Seed Vigour and Invigoration

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Abstract

Seed vigour is an important aspect of seed quality. It is a quantitative trait which is responsible for overall seed performance in terms of rate and uniformity of seed germination, seedling growth, emergence ability under unfavourable environments and post storage performance. Seed vigour is controlled by genetic factors, initial seed quality, production environments, harvesting and storage conditions. Seed vigour tests provide a more sensitive index of seed performance per se than the germination test. Efforts have been focused on developing novel or improving existing methods of vigour estimation in different crops. The vigour tests are tools routinely used for in-house seed quality control programs, especially for field and vegetable crops. Some treatments can improve seed vigour, although the treatment effects are more evident under sub-optimum than optimum growing conditions. This chapter deals with different aspects of seed vigour and its effects on plant growth and discusses physiological and biochemical parameters to understand underlying mechanisms.

Keywords

Crop production · Seed quality · Seed vigour · Seed vigour test · Seed invigouration

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1 Introduction

Quality-assured seed is essential for sustainable and profitable crop production in both agricultural and horticultural crops. Seed quality is determined by its genetic and physical purity, health status and physiological quality. Among the physiological quality parameters, seed vigour is an important and complex trait which is determined by interaction of genetic and environmental factors (Finch-Savage and Bassel 2016). Unlike germination and viability, seed vigour is not a single measurable parameter but a quantitative attribute, which is controlled by several factors associated with overall seed performance that includes rate and uniformity of seed germination, seedling growth; emergence under unfavourable environmental conditions; and performance after storage. The high seed vigour status of a lot ensures a more rapid and uniform crop establishment and growth across diverse environmental conditions. The vigour differences among seed lots may be due to variability in genotype, environment, maturity at harvest, mechanical integrity, seed treatments and seed ageing. Under optimum conditions, seeds from different sources may germinate at comparable rates. However, under stressful conditions in the field, the seeds can exhibit variable performance due to differential vigour status. High vigour seed lots may store well for longer duration without evident loss in their germination capacity in comparison to the low vigour seed lots, which deteriorate faster under similar conditions. Freshly primed seeds, for instance, can emerge rapidly and uniform compared with the original non-primed seeds, but the shelf life of primed seeds is most often reduced. As a consequence, the primed seeds have a high vigour for germination speed, but often a lower vigour with respect to seed storage. Another example is the physical sanitation of seeds, aimed at the removal of seed-borne pathogens, but which may also result in damage to the seed which needs repair upon rewetting of the seeds.

1.1 Definitions of Seed Vigour

As early as in 1876, Friedrich Nobbe described vigour as 'Triebkraft' a German word meaning 'the driving force'. Isely (1957) defined it as 'the sum total of all seed attributes which favour stand establishment under unfavourable field conditions'. Seeds which perform well are termed as 'high vigour' seeds. A later definition of seed vigour is 'the sum total those properties of seed that determine the potential level of activity and performance of the seed during germination and seedling emergence' (Perry 1978). Here we define seed vigour as 'the sum of seed properties that determine the ability of viable seeds to germinate fast and uniform, and to produce healthy seedlings with rapid and uniform emergence under both optimal and suboptimal environmental conditions', combining the definitions formed by the Association of Official Seed Analysts (AOSA) and International Seed Testing Association (ISTA) (AOSA 1983; ISTA 2021).

2 Factors Affecting Seed Vigour

Modern agriculture emphasizes on technology and high inputs for obtaining high yield. Successful seedling establishment is the first critical step towards successful crop production and efficient use of all inputs. Plant stand establishment and performance are strongly influenced by seed vigour, therefore seed vigour has an indirect effect on yield (Tekrony and Egli 1997; Finch-Savage 1995). Low vigour seed lots provide seedlings with a lower leaf area index, dry matter accumulation and crop growth rate. Seed vigour is primarily influenced by genetic and environmental factors, including seed treatments. A major cause of the loss in vigour is attributed to deterioration during development, harvesting, drying and storage which commences when the seed becomes physiologically mature and continues during storage. In direct seeded crops, there is a relationship between the number of plants established per unit area and total yield. Efforts on inputs, agronomic practices and technological interventions in post establishment stages are not able to compensate for the initial setback in plant stand due to low vigour. This impact is higher in non-tillering crops or where gap filling is not feasible. Also in the transplanted crops, low seed vigour affects the plant stand and population but to a lesser extent due to wastage of seed/ planting material, increased labour costs and reduced product quality due to low vigour. The impact of variation in seed vigour on both total and marketable yield varies among species and depends on the specific production practices and stage of crop harvest. The effect of vigour is highest in crops where the economic produce is harvested already at late seedling (micro greens and herbs) or vegetative stage (leafy vegetables and salad greens), followed by those crops which are harvested at fruiting (okra, beans, cucumber) and seed maturity (corn, cereals and pulses). Various factors have an impact on seed vigour.

