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Advances in seed science and technology

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Abstract
The seed is the most basic and important agricultural capital goods, and seed quality is important for agricultural production. Most methods presently used for seed quality detecting were destructive, slow and needed pretreatment, therefore, developing advanced methods has rapid, great significance for seed quality testing. The classical methods for detecting seed viability are time consuming and frequently cause seed damage and unwanted germination. It is a a novel micro-optrode technique (MOT) to measure seed viability in a quick and non-invasive manner by measuring the oxygen influxes of intact seeds, approximately 10 seconds to screen one seed. Thus, MOT is a reliable, quick, and low-cost seed viability detecting technique Multispectral imaging is a supervised classification model, based on nCDA transformation between viable and dead seeds, was built and tested on a new set of seeds. Here the prediction of viable and dead seeds resulted in 96% correct classified seeds, and confirm the potential of using multispectral vision technology in seed quality testing. The benefit of using multispectral imaging in comparison to instruments such as single seed NIR or IR, is the possibility to measure the multiple components by reflection from both visual and NIR wavelengths. Hence information of many valuable seed quality traits can be extracted in one single measurement. Cold plasma is an emerging non thermal technology primarily used for microbial disinfection and surface modification. The principle of plasma surface modification is exploited in food and agriculture for enhance of seed germination and other reasons. The aim of the present review is to give some insights on cold plasma technology exploitation for enhancement of seed germination. The seed germination rate can be increased on application of cold plasma by both direct and indirect treatments. The synergistic effect of cold plasma can replace the traditional seed disinfection solutions and chemical seed germination enhancers.

Keywords: seed science, agricultural, seed damage

Introduction
Real-time seed oxygen consumption provides a direct indication of metabolic status during germination. There are two basic approaches to measure oxygen consumption or production.
A novel technique allows long-term monitoring of real time oxygen consumption during seed germination in an open system. Most current techniques used to detect oxygen consumption by seeds measure the decrease in oxygen concentration in a closed chamber

Detecting seed viability by using oxygen influx
The classical methods for detecting seed viability like germination test, 2, 3, 5-triphenyltetrazolium hydrochloride dyeing, and electric conductivity measurement are time consuming and frequently cause seed damage and unwanted germination whereas an infrared thermography approach has been developed to non-invasively detect seed viability. However, this technique still requires approximately one hour to accurately test each sample. A novel micro-optrode technique (MOT) was developed to measure seed viability in a quick and non-invasive manner by measuring the oxygen influxes of intact seeds, approximately it takes 10 seconds to screen one seed.
The MOT is a highly sensitive and selective technique to measure oxygen concentrations and fluxes on the cell surface. Porterfield *et al.* invented this technique (known generally as self referencingoptrodic sensing) and applied the tool to basic research for the measurement of oxygen fluxes in animal and plant systems. Since that time, MOT has been utilized in a number of systems, including those for monitoring the effect of water quality on fish embryos by oxygen flux, detecting chemical toxicity to bacterial biofilms, determining plant root oxygen flux profiles, screening auxins transport mutants and a protocol has even been established for the Arabidopsis root system. The MOT is an ultrasensitive tool with high temporal and spatial resolution for detecting the physiological activity of live cells/ tissues. Use of this methodology will provide novel insights into seed science research.
Oxygen flux measurement with MOT. Prior to testing, seeds were soaked in measuring

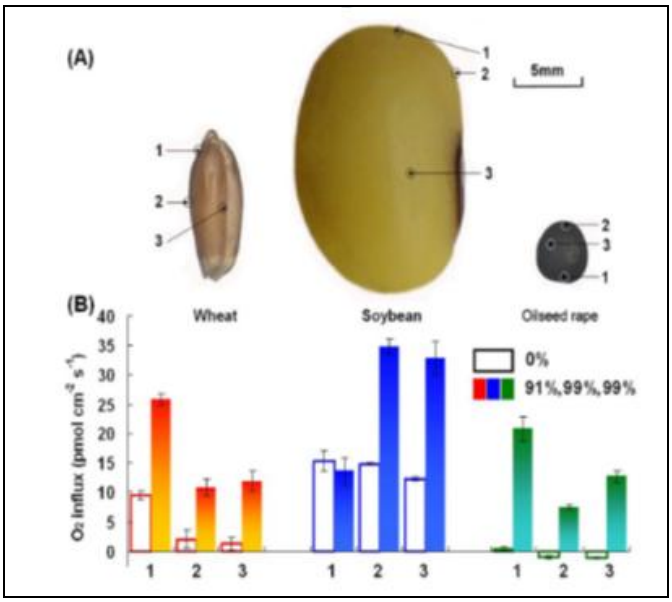
solution (0.1 mM $CaCl_2$, 0.1 mM KCl, 0.3 mM MES, pH 6.0) for three hours, and then transferred to a new solution to detect oxygen fluxes. Oxygen fluxes of seeds were measured in real-time and non-invasively using a micro-optrode based on NMT. The MOT was calibrated in measuring solution with known oxygen concentrations (0 and 21%) by nitrogen and air purging at 20^0C. The equation of oxygen flux based on the changes of phase angel (ψ) was:

