metzger, 2006; Finch-Savage and Footitt,
621 2012). The depth of dormancy on this scale alters the requirements for germination and
622 thus anything that alters the conditions that enable germination are altering
623 (inducing/relieving) dormancy. As dormancy is progressively relieved, for example by low
624 temperature or afterripening (Yamauchi et al., 2004; Holdsworth et al., 2008a), seeds
625 become able to germinate in a greater range of conditions, but seeds remain dormant
626 outside of those specific conditions. This residual dormancy imposes limitations to the
627 ability to complete germination. Residual dormancy that reduces the speed, but not
628 percentage germination, can be determined in the so-called germination-resistance test,
629 which essentially has repeated germination counts used to estimate MGT (Gordon, 1971).
630 For example, during afterripening maximum percentage germination may be reached, but
631 further treatment may still increase the speed of germination as residual dormancy is
632 relieved.

633

634 In an agricultural context dormancy throughout development is essential to prevent the pre-
635 harvest sprouting that can result in very severe economic loss in many crops, in particular
636 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.

677

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

692

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

701

702 **6. Mechanisms of seed vigour: what makes a seed vigorous in an agricultural** 703 **context?**

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

715

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,

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

762

763 A link has been made between genome integrity and seed quality so that early imbibitional
764 repair has been suggested as an essential component of seed vigour required to repair
765 germination-limiting damage accumulated during seed production and storage (Elder and
766 Osborne, 1993; Powell and Mathews, 2012; Waterworth et al., 2015). At a practical level
767 Mathews and Khajeh Hosseini (2007) suggest the extent of previous deterioration and the
768 time taken to repair it can determine the length of the lag period and therefore rate of
769 germination in maize. Furthermore, ageing and repair are suggested as the overall
770 physiological basis explaining the principles behind germination and vigour tests that
771 predict subsequent seed performance (Mathews et al, 2012). Specifically Waterworth et al.
772 (2015) argue that DNA repair represents an important limitation to seed vigour with
773 potential for the development of markers for predicting or improvement of seed vigour.

774

775 A wide range of other early events in the germination process have been outlined
776 (Weitbrecht et al., 2011) that must be completed and the progression through germination
777 is thought to be controlled by a series of repressors and checkpoints (Nonogaki et al.,
778 2006; Catusse et al., 2008). The presence of these sequential steps progressing through
779 the germination process has been demonstrated by gene expression profiling of
780 germinating *Arabidopsis* seeds (Bassel et al., 2011; Dekkers et al., 2013). Progressive
781 waves of co-ordinated gene expression are observed during the germination program,
782 which are observed as peaks of co-expressed genes over a time course of germination.
783 Dynamic shifts in the translation of expressed transcripts have also been observed
784 (Galland et al., 2014).

785

786 First it is necessary to identify the key checkpoints and where within seeds they are
787 executing their function. Important to this is the proteolytic degradation of repressor
788 proteins, which act to block events leading to the completion of germination. Central to the
789 repression of seed germination are the DELLA proteins (Lee et al., 2002, Bassel et al.,
790 2004), which are degraded in response to the perception of the hormone GA (Harberd et
791 al., 2009). The growth repression activity of DELLA is therefore relieved upon GA binding
792 its' receptor GID1 and the F-box protein SLEEPY. Removal of DELLA proteins in seeds
793 leads to a de-repression of cell wall remodeling gene expression and in turn growth of the
794 embryo (Stamm et al., 2012; Cao et al., 2006).

795

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

802

803 A role for microRNAs in the targeted removal of repressive transcripts has been
804 demonstrated as a mechanism involved in the control of seed germination (Martin et al.,
805 2010). The targeted removal of transcripts, which act to repress germination represents
806 another level of targeted removal of repressors and another possible mechanism by which
807 the sequential steps of germination are regulated. A role for miR159 targeting *MYB33* and
808 *MYB101* (Reyes and Chua, 2007) and miR160 targeting *ARF10* (Liu et al., 2007) represent
809 examples of how seed behaviour is influenced by miRNAs.