2.1 Acquisition of Seed Vigour and Seed Maturity

In general, seed vigour increases during seed maturation and is at its peak at the time of natural shedding, which is later than what agronomists call physiological maturity defined as the stage where maximum dry weight is achieved at the end of seed filling. During the phase of late seed maturation (LSM), also called maturation drying, the developing seeds switch their metabolic activity from the production of storage reserves (oil, starch and proteins), to the synthesis of protection mechanisms (which including production of late embryogenesis abundant proteins (LEAs), heat shock proteins (HSPs), sugars for a glassy cytoplasm, and a change in the nuclear DNA confirmation) and degradation of chlorophyll. During this maturation drying phase, there is a gradual increase in seed vigour. Seed maturity at harvesting plays a key role in the seed vigour status. Shaheb et al. (2015) reported that seed quality parameters, such as seed germination and vigour indices, were significantly influenced by harvesting time. Seed development is not uniform throughout the inflorescence in Malvaceae, Brassicaceae and Umbelliferae species, therefore a seed lot harvested at any one time from the mother plant contains seeds at various stages of development (Bewley et al. 2013). Cabbage seed quality and maturity have been assessed by measuring the amount of chlorophyll fluorescence (CF) from intact seeds. Seeds with the lowest amount of CF gave the highest percentage of germination and normal seedlings (Jalink et al. 1998). An improvement in seed quality of soybean seeds was observed by removing green seeds using the CF sorting technique (Cicero et al. 2009).

Although in nature, seeds are most often dispersed from the mother plant at maturity, during domestication of our agricultural crops there has been a selection for genotypes that do not shed the seeds. When crop seeds remain on the mother plant after reaching full maturity, they can start deteriorating due to high temperatures, humidity or UV light, resulting in vigour reduction. Rain prior to harvesting can induce germination in the seeds when they are not dormant. To enable initiation of metabolic activity during germination, part of the protection is removed, which may result in reduced longevity of the seeds, or ultimately loss of desiccation tolerance when preharvest sprouting occurs.

2.2 Environment

Seed lots may differ substantially in their vigour status depending on the environment during seed development, harvesting and post harvest handling. Seed development under environmental stress (drought, frost, low/high temperatures, pest incidence) is reported to have lower vigour than those produced under congenial conditions. Fast drying during harvesting and processing can cause damage and cracking when the cells in the outer layers shrink much faster compared with those more inside. With grain legumes such as peas and beans, this will result in an increase in the proportion of abnormal seedlings. Elevated temperature during early seed development can result in decrease in seed size, number and fertility, and reduction in seed vigour in cereals, legumes and vegetable crops. Hence specific geographic locations with favourable climatic conditions are selected for commercial seed production. Variability in ambient temperature has been reported to increase seed dormancy and reduce germination rates in crops which require lower temperature for germination and seedling establishment (Reed et al. 2022).

2.3 Seed Size

Seed size can affect the rate of germination or emergence, total germination and seedling growth. In wheat, seed size is positively correlated with seed vigour wherein larger seeds tend to produce more vigourous seedlings (Cookson et al. 2001; Muhsin et al. 2021). Small muskmelon seeds had the lowest germination, emergence and seedling growth showing the association of seed physical parameters with vigour (Nerson 2002). With increased seed size, higher germination and emergence was observed in *Triticale* (Kaydan and Yagmur 2008) and maize (Mir 2010). Bolder seeds were capable of emerging from greater planting depth and exhibited enhanced ability to penetrate ground cover, weeds and surface hindrances in both wild and cultivated types of different species (Fenner 1991; Massimi 2018). In chick pea, large-sized seeds recorded maximum germination percentage, seedling vigour and protein content, dehydrogenase content, alpha-amylase activity (Anuradha et al. 2009). Large seeds also performed better under salinity stress (Mehmet et al. 2011). Plants grown from large seeds were vigourous and produced higher dry matter as compared to those grown from smaller seeds in groundnut (Steiner et al. 2019). The generally higher vigour of larger seeds may relate to their maturity status at harvesting, immature seeds being smaller. With spinach, however, larger seeds with a thicker pericarp result in slow and lesser germination under moist conditions and warmer temperatures, because of poor oxygen diffusion through a thicker pericarp (Magnée et al. 2020). However, smaller spinach seeds with higher chlorophyll levels also performed poorer compared to more mature low chlorophyll seeds from the same size. So, the seed performance is based on parameters more than just size. Hence, it is difficult to establish a consistent association between the seed size and vigour, the relationship being more species-specific and dependent on growing conditions.

2.4 Seed Reserves

Early seed vigour trait in some crops could be related to higher reserve utilization efficiency and mobilization. Low vigour seeds are sensitive to stress and their lower tolerance was associated with reduced lipid and protein content and increased amino acids, carbohydrates and phosphorus compounds in the embryo (Andrade et al. 2020). Among maize genotypes, sweet corn seeds have poor seed germination and vigour due to enhanced solute leakage, and insufficient energy supply during seed germination attributed to lower starch and higher sugar content (Styer and Cantliffe 1983).

