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Potential use of the Q2 machine as an evaluator of the physiological quality (Germination and Vigour) of commercial seed-lots

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For the purposes of this review, '*germination*' is defined as "the production of a normal seedling as per ISTA definition and use in seed testing", and '*vigour*' as "the sum of those properties that determine the activity and performance of seed-lots of acceptable germination in a wide range of environments".

The suitability of the Q2 technology as a predictor of the physiological quality (germination and vigour) of a commercial seed-lot was evaluated here from the point of view of end-users, for example, in Seed Quality testing labs (QC in a conditioning plant, independent quality testing lab, Foundation Seed labs). Commercial seed-lots are therefore the focus.

Essentially a review would ask the question: does the Q2 technology accurately measure the respiration of a population of imbibing seeds, and do the measurements derived accurately estimate the germination potential, and the vigour, of that population of commercial seeds, since these are the claims made in promotional material on the technology?

<u>Background to potential use of oxygen measurements for seed quality evaluation</u>

There is much in current literature that covers respiration during imbibition, and some of it does attempt to relate respiration (whether it be oxygen uptake, activity of key respiratory pathways, or ATP production) to the quality of the seed-lot. However, almost all have explored the above while defining germination as radicle emergence and have used the speed of radicle emergence as the estimate of vigour to which correlations with the various measurements of respiration were sought. And indeed, the rate with which a population of seeds respires does, in many instances, correlate well with the speed at which the radicles of the seeds in that population emerge.

Previous attempts at quantifying respiration for seed quality evaluation, attempted to use physiological markers, such as ATP concentrations and ATP-requiring activities, but a review of the literature covering many different species concluded that in 90% of them, no such correlation was found (1).

In almost all papers exploring the relationship between respiration activity and seed vigour, very low quality seed-lots are included, or seed is artificially aged in order to provide a wide range of vigour levels. While this is ideal for research purposes, it does not represent a commercial approach to vigour testing, where it's mostly good quality seed-lots that are tested for vigour. A study of respiratory enzyme activities during germination in *Brassica* seed lots of differing vigour (2) found that although the differences in enzyme activity correlated with rate of germination in artificially aged seed, "the enzyme activity could not be used to distinguish between seed lots which had smaller vigour differences apparent only under stress". Respiratory enzyme activities would not therefore, be of much use in vigour determination of commercial seed-lots.

Temperature appears also to play a role in respiration during imbibition, especially at the beginning of imbibition. It also "greatly affected" the total CO_2 respired in order to reach the first germination event with sorghum seeds (3). While this ought not to pose too much of a problem for research studies, or within-sample comparisons, it cannot satisfy the need for absolute numbers as required for standardization of routine testing across multiple laboratories within a seed company.

Species-specificity also can play a role – sesame seeds for example, responded differently to low-oxygen and ambient-oxygen environments compared to pea seeds exposed to the same environments during imbibition (4)

For the evaluation of the germination performance of a population of seeds, ISTA Rules have included the germination of a normal seedling as the criterion of germination- for a reason. It is not necessary to delve into the body of evidence that lead to that decision, but we can assume that it was sufficiently compelling that simply looking at whether the seeds were dead or alive (whether or not a radicle emerged) did not provide enough information about that seed population. Among very few papers, there is one which sought to explore respiration rate and how it relates to "low temperature susceptibility" (essentially a Cold Test, involving field soil and rolled-paper towels), and the generation of 'normal' seedlings, using commercial maize seed-lots with good standard germination of 95 to 100% (5). The respiration of whole maize seeds was measured, but the differences in the magnitude of respiration rates did not relate to differences in the cold test results (i.e., vigour). Corbineau *et al* (6) also used commercial carrot seed-lots and concluded that germination responses depended on their size, particularly at sub- and supra-optimal temperatures - environments in which the expression of vigour would be expected to manifest.

In summary: There is much to support the relationship between respiration rate and the accompanying rate of radicle emergence, especially when seed-lots of very low quality are included. However, many variables have also been identified (e.g., temperature, species, seed-size, imbibition rate) which would necessarily impact implementation in a routine testing lab. Evidence that this relationship can successfully differentiate among commercial seed-lots where seed quality is usually considerably higher than those used in research, and where germination performance is measured by the number of normal seedlings generated, is, however, lacking.