810

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

816

817 In the context of seedling establishment and vigour, a rapidly establishing vigorous seed
818 will pass through these sequential steps more rapidly than a less vigorous seed (Figure
819 6c,d). As a result, it will take longer for the non-vigorous seed to reach the end of the
820 program and to complete germination. Identifying factors that control the overall
821 progression through the sequential series of checkpoints, rather than targeting the
822 individual checkpoints themselves may more effectively enhance the speed of germination.
823 A route to this may be found in understanding the mechanism underlying adaptive bet
824 hedging that results in a spread of germination times between seeds.

825

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

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

837

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

846

847 **7. Biophysics of germination and pre-emergence growth**

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

856

857 ***Turgor:*** Turgor is the internal pressure generated by the cell contents on cell walls, which
858 is the driving force of expansion. The generation of turgor by a germinating embryo cell
859 depends on the local availability of water within the heterogeneous soil matrix (see section
860 4 above). Depending on how tightly bound the water is by the soil and the overall
861 abundance of water, will determine the capacity of the seed to generate cellular turgor
862 promoting cell expansion. It has not yet been demonstrated what the solute is in
863 germinating embryos that generates cellular turgor pressure.

864

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

874 1982). Mechanisms to alter turgor in seeds and seedlings may also present an avenue to
875 enhance the generation of mechanical force and increase seed vigour.

876

877 **Cell wall modification:** The other half to the facilitation of plant growth is the weakening of
878 the mechanical properties of the cell wall (Cosgrove, 2005). This has been demonstrated to
879 occur through genetically encoded enzymes that are secreted from within cells to the cell
880 wall. This provides a link between cellular signaling and the control of plant growth through
881 changing the biophysical properties of the cell wall. Different classes of growth promoting
882 gene expression, which modify different components of the complex wall include expansin,
883 xyloglucan endotransglucosylase (XTH) (Rose et al., 2002) and some classes of pectin
884 modifying enzymes including pectin methylesterase (PME) (Peaucelle et al., 2015). A role
885 for PME inhibitor gene activity in the promotion of *Arabidopsis* seed germination has been
886 previously shown (Muller et al., 2013) demonstrating a role for cell wall modification in the
887 control of germination. Furthermore, PME activity has been demonstrated to regulate a
888 bipolar mechanical asymmetry during post-germinative hypocotyl growth (Peaucelle et al.,
889 2015), supporting a role for pectin modification in the control of seedling growth. A role for
890 ABA in the inhibition of cell wall loosening in *Brassica napus* has also been previously
891 reported (Schopfer and Plachy, 1985), indicating hormonal control of these biomechanical
892 changes. All signals controlling the germination process ultimately function through the
893 regulation of the cell wall remodelling enzymes (CWREs), which drive embryonic growth.
894 These CWREs represent the downstream targets of all upstream regulatory processes and
895 are the workhorses of the seed to seedling developmental transition. However, it remains
896 poorly understood how signaling pathways directly impact upon CWREs on a mechanistic
897 level and how these impact upon the physical properties of the embryo. These represent
898 key gaps in our understanding of seed germination and vigour.

899

900 **Cell wall and seed vigour:** Previous studies have demonstrated that the physical
901 properties of the cell wall control both seed germination and seedling establishment under
902 stress conditions in both *Arabidopsis* and tobacco (Li et al., 2011; Lü et al., 2013).
903 *Arabidopsis* plants ectopically expressing the cell wall loosening protein expansin (Lü et al.,
904 2013) have dramatically increased germination and seedling establishment under osmotic
905 stress. These observations suggest that a greater degree of cell wall loosening has the
906 capacity to confer vigour upon seedlings that have decreased turgor under osmotic stress.
907 The increased loosening of the wall may facilitate growth under osmotically limiting
908 conditions given the reduction in cellular turgor due to limited water availability. These
909 observations suggest that expansins represent downstream targets of seed quality and
910 vigour, and their high level induction can confer resistance to osmotic stress at the
911 germination and seedling establishment stages. Following seedling establishment, these
912 plants show a greater sensitivity of osmotic stress in terms of root growth and leaf