2.5 Positional Effect

The position of the fruit can influence seed vigour both directly and indirectly through its relationship with variation in the progression of seed development. Seeds from the lower regions of the canopies, main branches and primary tillers can be more vigourous than those from the intermediate and upper plant positions, secondary or tertiary tillers, which again may relate to difference in seed maturity. With Pinto bean (*Phaseolus vulgaris*) the largest seeds with higher vigour were obtained from the lower canopy (Ghassemi-Golezani et al. 2011). Contrasting observations were made with okra, where fruits obtained from middle nodes produced higher vigour seeds (Hedau et al. 2010). Seeds of pumpkin in middle and stylar segments had better seed vigour as compared to peduncular segment since the ovules at stylar and middle segments were first to get fertilized by high vigour pollen and have a temporal advantage in competing for resources from the mother plant

(Kumar et al. 2015). In cucumber, seeds from the top and peduncular fruit segments are delayed in reaching maximum quality compared with seeds from middle position, and this was correlated with a slower decline of chlorophyll fluorescence (Jing et al. 2000).

2.6 Seed Coat and Imbibition Damage

The outer cover or testa of a seed acts as a modulator between the internal seed structure and the environment, and is an important factor for vigour and field emergence, either by maintaining the integrity of internal seed components, protecting the embryo, allowing gas exchange between embryo and environment, and regulating the imbibition process. Imbibition damage has been shown to affect vigour in a range of temperate and tropical grain legumes (Powell 1985), but it may also occur in small seeded crops. Imbibition damage is caused by the rapid entry of water into the cotyledons during imbibition, which can result in disruption and disorganization of cell membranes, and leakage of sugars, amino acids and ions from the seeds. Especially under low temperature conditions the membrane is less flexible and more prone to disruption and imbibitional injury. The induction of imbibition damage is greater when seeds are very dry, creating a large difference in water potential upon imbibition and a strong flow of water through the membranes. Storing seeds overnight at a high humidity can reduce this problem. Fast drying of high moisture seeds can cause seed coat cracks that can increase the incidence of imbibition damage (Oliveira et al. 1984). The seed lots with extensive testa damage imbibed quickly with an induction of imbibition damage. These lots showed low vigour and emerged poorly in the field. Seed lots with little testa damage, on the other hand, imbibed slowly and exhibited lower imbibition damage, thus highlighting the importance of testa integrity in determining the vigour status in bold seeded legume. Measurement of electric conductance of seed leachate can be effective in identifying vigour differences among seed lots of such species. Equilibrating seeds to an acceptable range of moisture content is therefore suggested before performing EC test for seed vigour. Closure of hilar opening under conditions of excessive drying is a means of natural protection against imbibition damage, but this may significantly increase the proportion of so-called hard seeds, resulting in poor germination and field emergence.

Maternal inheritance also plays a role, as in the development of seed coat pigments and control of dormancy (Debeaujon et al. 2000). Sun et al. (2015) reported selection for a high permeability trait of the seed coat related to seed vigour during legume domestication.

2.7 Seed Ageing and Storage

Orthodox seeds deteriorate and age during storage under conditions of warm temperature, high seed moisture content and oxygen, resulting in a vigour decrease over time. Weathering of the seeds while still on the mother plant influences ageing to alter their vigour before harvest. Seed ageing or deterioration is the major cause of differences in seed vigour due to the accumulation of deleterious changes within the seed and ultimately the ability to germinate is lost. Reduced vigour of aged seeds can be linked to biophysical, biochemical and physiological changes associated with ageing (Bewley et al. 2013). During ageing under dry conditions, damage is induced by oxidation of DNA, RNA, proteins and membranes. DNA oxidation can result in strand breaks that need to be repaired before DNA replication. Limited DNA damage results in retardation of germination, while high levels of DNA damage could result in failure of germination or poor seedling quality. Oxidation of membrane lipids increases the risk of membrane leakage, hence aged seeds often show higher levels of electrical conductivity. Damage to the mitochondrial membranes, induced by oxidation, restricts the onset of aerobic respiration and thereby reduced seed vigour (Bewley et al. 2013). Vitamin E is the most important lipophilic anti-oxidant that protects the membranes. Seeds from Arabidopsis mutants deficient in vitamin E synthesis showed a relative very short shelf life (Sattler et al. 2004). Under the stressed storage environment (e.g., high temperature or relative humidity in uncontrolled storage) high vigour seed lots tolerate stress and lose vigour at a slower pace as compared to their low vigour counterparts. For an increasing number of crops, genetic variation in ageing tolerance has been observed under either dry or humid storage conditions. Sharma (2018) reported differential storage pattern in maize genotypes under ambient and controlled storage conditions wherein sweet corn and high protein maize showed greater decline in seed vigour and poor storability among genotypes compared.

2.8 Seed Processing

Seed processing is important in upgrading seed quality before use. However, processing seeds at high or very low moisture content may result in mechanical injuries on the seed coat and decline of seed vigour. Sweet corn seeds, for instance, are rather sensitive to mechanical damage by deshelling, especially after harvesting at relatively high seed moisture levels. During seed drying, moisture is removed initially from internal tissues of seed to seed surface and later from the seed surface into the atmosphere. High temperature or fast drying of seeds with high moisture content results in development of cracks on the surface, due to tissue shrinkage, which affects the seed vigour. Thus, selection of processing equipment, its slope and speed settings and seed moisture content during seed processing are important factors affecting seed quality.

