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Report on potential use of Q2 for evaluation of Seed Quality

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1 - Background to potential use of oxygen measurements for seed quality evaluation

Successful stand establishment requires high-quality seeds, i.e. seeds that (1) germinate completely, (2) germinate quickly and simultaneously, (3) produce normal and vigorous seedlings, and (4) have germination which shows little sensitivity to external factors, enabling them to germinate in a wide range of agro-climatic conditions (Corbineau and Côme, 2006 ; Corbineau, 2012). Methods of evaluation of seed quality, providing accurate prediction of seed performance under field condition are therefore important for seed companies.

Seed respiration increases dramatically during the first hours of imbibition which corresponds to the imbibition phase or phase I of the germination process, it stabilizes or increases more slowly during the germination *sensu stricto* phase (phase II) until radicle protrusion, and increases with seedling growth (phase III of the germination process)(Woodstock and Grabe, 1967; Dahal *et al*., 1996). Raymond and Pradet (1980) also demonstrated that the ATP/ADP ratio, which is related to the adenylate energy charge, is correlated with the rate of oxygen uptake. Among all the physiological, metabolic and cellular markers of seed vigour, the rate of oxygen uptake during seed imbibition before radicle emergence, has been often correlated with seed vigour in pea, *Zea mays*, *Capsicum annuum* and *Brassica* seeds (Woodstock and Grabe, 1967; Carver and Matthews, 1975; Halpin-Ingham and Sundstrom, 1992; Bettey and Finch-Savage, 1996). However it is not a good indicator in seeds exhibiting dormancy (Côme and Corbineau, 1989).

Oxygen uptake by a seed can result from other biochemical reactions than respiratory activity, like oxidation of phenolic compounds or/lipids, and microorganisms growing on the surface of many seeds might also be involved. In dormant barley seeds, for example, oxygen fixed by phenolic compounds of the glumellae corresponds to 37 and 53% of the total oxygen uptake by the whole grains at 20 and 30°C, respectively (Lenoir *et al*., 1983); the same phenomenon has been observed in oat (Corbineau *et al*., 1986). It is necessary to determine the respective role played by the various compartment of the

seed, namely the embryo itself which will give rise to the seedling, the endosperm if it is present and the seed surrounding structures.

Numerous studies have shown that decrease in seed quality during seed ageing, or improved seed quality by priming were correlated with changes in respiratory activity (Chojnowski *et al*., 1997; Li *et al*., 2010; Bradford *et al*., 2013). In addition, the respiratory quotient (RQ), which represents the CO_2 evolved divided by the oxygen uptake, could be also a good index of seed deterioration; high RQ have been reported in deteriorated seeds (Woodstock and Grabe, 1967; Woodstock *et al*., 1984). If the correlation between the respiratory activity and viability is established by numerous data, only few studies have clearly demonstrated a relation between seed respiration during the initial hours of germination and later stage of germination and seedling growth. For example, Woodstock and Grabe (1967) have shown in *Zea mays* L. a linear relationships between RQ and O2 uptake measured 2 and 6 h after imbibition and later shoot growth, which were negative and positive, respectively. Tetrazolium test based on the respiratory activity, which results in an increase in reduced cofactors, also informs on the seed viability, however its use for evaluating seed quality is debatable (Baalbaki *et al*., 2009; Elias *et al*., 2012).

2 - Potential methods for oxygen evaluation

Measurement of seed oxygen uptake involved various techniques (gas-chromatography; polarographic determination using Clarc type electrode; manometric measurements using Warburg equipment or Gilson type respirometer; biochemical determination of O_2 uptake or CO_2 release), but all these methods are not adapted for measuring respiratory rates of individual seed within a population.

A new technology, using membrane the fluorescence of which is oxygen dependent Draaijer *et al*., 1999), has been developed by ASTEC-Global (WWW.astec-global.com). This equipment, called Q2, allows the measurement of respiration rate of large numbers of individual seeds following imbibition. Individual seeds are placed into the wells of a 96-well microtiter plate, each well containing agar (0.5 to 1% w/v) or a filter paper disk in order to provide water for seed imbibition. The volume of each well is about 400 ml, but it can be modulated by the volume of agar in the bottom depending of the seed size of the species studied, and the temperature during measurement can be regulated between 10 and 35°C (± 0.5 °C) (Bradford *et al*., 2013).