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

915

916 Seed germination and seedling establishment occur within the soil seedbed matrix, which is
917 a very different environment from the agar plate and filter paper-based assays that are
918 widely used in laboratory experiments (Section 4). Mechanical impediment by increased
919 soil strength through seedbed deterioration and at its worst, a soil crust, must be physically
920 overcome by greater force generated by the seedling. Stress experienced by plant organs
921 has been shown to result in decreased extensibility of the cell wall. The application of
922 compaction stress to growing pea roots by placing bricks on top of soil led to increased
923 stiffening of the cell wall in this organ (Croser et al., 2000). An increase of 36% in hypocotyl
924 cross-sectional area occurred in snap beans after physical impedance (Taylor and Tan
925 Broeck, 1988). In carrot and onion redistribution of seed reserves to thicken both roots and
926 hypocotyls was observed when grown under increased mechanical resistance (Whalley et
927 al., 1999). This was accompanied by reduced growth rates that were enhanced by
928 subsequent removal of the mechanical impedance. Unpublished work in our laboratory
929 indicates that seeds give off similar quantities of CO₂ per unit length of growth when rate of
930 growth is progressively reduced by mechanical impedance (Finch-Savage and Whalley
931 unpublished). Thus seed reserves are not used up faster by enhanced respiration during
932 growth under stress. However, more seed reserves would be used per unit length during
933 the thickening of the hypocotyl as observed by Whalley et al. (1999). Consequently this
934 would reduce the soil depth from which the seedling can emerge. At greater sowing depths
935 larger seeds can therefore appear to enhance vigour (see below).

936

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

953 enhance the rate of germination. A second gluconeogenic pathway that uses pyruvate, a
954 breakdown product of storage protein catabolism, has been described (Eastmond et al.,
955 2015). This provides an alternative energy source from stored seed reserves that may be
956 used to drive seedling growth.

957

958 **Cell cycle and seed vigour:** The cell cycle and endoreduplication have been strongly
959 correlated with plant cell expansion. Following the induction of germination there is a
960 discrete activation of the cell cycle from G1 concurrent with the cell expansion events that
961 drive germination and seedling establishment (Vázquez-Ramos and de la Paz Sánchez,
962 2003; Sliwinska et al., 2009). A role for the cell cycle regulatory protein KRP5 in the control
963 of endoreduplication and promotion of cell expansion has been demonstrated in the
964 embryonic axis in *Arabidopsis* (Wen et al., 2013). The *krp5* mutant shows impaired
965 germination and represents a molecular link between the cell cycle and the regulation of
966 seed germination. The TCP14 and TCP15 transcription factors have also been shown to
967 promote germination downstream of GA and cell divisions within the radicle meristem
968 (Resentini et al., 2015).

969

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

976

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

982

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

990

991 Seed size can differ greatly within a species dependent on the seed production
992 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

1033 generate force from an equivalent amount of gene expression relative to smaller cells, this
1034 suggests that greater forces driving seedling establishment come from the hypocotyl rather
1035 than the radicle. This presents the hypocotyl as the prime cellular subdomain for the
1036 manipulation of seed vigour, as manipulation of these larger cells will have a greater
1037 potential to impact on embryo and seedling growth force than within the small celled
1038 radicle.

1039

1040 **8. Strategies to improve seed vigour**

1041 The limited knowledge of the underlying mechanisms that drive seed vigour makes
1042 developing approaches to enhancing this crop trait challenging. Here we discuss
1043 approaches that have been taken, and may be taken in the future to approach this problem.