2.9 Physical Sanitation Treatment

Some pathogens move with the seeds to the next generation interfering with the health of the new seedling. Sanitation treatments are performed to prevent seedling diseases. With increasing restrictions on the use of chemical disinfection, physical sanitation methods and the use of natural components are becoming more popular. While the aim of these treatments is to kill the pathogen, they may often have a deteriorating effect on seed vigour. Seed technologists have to balance between a treatment harsh enough to irradicate the pathogen or its pathogenicity, while mild enough to maintain seed vigour. However, the initial seed vigour also influences the sensitivity of the seeds to sanitation treatments. Less mature *Brassica oleracea* or *Daucus carota* seeds are more sensitive to hot water or aerated steam treatments compared with mature seeds (Groot et al. 2006). During seed priming part of the protection, that was induced during seed maturation, is removed to enable initiation of metabolic activity. Likely for that reason primed seeds are more sensitive to these physical sanitation treatments (Groot et al. 2008).

2.10 Genetic Variation

Seed vigour is a complex quantitative trait affected by multiple factors. With the use of high-throughput sequencing and genomic techniques quantitative trait loci (QTL) related to seed vigour have been identified in different crops (Reed et al. 2022). QTL analysis for seed vigour-related traits has been studied in different crops like *Arabidopsis*, rice, maize, *Brassica* sp. and lettuce. Li et al. (2017) and Shi et al. (2020) identified 19 and 26 QTLs respectively related to seed vigour in rice and wheat. Liu et al. (2011) identified QTLs for seed vigour-related traits which could provide information on early seedling vigour of maize. The genetic information on QTLs related to early vigour in different crops could be explored for developing breeding lines/genetic stocks with early vigour traits which could be utilized for developing high vigour lines through breeding. Morris et al. (2016) identified two genes related to seed vigour in *Brassica oleracea*. Alleles from the BolCVIG1 gene gave different splicing variants that coincided with variation in abscisic acid sensitivity, while the second gene BoLCVIG2 was a homologue of an Arabidopsis alternative-splicing regulator gene (AtPTB1).

3 Seed Vigour Assessment

Seed vigour is accepted as an important seed quality component, but official vigour testing is carried out for only a few crops. Numerous tests that have been attempted in different crops are not accepted universally due to variability in results. Still, development of reliable vigour tests for specific crop species for determining their planting value is desirable (Lopes et al. 2012; Marcos-Filho 2015; Reed et al. 2022).

Seed vigour tests aim to provide a more sensitive index of seed performance per se than the germination test. Efforts have been focused on developing novel methods or improving the existing methods of vigour estimation in different crops, by measuring the rate and uniformity of seed germination and seedling growth, emergence ability of seeds under adverse environmental conditions and performance after storage. Such vigour tests are routinely used for in-house seed quality control programmes, especially for field and vegetable crops.

While researchers have applied a large number of vigour tests in different crop species which were found useful for the assessment of seed vigour, there is lack of consistency and reproducibility of tests across species. However, the most commonly applied tests can be grouped based on physical attributes; germination and seedling growth; performance during or after subjecting to stress conditions; and physiological and biochemical parameters.

- **Physical tests:** based on seed density; seed size; seed colour; embryo size and image.
- **Performance tests:** based on measurable parameters of germination, such as rate of germination; radicle emergence; and vigour indices (VI) based on seedling growth (weight or length) and germination.
- **Stress tests:** based on seed germination after subjecting the seed to a defined condition of stress, such as low temperature; high temperature combined with high humidity or seed moisture; or a combination of multiple stresses.
- **Physiological and biochemical parameters**: based on the permeability of cellular membranes; changes in respiratory functions; key respiratory enzymes.

However, only some of these are used for official purposes as have been recommended by the ISTA and/or AOSA.

ISTA having a vision of 'uniformity in seed quality evaluation worldwide' provides a framework within which quality may be evaluated and compared by vigour tests. The ISTA seed vigour committee evaluates the performance of seed vigour test methods by the reproducibility of vigour method and the relationship between vigour test results and seedling emergence in the field.

The following vigour tests have been validated and recommended by ISTA for seed vigour estimation (for details please see ISTA Rules 2021):

- Conductivity test: *Cicer arietinum*, *Glycine max*, *Phaseolus vulgaris*, *Pisum sativum* (garden peas only, excluding petit pois varieties) and *Raphanus sativus*
- Accelerated ageing test: *Glycine max*
- Controlled deterioration test: *Brassica* spp.
- Radicle emergence test: *Zea mays*, *Brassica napus* (oilseed rape, Argentine canola), *Raphanus sativus*, *Triticum aestivum* L. subsp. *aestivum*
- Tetrazolium vigour test: *Glycine max*

AOSA also recommends most of the above-mentioned tests.

3.1 Seed Vigour Tests

3.1.1 Seed Size/Density

Seed vigour estimation may simply be based on seed size, and seed density. In general, the larger seeds with higher density have higher seed vigour than smaller and lighter seeds.

3.1.2 Performance-Based Tests

Rapid germination is an important component of the seed vigour concept since it usually corresponds to rapid seedling growth and emergence in the field. Tests based on seedling growth parameters are simple to perform and usually do not require special equipment besides those used for germination. Such vigour tests can be based on seedling performance including first count of the germination test, rate of germination or seedling emergence, mean germination time, seedling growth (length or dry weight), seedling vigour indices, and more recently the emergence rate of the primary root (radicle emergence).