$$J_{O_2} = -D\frac{\Delta C}{\Delta X} = -D\frac{\left(\frac{\varphi_1 - \varphi_2}{-m}\right)}{\Delta X}$$

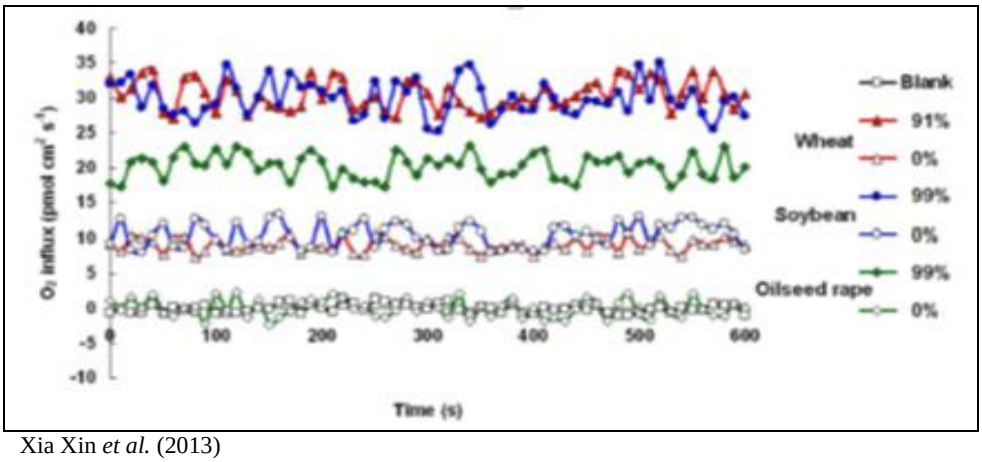
Where J_{O2} is oxygen flux (pmol cm^{-2} s^{-1}), D is molecular diffusion coefficient for oxygen in measuring solution (2.1 x 10^{-5} cm^2 s^{-1} at 20ºC), ψ_1 is phase angle at near pole, ψ_2 is phase angle at far pole, and m is linear slope of calibration plot within ambient conditions (0–21% O_2). ΔX is the distance moving the optrode between two positions close to the seed surface in a pre-set excursion at a programmable frequency of about 0.1 Hz.

A seed was fixed on the bottom of a Petri dish with a plastic colloidal cloth. Measuring solution was then added to the Petri dish to cover the entire seed. The measuring point (embryo/embryonic axis) was located by microscope and the microoptrode was adjusted to the point using a three dimensional motor. The oxygen fluxes were recorded every 10 seconds and measured for at least 5 minutes. Finally, the data of oxygen fluxes and the images were acquired and recorded in real-time using the imFlux software.

Different oxygen fluxes were observed among the testing sites in wheat, soybean and oilseed rape, the biggest oxygen fluxes were detected at site1, site2 and site1, respectively, i.e. at the center of the embryo/embryonic axis. The oxygen fluxes at the endosperm or cotyledon were much lower. The oxygen flux rate in live wheat seeds (91.0% viability) and dead wheat seeds (0% viability) were determined in the working solution. This is an obvious difference in the oxygen flux rate between the live and dead wheat, soybean, and oilseed rape demonstrating that oxygen consumption varied with seed viability. Xia Xin *et al.* (2013)



Xia Xin *et al.* (2013)