1044

1045 ***Role of the maternal environment:*** The environment in which a seed develops impacts its
1046 behaviour in subsequent generations. As we describe above seed producers carefully
1047 select production areas for their beneficial climates, however a mechanistic understanding
1048 between the maternal environment and seed quality remains to be clearly demonstrated. It
1049 has been shown that flavonoid production in the mature seed coat is influenced by
1050 maternal temperatures (MacGregor et al., 2014) and a role for the flowering time regulator
1051 *FT* is linked to this process (Chen et al., 2014). A role for seed coat pigments in the control
1052 of dormancy and germination has also been demonstrated (Debeaujon et al., 2000)
1053 providing a potential link between the maternal environment and seed quality. High
1054 permeability of the seed coat was selected for during legume domestication possibly due to
1055 seed quality properties (Sun et al., 2015). In soybean, seed coat permeability was shown to
1056 be controlled by a single transmembrane protein GmHs1-1 (Sun et al., 2015). The
1057 mechanisms by which seed coat properties influence seed behaviour remain unknown.

1058

1059 A positive correlation between the quantitative expression of the *Arabidopsis* dormancy
1060 QTL DOG1 and depth of seed dormancy has been previously demonstrated (Chiang et al.,
1061 2011; Kendall et al. 2011). The expression level of this gene is also strongly influenced by
1062 the maternal environment strengthening this correlation. The mechanistic mode of action
1063 for DOG1 remains elusive, and the link between an increased abundance of this gene or
1064 protein and the maternal control of seed dormancy remains unknown (Dekkers and
1065 Bentsink, 2015).

1066

1067 ***The impact of harvest time on seed vigour:*** In many species, including *Brassicaceae*
1068 and *Umbelliferae*, seed development is not uniform within the inflorescence and so a
1069 seedlot harvested at any single time from the mother plant can contain seeds that have
1070 different developmental stages and even seeds that have begun to deteriorate (e.g. Still
1071 and Bradford, 1998; Still, 1999; Copeland and McDonald, 2001; Bewley et al., 2013). Seed
1072 companies employ strategies to limit these problems, but they have not been eradicated.

1073 Thus individual seeds will vary in vigour and other seed characteristics resulting in
1074 heterogeneity. In wild species, seeds would be shed at different times as they mature, but
1075 may still vary since they would have experienced different environments during
1076 development. This is natural bet hedging adaptive behaviour. In many crops domestication
1077 has selected characteristics that avoid seeds being shed at different times to maximize
1078 yield. Therefore a dilemma exists in some species when to harvest plants for seeds
1079 destined to sow the next generation, rather than yield. Where plants fully senesce before
1080 harvest, the effect of different developmental stages is minimized, but this impact varies
1081 between species.

1082

1083 We have discussed above that seeds deteriorate both on and off the plant following
1084 physiological maturity (Figure 3). For this reason in commerce seeds may be harvested
1085 early before full HM and dried rapidly under controlled conditions to maintain initial quality
1086 by reducing pre-HM deterioration (Bewley et al., 2013). There is clearly a balance to be
1087 drawn since it is well documented that following disconnection from the mother plant there
1088 is a post MM developmental programme that enhances seed vigour, which is terminated by
1089 continued loss of moisture from the seed (summarized by Bewley et al., 2013). Once dried,
1090 seed performance can also be enhanced, by holding seeds in the lag phase of subsequent
1091 seed imbibition (priming) either by limiting water availability (Heydecker and Coolbear,
1092 1977) or cell wall extension (ABA: Finch-Savage and McQuistan, 1988b, 1991) to prevent
1093 germination completion. We discuss this below, however, there may be other opportunities
1094 to manipulate seed performance. Seeds could be harvested before HM, (i.e. still moist), but
1095 then held under appropriate conditions to avoid drying, potentially enhancing seed lot
1096 uniformity by allowing less developed seeds to complete their developmental program.
1097 Generally this may not be practical on a crop scale due to physical harvest damage and
1098 difficulty in extraction from the surrounding fruit structures. Nevertheless, a range of
1099 techniques to improve seeds physiologically are being developed (Halmer, 2004). We
1100 discuss below strategies to alter seed performance both before and after seed drying as a
1101 means of overcoming adaptive variation not lost during domestication. Although we do not
1102 describe them here there is also a number of physical techniques under the heading of
1103 conditioning or processing (e.g. mechanical size and density grading) that are routinely
1104 carried out by seed companies following harvest to refine the quality of bulk seed lots
1105 (Halmer, 2004).