1. **Radicle emergence test:** The slow rate of germination is an early physiological expression of seed ageing, the major cause of reduced vigour. The standard germination tests evaluate radicle emergence with an early and late count of normal seedlings at a fixed period after imbibition. The duration of the test can be long and may for some species extend beyond 1 month. The radicle emergence test is relatively rapid with an early count of emergence of radicle (2 mm) for vigour estimation. It is an accepted vigour test for maize (*Zea mays* L.), aubergine (brinjal), oilseed rape and radish (ISTA 2017). Differences in radicle emergence rates have been attributed to the length of the delay from the start of imbibition to radicle emergence (Matthews and Khajeh-Hosseini 2007), which is dependent upon the time required for damage repair before radicle emergence. Fast radicle emergence is indicative of high vigour.

3.1.3 Stress Tests

The following tests are stress tests which impose stress conditions on the seed and based on the performance of the seeds under stress conditions the quality of seed lot in terms of vigour is measured.

1. **Cool test:** The test is limited to measuring the effect of cool temperature (18 °C) on the germination of cotton seed and the growth rate of cotton seedlings (Hampton and TeKrony 1995). Since the seeds are subjected to germination at a temperature below its optimum of 25 °C, their ability to produce normal seedlings and speed of germination are taken as indication of vigour. Though not a recommended test, it may provide useful indication of vigour in some cases.
2. **Cold test:** Cold test estimates the ability of the seed to withstand the low temperature stress when the soil is humid and cold which hampers the growth of weak seedlings experienced during early spring planting. This test is commonly used for assessing seed vigour in maize (Caseiro and Marcos-Filho 2000).

The seeds are sown in soil collected from maize field (to simulate field conditions and microbial load) and exposed to low temperature (10 °C) for 7 days followed by assessment of seed germination. The seed lots exhibiting high germination after cold test are reported to be vigourous. This test was reported both by ISTA (Hampton and TeKrony 1995) and AOSA (1983), but due to difficulty in standardization of test procedure, this is not recommended officially, though used by seed companies for internal quality control in the maize growing belts of North America and Europe (Matthews and Powell 2009).

3. **Hiltner (Brick-gravel) test:** Perhaps the oldest test and developed by Hiltner in Germany in 1917. Weak seedlings are not able to generate enough force to overcome the mechanical stress imposed by the pressure of brick gravels. The higher the number of seedlings emerged through the brick gravel layer, the greater is the vigour of the seed lot. This method was suitable to study the vigour levels of cereal seeds. A paper piercing test (Fritz 1965) is in principle similar to the Hiltner brick gravel test. In this test the germination is measured in sand covered by a layer of moist paper, the vigourous seed lots emerge by piercing the paper.
4. **Accelerated aging test:** The accelerated ageing (AA) test is the most commonly used vigour test. The test was developed by Delouche and Baskin (1973) to assess the storage potential of a number of species, and recommended as a vigour test by McDonald and Phannendranath (1978). Seeds are exposed to high temperature (40–45 °C) and humidity (90–100% RH) for a specified time which causes metabolic inactivation by deterioration, especially in low vigour seeds. The lots showing higher germination percentage after AA have higher vigour as compared to the lots with lower vigour post AA test. ISTA has validated this vigour test for *Glycine max* only (ISTA 2015).
5. **Controlled deterioration test:** Controlled deterioration test (CD test) was developed by Matthews (1980) and predicts field planting potential of seed. This test is quite similar to the AA test, in subjecting seeds to rapid ageing at high m.c. and high temperature conditions. However, while in AA test the seed m.c. increases gradually from the start of test, in CD the seeds are first equilibrated to a high m.c. and then subjected to high temperature stress. In *Brassica* species seeds are brought to 20% moisture and then exposed to 45 °C for 24 h. The high vigour seeds retain high germination after deterioration. ISTA has validated this test (ISTA 2015). Comparable CD tests have been published for other crops, with some modifications in seed moisture content, temperature and duration, in which a positive relationship has been observed between the CD test tolerance and the field emergence for several crops (Kazim and Ibrahim 2005). The AA and CD test shows a poor correlation with storability under dry conditions, due to the difference in the physiological status of the seeds under humid conditions, with a liquid cytoplasm in contrast to a glassy cytoplasm under dry storage conditions. However, it may show a reasonable correlation with storage under humid tropical conditions at comparable relative humidity levels.

3.1.4 Physiological and Biochemical Tests

The physiological and biochemical tests measure the physio-chemical changes that occur during ageing providing indirect vigour estimation.