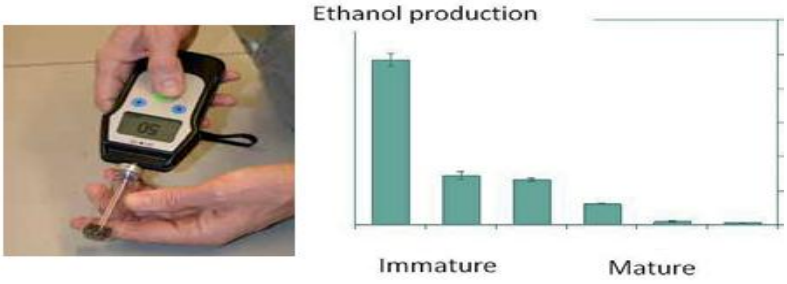


Xia Xin *et al.* (2013)

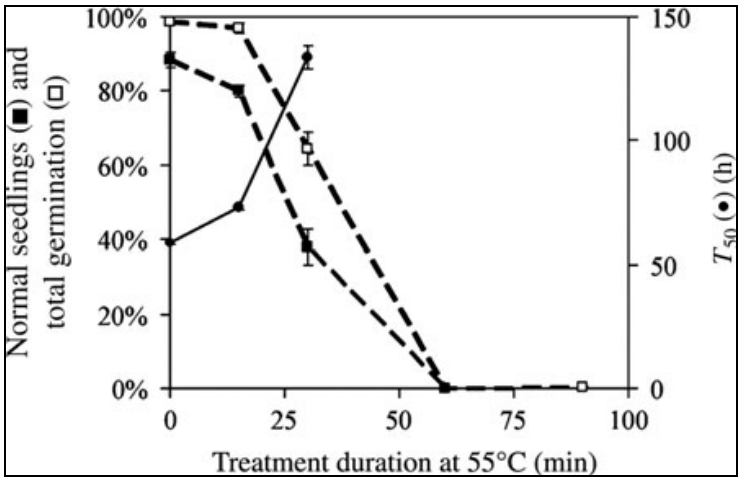
This very new technology is based on the fact that when seeds deteriorate they disintegrate slowly under the production-of ethanol. Immature seeds produce more ethanol than the mature seeds. Simple, Fast and Cheaper. Useful for seed storage. The method uses a modified breath analyser using elevated temperatures 40^0C instead of 20^0C shortened the assay time and improved its sensitivity.

Five hundred milligrams of seeds (treated, sorted or control) equilibrated at 32% RH were placed in a 20ml glass vial. Water was added to achieve the desired seed moisture percentage, taking into account the initial seed moisture content of 6%. The vials were sealed with aluminium crimp caps with 3-mm-thick butyl rubber and Teflon septa immediately after adding water. The vials containing wetted seeds were incubated at either 20^0C or 408C. Ethanol concentration in the headspace was measured at different time points using the modified breath analyser. To avoid temporary cooling, sample measurement outside the incubator was performed within 5s.

The original breath analyser was modified by replacing the breath sampling port with a Luer fitting and by making adjustments to the software. A disposable hypodermic needle (18 gauge, 5 cm long) was used to collect gas samples from the vials. The needle was shortened to approximately 2.5 cm and cut half through approximately 1.5 cm from the Luer hub to allow for pressure equilibration in the vial during its insertion. The device collected a subsample of approximately 0.3 ml from the headspace for every measurement. The analytical range of the breath analyser was 10–2500 mg ethanol per litre of gas mixture. Jan Kodde *et al.* (2011)



The analysis showed an inverse correlation between ethanol production and seed quality. The increase in ethanol production was observed when cabbage seeds were deteriorated by storage under ambient conditions or hot water treatments, both of which reduced the number of normal seedlings. Premature seeds produced more ethanol upon imbibition than mature seeds. Ethanol production occurred simultaneously with oxygen consumption, indicating that lack of oxygen is not the major trigger for ethanol production. Jan Kodde *et al*. (2011)