1106

1107 ***Moist seed treatment before drying (continued seed development?): Prunus avium***
1108 (cherry) seed performance differs between individual seeds, harvest occasions, areas of
1109 production and mother trees due to differences in the extent of seed development (Finch-
1110 Savage et al., 2002). *P. avium* is a wild species that has domesticated cultivars selected for
1111 horticultural qualities in the harvested fruit. The seed is deeply dormant (Suszka, 1962)
1112 and so represents an extreme of commercial crop seed, but illustrates how variation in

1113 seed performance is generated and can be overcome by understanding the source of
1114 variation and adopting natural treatments. *P. avium* seeds mature at different times on the
1115 mother tree, but for practical reasons seeds are harvested on a single occasion and so
1116 many remain immature. The most mature seeds respond to relatively simple dormancy
1117 breaking treatments; the least mature require complex treatments lasting up to 26 weeks
1118 that have repeated warm and cold periods to mimic repeated winter and summer seasons
1119 (Suszka et al., 1996).

1120

1121 Finch-Savage et al. (2002) show that if freshly harvested seeds are cleaned (fruit
1122 removed), prevented from drying by storing in moist sand which allows aeration, and held
1123 at 15°C (neutral, neither dormancy inducing or relieving) for up to 8 weeks the performance
1124 of individuals in and between the seed lots progressively becomes more homogeneous and
1125 responds to simple treatments to break dormancy. Thus seed development appears to
1126 continue and all seeds acquire the characteristics of fully mature seeds. However, further
1127 detailed work is required to confirm there is no other explanation for the seed improvement.
1128 The benefit is retained upon drying. The extent to which this is possible with optimization in
1129 any other species is not known, but there is little logic in allowing seeds to dry and
1130 potentially age before physiological treatment (e.g. priming).

1131

1132 ***Moist seed treatment after drying (imbibition, hardening, priming: continued***
1133 ***physiological advancement?)***: Several methods have been developed to improve seed
1134 performance physiologically by manipulating imbibition (Halmer, 2004). These include
1135 short-term imbibition allowing repair as a result of ageing (Walters, 1998; Powell et al.,
1136 2000); the process of seed hardening, which involved repeatedly wetting and drying seed
1137 (Hegarty, 1978), and several approaches under the banner of seed priming (Heydecker
1138 and Coolbear, 1977).

1139

1140 Seed priming techniques limit the availability of water to the seed so there is sufficient to
1141 progress metabolism, but insufficient for radicle extension and completion of germination;
1142 seeds therefore remain desiccation tolerant. Water can be limited by placing seeds on
1143 absorbent paper soaked in an osmoticum of appropriate strength (osmotic priming;
1144 Heydecker et al., 1975); or in aerated osmoticum (Bujalski and Nienow, 1991); or seeds
1145 separated from the osmoticum by dialysis membrane (membrane priming; Rowse et al.,
1146 2001). Water can also be limited by mixing it with various solids to create an appropriate
1147 matrix potential (solid matrix priming; Taylor et al., 1988). Water may be applied in limited
1148 quantity in a rotating drum (drum priming; Rowse and McKee, 1999) or by other means
1149 (Halmer, 2004). Alternatively, germination completion may be inhibited by placing seeds in
1150 an ABA solution, followed by washing to remove the inhibitor (ABA priming; Finch-Savage
1151 and McQuistan, 1988b, 1991). In all cases seeds are then dried and stored or sown.