Tests based on respiratory parameters

1. **GADA test:** The glutamic acid decarboxylase activity (GADA) test was developed by Grabe (1964). GADA is a key respiratory enzyme during germination. Hence, the activity of this enzyme helps in the estimation of vigour.
2. **Tetrazolium vigour test:** Tetrazolium test is primarily used as a quick viability test. But Kittock and Law (1968) used it for the vigour estimation of seeds based on the colour intensity of stained embryos or cotyledons. The colourless 2,3,5-triphenyl tetrazolium chloride can pass intact membranes and turns into red-coloured formazan in living cells catalysed by dehydrogenase enzymes involved in aerobic respiration. In seeds the metabolically active cells with intact membranes stain pink or red. ISTA has validated the Tetrazolium test for the assessment of seed viability of a large number of crops (Leist et al. 2003) and for *Glycine max* as test for seed vigour (ISTA 2015).
3. **Respiration (RQ) test:** During the process of respiration, oxygen is taken up by seeds and carbon dioxide is released. The ratio of the volume of carbon dioxide evolved to the volume of oxygen consumed per unit time is called respiratory quotient (RQ). The RQ was found to be more often related to the vigour than oxygen uptake alone. This test was used for vigour estimation in maize (Woodstock 1988).
4. **Respiratory activity test:** The respiratory activity test measures the amount of carbon dioxide released from seeds during the respiration process, which results from the oxidation of organic substances in the cellular system after the start of the imbibition process. Thus, high respiratory rates are associated with vigourous seeds due to the oxidation of a large amount of reserve tissues present in their cells. This test is useful in vigour estimation of soybean (Dode et al. 2013) and okra (De Sousa et al. 2018). In GADA test, vigour assessment is based on estimation of glutamic acid decarboxylase enzyme activity whereas in this test the seed vigour is proportional to the respiration rate of the seeds, higher the respiration rate, higher the carbon dioxide release and thus the vigour.
5. **Conductivity test:** Conductivity test developed by Matthews and Bradnock (1967) evaluates the integrity of cell membranes and their ability to repair them during the ‘soak period’. The test is based on the hypothesis that biomembranes undergo disintegration during seed ageing, rendering low vigour seeds with poor membrane integrity, and hence causing release of more solutes to the steep water, increasing the electrical conductivity of the solution. Seeds are soaked in de-ionized water for a stipulated time to allow seed leachates to come out in the soak water. The conductivity of steep water is inversely proportional to the seed vigour. Woodstock (1988) reported that the rate of electrolyte leakage in seeds was negatively correlated with membrane intactness. The extent of solute leakage during seed imbibition has been reported to be associated with the level of seed vigour by researchers (Wilson and Mohan 1998; Zhao and Wang 2005). ISTA

has recommended the conductivity test for vigour estimation in *Cicer arietinum*, *Glycine max*, *Phaseolus vulgaris*, *Pisum sativum* (garden peas only, excluding petit pois varieties) and *Raphanus sativus*.

3.2 Some Novel Methods of Vigour Assessment

1. **Ethanol test:** The ethanol test is a promising method that can be used to differentiate seed lots with different vigour levels. Decline in seed vigour due to ageing results in damage of the membranes, including in mitochondria, which impairs aerobic energy metabolism. Loss of mitochondrial integrity due to ageing directs the seeds to anaerobic respiration and ethanol release, which has been associated with seed vigour in various crop seeds (Buckley and Huang 2011). The test is based on the alcoholic fermentation theory, where the enzymes pyruvate decarboxylase and alcohol dehydrogenase act on pyruvate, producing ethanol and CO_2 as well as oxidizing NADH during this process. Ethanol release measured using a modified breath analyser was successfully used as a rapid test to rank the vigour of seed lots of canola (Buckley and Buckley 2009) and cabbage (Kodde et al. 2012).
2. **Single seed oxygen consumption** at the beginning of germination has been considered as an indicator of seed vigour (Reed et al. 2022) and is largely correlated to the activation of metabolic activity. Oxygen consumption is directly related to seed respiration and energy production. The Q2 machine, with oxygen-sensing technology, provides a fast and automatic measurement of oxygen consumption and respiration efficiency of individual seeds and has been found as a measure of seed vigour in several species, including *Beta vulgaris* L. and *Pinus massoniana* Lamb. (Zhao and Zhong 2012) and is recommended for seed vigour assessment, although this has been debated by Powell (2017).
3. **Volatile organic compounds production**: Seeds undergo deterioration due to lipid peroxidation leading to emission of volatile organic compounds (VOC). Since it has been established by many researchers that there is a significant difference between high and low vigour seeds with respect to quantity and profile of VOCs emitted, there is great potential for utilizing the VOC profile to obtain a quick and reproducible test of seed vigour (Umarani et al. 2020). Further research is needed to develop standard and reproducible protocols for fingerprinting of VOCs for seed vigour assessment and to identify the standard volatile biomarker (s) specific to crop species.
4. **Seed quality analysis by spectral properties:** Rapid discolouration and micro-organism contamination during production and storage is the main cause of seed discolouration. The colour sorter uses an electronic eye that can pick up different colours according to the way the machine is adjusted. As seed falls down a shoot, it passes in front of the electric eye. If the colour of the seed is different than the desired colour, the electric eye will activate a sudden burst of air that pushes that seed into a reject bin while the rest of the seed passes through to another bin.

Colour sorting improved germination and vigour in eastern Gamma grass (*Tripsacum dactyloides*) seeds (Klein et al. 2008).