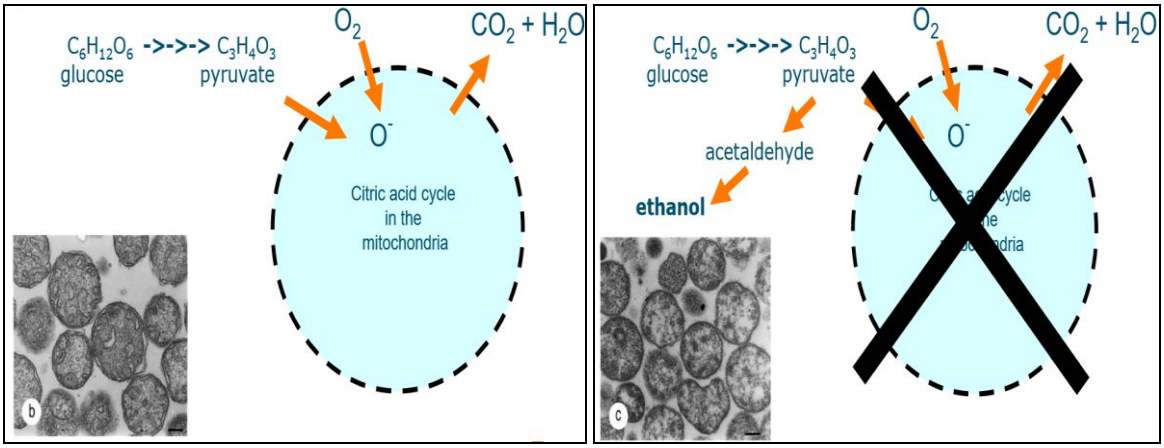


Image-Spectroscopy Technology

Multispectral imaging

Multispectral imaging is an emerging non-destructive technology in seed science, which integrates the conventional vision and spectroscopy technique to attain both spatial and spectral information from the target objects simultaneously. Multispectral imaging requires no sample pre-treatments, making it more suited for process monitoring and quality control. More importantly, this technique has a great potential to measure the multiple components by reflection from both visual (visual colour compounds) and near-infrared wavelengths (non-visual chemical compounds) at the same time for quality assurance. Vision and spectral technologies have shown promising results in different aspects of determining seed quality features. The spectral imaging system VideometerLab instrument (Videometer A/S) consists of a 5 mega pixel CCD camera, mounted inside the top of an integrating sphere, coated with highly white and diffusing paint and illumination by narrowband high-power LED (light-emitting diodes) placed at the rim, and thereby ensure a uniform and diffuse illumination of the sample at the bottom port of the sphere reflection. The LED's provide light in succession (sequential strobes) at the following 19 wavelengths: 375, 405, 435, 450, 470, 505, 525, 570, 590, 630, 645, 660, 700, 780, 850, 870, 890, 940 and 970 (and backlight at 625 nm beneath the sample holder). Before capturing images the instrument is calibrated to absolute reflectance using a bright and dark reference object (NIST traceable targets), and geometrically aligned using dotted plate. The seed sample is placed at the bottom of the integrating sphere and within 5–10 s a high-resolution multispectral image of 2056 × 2056 pixels were captured. The technology behind the system has originally been developed at the Technical University of Denmark and is described in

details in a patent from 2006.

Data analyses will be done by using Videometer Lab software version 2.13.73 (Videometer A/S). The multispectral images (MSI) were transformed using normalized canonical discriminant analyses (nCDA) in order to minimize the distance to observations within the seed colour classes and to maximize the distance to observations between classes. The first part of the analysis was to build a mask to segment the seeds from the background, which was based on an nCDA transformation of seeds and filter paper and a simple threshold. Next all seeds were collected in a blob database from which different colour, texture and shape features of the individual seeds could be employed. The colour feature MulticolourMean extract the mean intensity of the reflected light for each single wavelength. It was extracted for the two seed sets (calibration and validation sets), and presented as a mean intensity spectrum. The feature Region MSI mean, calculate a trimmed mean of MSI transformed pixel values within the blob (each single seed). It was extracted from seeds in the two seed sets. In the calibration set (seed set 1) the MSI was based on an nCDA transformation between the 94 seeds that were stained red in the tetrazolium test (viable seeds) and the 26 seeds that remained unstained (dead seeds). The threshold value was set to zero, so negative values correlated for viable seed and positive values correlated for dead seeds. The same MSI transformation was employed to seed set 2 for validation of data (containing 4 × 75 seeds from Arak and Ahvaz, grown under control and non-irrigated conditions respectively). Extracted data were further handled in Excel, where the RegionMSImean values were correlated to the germination capacity. Differences among reflection spectra were analyzed using linear mixed models (fixed effects: wavelength and seed class (colour or germination capacity); random effect is the replicates) using Rv 3.0.2 (RStudio. ink). Model fits were assessed by visual inspection of residual and normal probability plots.