1152

1153 Seeds after all these treatments are metabolically advanced leading to a shortened lag
1154 phase on re-imbibition and thus more rapid and uniform germination. The mechanism is not
1155 fully understood, but may result partly from repair (Powell et al., 2000) and interestingly
1156 priming can result in an increase in cell wall elasticity (Karssen et al., 1989) suggesting that
1157 cell wall remodifying gene expression is induced under this treatment. It has been
1158 questioned whether priming this improves vigour *per se*, or whether it merely advances the
1159 stage of germination of the seed. Conceptually it seems plausible that this protracted
1160 process takes both fast and slow germinating seeds to an advanced checkpoint in the
1161 germination process such that their subsequent germination requires only minor additional
1162 advances before germination is completed (Figure 6).

1163

1164 Priming treatments are typically carried out upon dry “mature” seed usually of high-value
1165 vegetable and flower species. It is interesting to note that seeds of species that benefit
1166 most from these treatments tend to be those with indeterminate floral development and
1167 thus heterogeneous seed development, often containing under-developed seeds such as
1168 those in the *Umbelliferae* and many ornamental species. In commerce, poor quality aged
1169 seeds do not respond as well to seed priming as do higher quality fresh seeds, and thus it
1170 is not generally economic to use the technique to reverse the deterioration of ageing, rather
1171 overcome the problems of heterogeneous development.

1172

1173 Most experimental results present laboratory germination and there are relatively few fully
1174 replicated field experiments, which are needed to properly evaluate the efficacy of seed-
1175 enhancing treatments. In one such experiment, the seedling emergence performance of
1176 primed and unprimed carrot seeds were compared in 37 seedbed environments (Finch-
1177 Savage, 1990). Priming seeds reduced the variation in percentage seedling emergence
1178 (Figure 7; primed 75%, SD 11; untreated 65%, SD 15) and so by the definitions quoted
1179 above can be considered to have improved vigour. However, the data was analysed on
1180 thermal time scales to determine predictability of timing across environments. Thermal time
1181 to emergence in non-stressed conditions was subtracted in all environments to indicate the
1182 relative effect of the seedbed. Although the results showed mean seedling emergence was
1183 earlier from primed (210 °Cd) than untreated seeds (244 °Cd) the timing of seedling
1184 emergence across environments (standard deviation (SD) of the means; 81.8 and 63.2
1185 respectively) was not significantly different and so the result was no more predictable.
1186 Much of the variation in both cases across environments was due to the timing of water
1187 availability in the seedbed, and not germination *per se*.

1188

1189 Priming treatments can also be used to overcome specific dormancy issues. Examples of
1190 this include advancing the stage of germination in seeds to a point beyond when they are
1191 sensitive to thermoinhibition, a particularly important issue in lettuce, by exposing them to

1192 light beyond the point of sensitivity, and by enabling immature embryos to complete their
1193 development, which happens in seeds such as celery (Halmer, 2004).

1194

1195 Another issue in seed quality is that of imbibitional damage (Powell, 2006). This occurs
1196 when the water potential of embryo cells is very low and upon imbibition in pure water, cells
1197 lyse due to the rapid generation of high pressure, and is typically associated with planting in
1198 low temperature soils (Bennett and Waters, 1987). Controlled hydration rates can help
1199 alleviate this problem (Taylor et al., 1992). For example, seed priming at low osmotic
1200 potential can minimize the effects of imbibitional damage by giving cells the opportunity to
1201 adjust their osmolytes accordingly.

1202

1203 In order to develop novel approaches to prime seeds, we must first understand the
1204 molecular nature of the sequential steps of germination, and how arrest at various stages
1205 relates to the overall developmental program. Once this is understood, novel opportunities
1206 to manipulate this system will emerge by targeting checkpoints or the processes that
1207 control the overall rate of progression through these checkpoints. In the context of priming,
1208 arresting the germination program at the latest possible checkpoint would be advantageous
1209 in order to have the most rapid completion of germination following subsequent imbibition.