<u>Manufacturer's claims: evaluation of seed quality (Germination and Vigour)</u>

In order to be considered suitable for the evaluation of the germination, and vigour, of a seed-lot, the technology would have to satisfy the same ease of use of germination and vigour tests currently employed – generally according to ISTA Rules and recommendations – and criteria for evaluating the same. The Q2 measurements would have to provide information on the ability of a seed-lot to generate normal seedlings (germination %) and how that seed-lot would perform under non-favourable conditions (vigour). The Q2 measurements could also evaluate attributes of vigour – for example, speed of germination, or uniformity of germination.

<u>Specific claims for the Q2:</u>

Measures yield potential: For field crops (where the reproductive units are harvested), Yield is a complex, final, attribute of a crop, and given a certain threshold of emergence, is determined by environmental conditions at growth stages after stand establishment can potentially be influenced by seed quality. This is therefore just not theoretically possible, and not backed by data.

Measure stress tolerance: it is not explained how the data from the Q2 measure this, nor are any data gathered under conditions of stress provided.

It is an extremely fast test: this is certainly accurate.

Provides a holistic view on vigour: this is exactly what it does not do. The Q2 measures selected potential vigour attributes such as respiration rate. It does not provide any information on how the seed-lot will perform under non-favourable conditions, and does not provide information on germination performance respiration rate – for commercial seed-lots, the effect of stress on the generation of normal seedlings would be important.

The Q2 is a single seed oxygen consumption system. Because oxygen consumption is directly related to energy production, this technology gives a perfect view on different aspects of seeds such as imbibition time, speed of germination, homogeneity and energy availability during the germination: Energy production needs to be defined. As already noted, an examination of the correlation between seed vigour and ATP accumulation in the early stages of germination was examined in over a dozen species and in 90% of them no such correlation was found (1).

In addition Q2 data is more robust and defining than traditional germination tests. You will easily determine dead, dormant or actively germination seeds: it is not immediately apparent how the data are more robust and defining. Simply saying so does not make it so.

Although it currently does not provide specific details on seedling abnormalities, the Q2 data can give quicker and more accurate indications of the vigour and homogeneity of a seed-lot: since single seeds are measured, homogeneity can indeed be assessed. How the Q2 data more accurately indicate vigour is left to trust. And therein lies the problem: nowhere are we ever provided with comparisons between Q2 data and those of traditional seed quality tests. Given that the technology has been out there for well more than 10 years, the absence of any data with which to back up the claims with regard to vigour is disquieting.

<u>Impediments to implementation in a commercial seed-testing lab:</u>

- Species-specific, and size-specific, protocols are needed; this is not a situation in which one-size-fits-all. In a lab which tests multiple crop species, with multiple thousand seed weights, this would not be practical.

- The Q2 values and their correlations with regard to germination percentage are also species specific. Some of the values apply to some species, and not others. This difficulty can perhaps be accounted for in a research study, but not in routine testing.
- Seeds are almost always treated nowadays, often with insecticides. This technology relies on the seeds being imbibed on an agar substrate. From experience of insecticide-treated seeds – an agar substrate in a micro-titre plate can impact germination % (compared to rolled-paper towels) by 60%.
- Plates need to seal well – for research, tubes with screw caps might be acceptable, but not in a high through-put testing lab.
- Evidence (data) that Q2 measurements are comparable to current traditional tests are still needed
- Temperature sensitivity – this is impossible to standardize across commercial testing labs without positioning the Q2 machine in a temperature-controlled environment.

In summary: while the technology might be a useful tool for research purposes where respiration during early imbibition might be an important measurement, especially for small-seeded species, it would not currently be a suitable replacement for commercial seed quality testing, especially of field crop species. Many of the claims made are simply not backed by the necessary supportive data, and given the length of time the technology has been available for testing of this nature, a question mark has to be placed over this absence.

Conclusions:

The Q2 technology can no doubt accurately measure the respiration rate of single seeds at the very initial stages of imbibition. This would be valuable in a research capacity. As a replacement (or even additional) testing tool for the testing of the seed quality of field crop species (especially testing as defined by ISTA), there is certainly not sufficient evidence that it would accurately do so.

References

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