Visually, 120 castor seeds were divided into three classes: yellow, grey and black seeds. images were taken at 19 different wavelengths ranging from 375–970 nm were captured of all the seeds. Mean intensity for each single seed was extracted from the images, and a significant difference between the three colour classes was observed, with the best separation in the near-infrared wavelengths. A specified feature (Region MSI mean) based on normalized canonical discriminant analysis, were employed and viable seeds were distinguished from dead seeds with 92% accuracy. The same model was tested on a validation set of seeds. These seeds were divided into two groups depending on germination ability, 241 were predicted as viable and expected to germinate and 59 were predicted as dead or non-germinated seeds. This validation of the model resulted in 96% correct classification of the seeds. Merete *et al*. (2015).

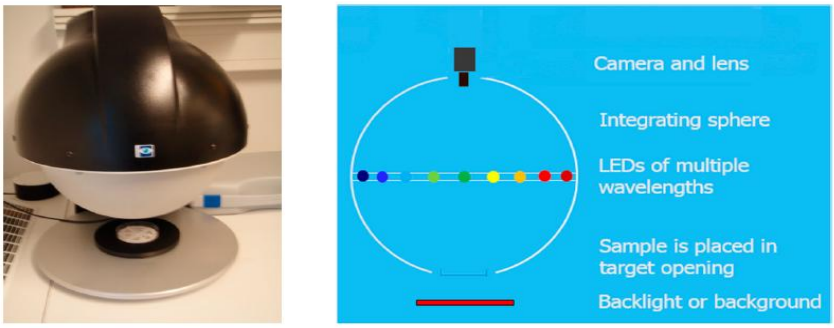


Figure 1. (**A**) Picture of the VideometerLab instrument and (**B**) is the outlines setup of the VideometerLab instrument.

Merete *et al*. (2015).

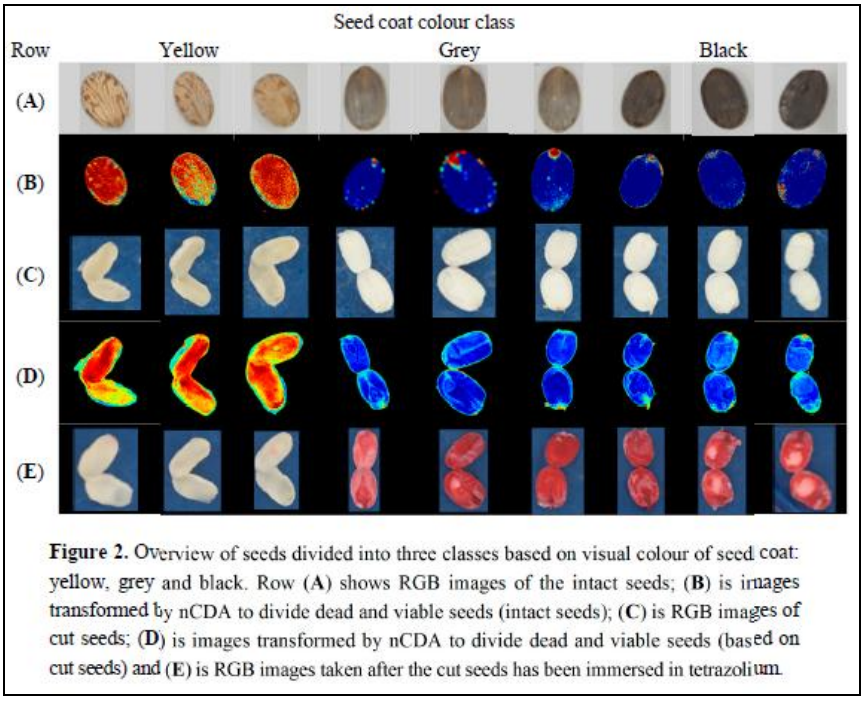


Figure 2. Overview of seeds divided into three classes based on visual colour of seed coat: yellow, grey and black. Row (**A**) shows RGB images of the intact seeds; (**B**) is images transformed by nCDA to divide dead and viable seeds (intact seeds); (**C**) is RGB images of cut seeds; (**D**) is images transformed by nCDA to divide dead and viable seeds (based on cut seeds) and (**E**) is RGB images taken after the cut seeds has been immersed in tetrazolium.