1210

1211 ***Pre-germinated seed treatments:*** A further opportunity to enhance seed performance
1212 exists where seeds are allowed to progress beyond emergence of the radicle to sow moist
1213 (Fluid Drilling; Gray, 1981), or to dry before desiccation tolerance is lost (Finch-Savage,
1214 1988; Finch-Savage and McKee, 1988); or when desiccation tolerance is re-introduced
1215 (Finch-Savage, 1989; Bruggink and van der Toorn, 1995, 1997). Although the basis of such
1216 commercial treatments and products has been developed, to date practical issues have
1217 limited use in commerce. The key advantage in this approach is that seeds can be selected
1218 from a seed lot as germinated (100% viable) or the first to germinate (most vigorous).
1219 Indeed there is evidence that first germinators from seed lots of different quality have
1220 similar high vigour (Finch-Savage, 1986; Finch-Savage and McQuistan, 1988a).

1221

1222 The concept of synthetic seeds allowing the direct genetic manipulation of the embryo
1223 through somatic embryogenesis offers potential for the production of superior performing
1224 seeds (Redenbaugh 1993), but the technology is not currently sufficiently developed or
1225 economic.

1226

1227 ***Conventional breeding:*** In the pursuit of vigour we seek to push plants to perform in ways
1228 they would not naturally, and to adopt a more agronomic behaviour than that they have
1229 adapted to. A diversity of genetic backgrounds may have the capacity to be vigorous, and it
1230 becomes a question of unlocking this genetic potential and realizing the full capacity of

1231 plant performance in the field. One approach is that of Quantitative Trait Loci (QTL)
1232 analysis we discuss further below (section 9)

1233

1234 Altering the timing of dormancy loss following physiological maturity and harvest is an
1235 active area to reduce the problems of residual dormancy while preventing pre-harvest
1236 sprouting in grain crops. It is suggested that studies linking genetics with our physiological
1237 understanding appear to be most promising, for example genes controlling sensitivity to
1238 ABA and GA may be radically altered by desiccation marking the end of seed development
1239 (Benech-Arnold, 2004) as has been shown previously in tomato (Kermode and Bewley,
1240 1985).

1241

1242 There are examples of specific potential genetic improvements through breeding that affect
1243 seed performance, for example hard-seediness in beans, or mechanical damage in navy
1244 beans (Copeland and McDonald, 2001). Other modification to seed coats that alter the rate
1245 of water uptake, and minimize the negative impact of imbibitional damage on seed vigour
1246 may be possible (Powell, 2006). Furthermore, the advantage of hybrid vigour in crops
1247 where hybrid production is practiced like maize can extend to the quality of the seeds
1248 produced (Copeland and McDonald, 2001). The extent of quality improvement is influenced
1249 by the method of crossing, but the enhanced quality can lead to improved stands under
1250 adverse conditions following sowing.

1251

1252 Increasing understanding of the regulation of germination and pre-emergence seedling
1253 growth may facilitate more targeted selections and screens in breeding programs for
1254 specific attributes and vigorous phenotypes. This is particularly true for our understanding
1255 of the cellular basis of vigour and where within embryos this trait is conferred.

1256

1257 **Genetic manipulation:** Transgenic approaches may also be taken to improve seed vigour.
1258 Targeted alteration of a positive ABA signalling factor, or negatively acting GA signalling
1259 factor results in the more rapid germination of seeds and enables germination to occur
1260 within stress conditions. While these manipulations conform to the definition of increased
1261 seed vigour, there is also a loss in stress tolerance within these seedlings (Achard et al.,
1262 2006). This appears to be a common trend when targeting loci that are central to
1263 fundamental signalling pathways in plants. “Green revolution” alleles in wheat DELLA
1264 proteins were capable of enhancing yield, while a cost to seed vigour at deeper planting
1265 depths has recently been reported (Amram et al. 2015).

1266

1267 Systems-based approaches to understanding the interactions between these key loci and
1268 their downstream targets may provide a means for the rational manipulation of the system
1269 in an advantageous fashion. More sophisticated and subtle approaches to manipulating
1270 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

1311 about seed vigour, its impact on crop production, and the various approaches that could be
1312 taken to improve it. Further research and novel approaches into understanding this
1313 enigmatic and complex trait are needed and will help to ensure a more reliable food supply
1314 into the future.

1315

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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.