Measurements of oxygen relative content in the well are automatically carried out regularly at desired time interval (generally 1 to 2 h) in order to determine time courses of oxygen uptake in each well by individual seed. Data are collected in a data base and analyzed by the Q2 analysis software which calculates different parameters called ASTEC values:

SMR: Starting Metabolism Rate (initial slope of oxygen consumption)

IMT: Increased Metabolism Time (time when the initial respiration rate increases)

OMR: Oxygen Metabolism Rate (the maximal respiration rate when the oxygen availability is not limited)

COP: Critical Oxygen Pressure

RGT: Relative Germination Time

Bradford *et al.* (2013) have added 2 other parameters: the R50 (time for a seed to reduce the initial oxygen level by 50%) and AUC50 (Area Under Curve to 50% Oxygen) which are not calculated by the software.

The software can also calculate RGT homogeneity in population, which is the variance in RGT values among individual seeds.

It is no doubt that the Q2 instrument is a very convenient method to assay oxygen uptake of many individual seeds during germination. However: (1) it is a global measurement that cannot discriminate the oxygen uptake associated with the embryo respiratory activity from that resulted from lipid or/and phenolic compounds oxidation;
(2) the measurements are done in a closed compartment, the atmosphere of which decreases as a function of time. After 2 days for example, the atmosphere in the well reaches less than 30% of the initial oxygen tension, i.e. less than 5% oxygen; (3) measurements do not seem to take into account the weight of seed.

3 – Application of Q2 to evaluation of seed germination and vigour

Evaluation of seed vigour

Bradford *et al*. (2012, 2013) applied the Q2 to predictions of rate of germination and vigour. They demonstrated (2013) that some ASTEC values (IMT, RGT and OMR) correlated with the rate of germination in the Q2 (QT50) across numerous batches of Brassica seeds (cabbage (17), turnip (4), Chinese cabbage (6) and cauliflower (4). Significant correlations were seen between RGT and HOM with first counts in an SG test

(vigor) but there was poor prediction of vigor, since r^2 values were low, SMR and COP were not significantly correlated with vigor. IMT and RGT were also good markers of onion seed rate of germination (QT50), or vigour, when OMR was less correlated to seed vigor (Bradford et al., 2013).

However Q2 data are obtained in favourable conditions of oxygen supply and at optimal temperature, they do not provide therefore data on subsequent seedling growth and/or the emergence ability of seeds under unfavourable environmental conditions. The ASTEC values correlated to germination rate in the Q2 depends on the species, and must be determined for each species.

Evaluation of priming efficiency and effects of ageing

Improvement of germination rate by priming and its delay after ageing are correlated with changes in the respiratory activity, in particular with IMT, RGT and AUC50 (Bradford *et al*., 2012, 2013) which is not surprising. In contrast the maximal respiratory rate (OMR) was poorly correlated with seed vigour.

Q2 can provide data which rapidly inform on the efficiency of priming and can substitute for analyst's work. Data obtained and measures of seed-to-seed variation in respiration (HOM) also characterize the heterogeneity of the batch.

Conclusion

Q2 technology can measure respiration rates of individual seeds; it can then characterize the homogeneity of a seed batch. It can be used to assess the rate of germination expressed as QT50, which correlates with some ASTEC values, at least for brassica, onion, tomato and lettuce seeds. However, additional research is required in order to determine whether ASTEC values are correlated with germination rate (QT50) in other seed species with different proportions of tissues (embryo, endosperm and seedcoat).

However, data published (Bradford *et al*., 2013) do not permit to claim that Q2 can be used for vigour evaluation as defined by ISTA, i.e. "the emergence ability of seeds in unfavourable conditions" or/and "performance after storage". It would be important to

determine whether respiratory activity measured in unfavourable conditions could provide information on seed vigour.

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