Merete *et al*. (2015).

Multispectral imaging and single kernel near-infrared spectroscopy are used for determination of seed health and variety separation of 27 winter wheat and nine winter triticale varieities due to variation in the chemical composition. Fusarium sp. and black point disease-infected parts of the seed surface was successfully distinguished from uninfected parts with use of a multispectral imaging device (405–970 nm wavelengths). The study produced an interesting result of successful distinguishing between the infected and uninfected parts of the seed surface. Furthermore, the study was able to distinguish between varieties. Martina *et al*. (2016).

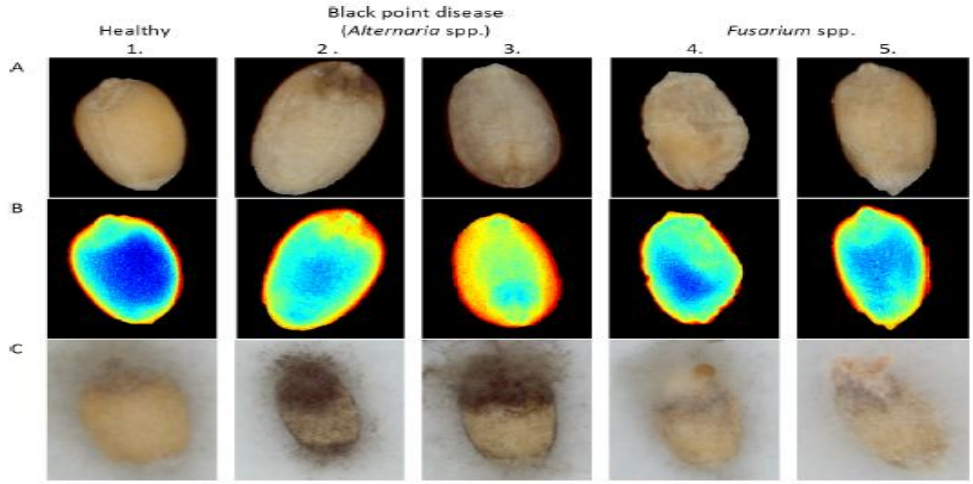
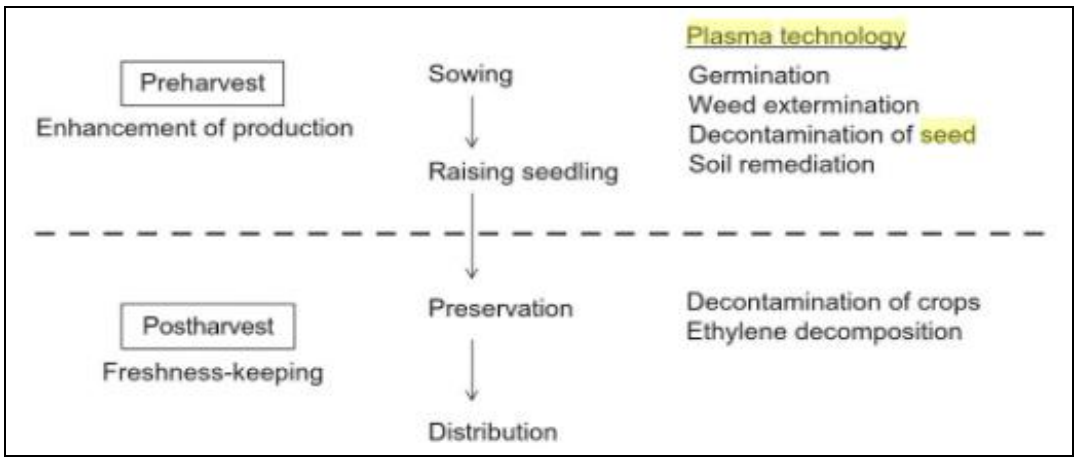


Fig 1. Image comparison of healthy seeds with infected seeds. Seeds on image were infected by black point disease (*Alternaria* sp.) and *Fusarium* sp. (A) RGB-captured images. (B) nCDA-transformed images. (C) RGB images after seed incubation.

Martina *et al*. (2016).