5. **Seed maturity analysis by chlorophyll fluorescence (CF):** Seed within a lot that has not reached full maturity will most likely be less vigourous with impaired germination capabilities. Sometimes the seeds have finished the seed filling phase, but not the seed maturation phase. Such seeds are difficult to separate using normal density, size or shape separations. Chlorophyll fluorescence measures the amount of green chlorophyll present in each seed or the seed coat. The higher the chlorophyll in the seed the lesser mature and vigourous the seed (Jalink et al. 1999). The principle is used in commercial seed sorters.
6. **Seed separation by near-infrared (NIR):** Near-infrared light transmission through the seed gives off a signal that is linked to the internal components of the seed, which can be related to seed vigour and germination. Low vigour, dead and contaminated seed with fungal pathogen gives specific NIR signal which can be used to separate them from healthy and vigourous seeds. Near-infrared spectroscopy could predict seed germination and vigour status of soybean seeds (Al-Amery et al. 2018).
7. **Multispectral and X-ray imaging:** This could be used for rapid and non-destructive evaluation of seed quality, overcoming intrinsic subjectivities of seed testing. X-ray-based seed vigour estimation in tomato was undertaken with X-ray digital imaging (Bruggink 2017). These techniques help to sort broken, undersized, diseased seeds from healthy seeds. Bianchini et al. (2021) used multispectral and X-ray images for the characterization of *Jatropha curcas* L. seed quality and de Medeiros André et al. (2020) used FT-NIR spectroscopy and X-ray imaging for vigour estimation. Mahajan et al. (2018) reported the use of machine vision-based alternative testing approach for vigour testing of soybean seeds (*Glycine max*) wherein multispectral and X-ray imaging system rapidly and efficiently undertook non-destructive characterization of seed quality.
8. **Seed imaging systems:** The utilization of computers for seed vigour assessment had increased significantly in recent times. The SeedVigor Imaging System (SVIS®) developed by Sako et al. (2001) is being used successfully for vigour assessment in soybeans and corn (Marcos-Filho et al. 2009). The image analysis is performed on scanned images of seedlings, whose parts were identified and marked by developed software. After the computer image processing, data was obtained on the root length, hypocotyl and indices of vigour and uniformity of growth. Marcos-Filho (2015) reported that the vigour index, uniformity of growth and seedling length were consistently comparable with the results of recommended vigour tests. Another programme, SeedVigor Automated Analysis System (Vigor-S), was designed based on similar principles to the SVIS® to provide an efficient evaluation of seed physiological potential (Rodrigues et al. 2020).

4 Seed Invigoration

After harvest seeds are not always at highest level of vigour, and they may further deteriorate during storage. The seed invigoration treatments play a pivotal role in seed quality improvement where these treatments enhance the germinability, planting value, field performance, and yield via physiological and cellular repair. They may subsequently rejuvenate post storage seed quality. The selection of seed invigouration treatments is crop and problem specific and has variable applicability for enhancement of seed quality and planting value upgrading. In this chapter, we primarily put emphasis on 'seed priming' as the most widely reported invigouration treatment to reinvigourate low vigour seed lots. Seed invigouration treatments involving partial hydration of seeds are metabolically more advanced towards radicle protrusion (germination *sensu stricto*) than un-treated seeds. Therefore, these seeds are exhibiting rapid and uniform germination.

Rapid seed germination and stand establishment is critical for crop production especially under sub-optimum environmental conditions. Various seed priming treatments have been devised to improve the rate and uniformity of seedling emergence and crop performance. The priming treatments provide controlled hydration of seeds that trigger several metabolic, biochemical, and molecular alterations enabling seeds to germinate more efficiently, exhibit faster and synchronized germination, and impose vigour and stress tolerance to young seedlings (Varier et al. 2010).

4.1 Effect of Seed Priming

4.1.1 Biophysical and Structural Changes

Seed water status refers to the measurement of state of water in relation to seed. Water in seed is available in three forms, i.e. bound, free and adsorbed states. The free water is bound by weak hydrogen bond and is available for metabolic activities. The physical state of water is affected by hydrophilic compounds, viz. protein and sugars in plant tissues. Better performances of primed seeds are attributed to the modifications of seed water availability and binding properties (Nagarajan et al. 1993). Matsunami et al. (2021) studied the role of aquaporins (AQPs), transmembrane proteins that serve as water channels allowing rapid and passive movement of water in seed water metabolism. Among the AQPs, plasma membrane intrinsic proteins (PIPs) and the tonoplast intrinsic proteins (TIPs), expressed in the plasma and vacuole membranes respectively, are known to play a key role in transcellular and intracellular plant water transport.

Internal seed morphology of primed seed's free space (empty area between the embryo and the endosperm or between the seed contents and the integument within a seed) plays an important role in seed germination of primed seeds. The X-ray studies of primed tomato seeds reported that priming allowed greater water uptake by the embryo resulting in rapid growth and penetration of radicle tip. SEM observations on internal microstructural changes of osmoprimed okra seeds showed abundance of starch granules in primed seeds with pitted surface of starch granules and visible changes in their surface roughness, indicating activation of hydrolytic enzymes during the priming treatment. These seeds also showed an increased amount of amorphous material, probably proteins or other biopolymers that indicated the synthesis of protein bodies in primed seeds (Dhananjaya 2014).

4.1.2 Cellular and Metabolic Changes

Seed priming initiated activities of cell wall hydrolases as endo-β-mannanase that help in lowering mechanical constraints during the initial period of germination and radical protrusion (Toorop et al. 1998). The α and β tubulin subunits, a constituent of microtubules, are reported to help in the maintenance of the cellular cytoskeleton. An increase in the abundance of α and β subunits of tubulin has been recorded during priming in tomato seeds (de Castro et al. 2000). During priming under abiotic stresses, the cellular structures and proteins accumulated during the course of water uptake are known to be protected by specific proteins such as late embryogenesis abundant (LEA) protein and dehydrins (Chen et al. 2012). The expression of LEA proteins undergoes sequential changes with a decline during the imbibition phase, upregulation in the dehydration phase followed by degradation during the germination phase (Soeda et al. 2005).

The priming-induced enhancement in seeds is due to activation of DNA repair mechanisms, synchronization of the cell cycle in G_2 and preparation to cell division (Bewley et al. 2013). Cell division starts just after radical protrusion, thus, seed priming, which prolongs Phase II of seed germination and is finished just before Phase III (radicle protrusion), thus does not affect cell division in itself but prepares the seed for cell division (Varier et al. 2010). Seed priming also increases the ratio of cells in the G_2 phase to the G_1 phase, an expression of the beneficial effects of priming on seedling performance (Bino et al. 1992). Mir et al. (2021) reported the beneficial effect of priming on seedling performance due to the action of replicative DNA synthesis processes prior to seed germination in hydroprimed maize seeds.

4.1.3 Physiological and Biochemical Changes

Enhanced vigour and germination of primed seeds is attributed to a range of physiological and biochemical changes viz., damage repair, better mobilization of reserves into growing seedling, reduced time of imbibitions required for the onset of RNA and protein synthesis, polyribosome formation, increase in total RNA and total protein content, improved membrane integrity, control of lipid peroxidation, increased sugar content, protein and nucleic acid synthesis, removal of inhibitors like abscisic acid, efficient production and utilization of germination metabolites, increased activity of enzymes viz. alpha amylase, acid phosphatase, esterases, dehydrogenases, isocitrate lyase, protease, peroxidase, catalase, glutathione reductase, superoxide dismutase and ROS production (Pandita et al. 2007; Vashisth and Nagarajan 2010; Lutts et al. 2016).

The reactive oxygen species (ROS) functions as signalling molecules in plants thus regulating growth and development, programmed cell death and hormone signalling. Reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) are generated as a result of redox reactions in seeds. Seed priming treatment strengthens the ROS mechanism in the seeds facilitating better performance of primed seeds (Apel and Hirt 2004).

4.1.4 Stress Resistance

Seed priming is an effective way to alleviate the inhibition of seed germination and seedling growth under stress conditions as it provides tolerance against abiotic stress (drought, heat, salinity, nutrient). Halopriming improved field emergence in chilli seeds under salinity stress (Khan et al. 2009) whereas ascorbic acid priming reduced aluminium stress in maize (Kussumoto et al. 2015). Hydropriming in paddy seeds enhanced resistance against carbon dioxide stress and oxidative damage, while osmopriming with $CaCl_2$ in wheat seeds provided resistance against drought stress (Nedunchezhiyan et al. 2020). Seed priming with cytokinins (plant growth substance) also imparted salinity and drought tolerance (Bryksova et al. 2020). Beneficial microorganisms or plant growth-promoting microorganisms used for seed-enhanced plant stress tolerance against drought was also recorded due to bacterial priming (*Bacillus* sp.) (Amruta et al. 2019; Singh et al. 2016). Magnetic field treatments imparted tolerance to biotic and abiotic stresses (Bhardwaj et al. 2012).

4.2 Storage of Primed Seeds

Priming often adversely affects the longevity of seeds. However, the extent of impairment depends on priming protocol, storage temperature, duration and moisture, storage period and crop species (Parrera and Cantliffe 1994). During priming, DNA replication is initiated in the embryonic axis and progresses from G_1 to S phase and subsequently to G_2 phase. When primed seeds are dried and stored, these nuclei get arrested in G_2 phase, and are vulnerable to cellular damage (Bewley et al. 2013). The longevity of primed *Digitalis purpurea* seeds could be preserved by slow drying at low temperature and moisture level followed by storing under low temperatures (Butler et al. 2009), while in commercial priming slow drying at a higher temperature is often used to improve shelf life (Bruggink et al. 1999). Gene expression studies showed a decline in the expression of LEA genes during *Brassica* seed priming and a reinduction of expression of LEA genes during slow and warm drying of the primed seeds (Soeda et al. 2005). Notwithstanding the decreased longevity of primed seeds in many species, priming offers high commercial value by practising storage at low temperature, controlled and slow/fast drying post priming or other chemical methods.

5 Conclusion

Seed vigour is an important trait of seed quality assessment which ensures high performance under variable environmental conditions. The early vigour trait is important for optimum field establishment. Numerous tests have been attempted in different crops but most are not universally accepted due to high variability in their results. Novel and innovative methods of seed vigour estimation have been developed for reliable assessment of seed quality which needs further refinement. Seed invigoration treatments are effective for improving the vigour status of low vigour seeds but post-treatment storability is a cause of concern.

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