Cold plasma technology

cold plasma: Here high pressure gas discharge the collision between electrons and gas molecules occurs frequently. This causes thermal equilibrium between the electrons and gas molecules. Even in cold plasma the electron temperature is still typically several thousand centigrade. A cold plasma is one in which the thermal motion of the ions can be ignored. Consequently there is no pressure force, the magnetic force can be ignored, and only the electric force is considered to act on the particles. Examples of cold plasmas include the Earth's ionosphere (about 1000K compared to the Earth's ring current temperature of about 108K). The flow discharge in a fluorescent tube. Cold plasma processing methods possess many advantages in agriculture, owing to their operation at low- temperatures and short processing times, without inducing damage to crops, foods, seeds, humans and the environment. Plasma discharges produce reactive neutral species, charged species, electric fields and ultraviolet radiation. These factors cause the change in density of reactive oxygen species (ROS), reactive nitrogen species (RNS),Oxidation-reduction potential, electrical conductivity and so on, and affect seed germination, plant growth and the quality of agricultural product.



Plasma applications to agricultural production operations

Cold Plasma in Food and Agriculture

The contribution of plasma technologies in agricultural operations are limited to the decontamination of seeds or crops intended for sowing or storage, disinfection of processing surfaces or tools, the enhancement of seed germination or growth, production of nitrogen based fertilizers, soil remediation, reduction of pathogen invasion, and the removal of ethylene from air to reduce the rate of ageing.

Seed germination effect of cold plasma

Sirova *et al*. (2011) reported that the seedling growth during germination involves two key steps

1. primary cell elongation of the axial part of the embryo,
2. Simultaneous or delayed cell division in the radicle meristem.

Ji *et al*. (2016) reported that the seed germination is initiation of embryo breaking the dormant stage and always start with imbibition of water. Seed germination activity involves several physiological and biochemical changes such as protein synthesis, enzyme activation and starch metabolisms. The authors have also reported that the seed germination always hindered by seed dormancy factors which is undesirable process. The cold plasma can be applied by two different ways

I. Direct treatment of seeds,

II. Indirectly treating the seeds with plasma activated water (PAW) or plasma acid.

Direct treatment

In direct treatment method the seeds are directly placed in between the electrodes or placed under the plasma regime like in plasma jets. Direct exposure of chickpea seeds to atmospheric cold air plasma for 5 min by Mitra *et al*. (2014) observed an overall increase in seed germination by 89.2%. The authors have reported that the increase in the seed conductivity and seed roughness after the plasma treatment is the main reason for enhancement. The increase in seed roughness or etching caused by bombardment of reactive species may be the reason for increase in hydrophilicity of seeds. Violleau *et al*. (2008) reported that the oxygen plasma treatment of corn seeds increased the germination rate and higher yields. Another study conducted by Puligundla *et al.* (2017) on effect of corona discharge plasma jet on sprouting of rapeseeds. Exposure of rapeseeds for 1 min increased the germination rate by 7.7% compared to untreated seeds. The authors have reported that exposure of seeds resulted in scarification; formation of deep longitudinal cracks on the seed surface, increase in surface area attributing to increase in surface energy

Indirect treatment

In this type of treatments the seeds are treated with plasma activated water (PAW). The PAW is generated by application of cold plasma on the water surface or underneath water using different plasma sources.

Volin *et al.* (2000) reported a significant delay in the germination speed of seeds treated by Fluorocarbon plasmas. Their findings are just the opposite to our results. When they exploited fluorocarbon plasmas, the seed coat characteristics were modified via plasma deposition of hydrophobic materials, which would decrease water absorption and thus result in delayed germination. In our experiments, the helium plasma was exploited, which might make improvement in the wettability of seeds and eventually influence their germination speed.

Jiang *et al.* (2014) showed that the treated plant had a better growth than the control at booting stage. Compared to the control, the treated wheat seedlings had longer root, higher height and heavier weight, therefore they were better at absorbing water and nutrition, and could get more light for photosynthesizing. The effect of cold plasma treatment could improve the growth of wheat not only at seedling stage but also at booting stage. At the same time, we found that chlorophyll content of the treated wheat was higher than that of the control, indicating that cold plasma treatment could increase the physiological activities of wheat.

Li *et al*. (2014) reported that cold plasma had an active effect on soybean seed germination. The germination and vigour indices were increased by cold plasma treatments. The T2 cold plasma treatment produced the highest stimulatory effect among the different treatments; however, a cold plasma treatment with lower or higher energy levels had no significant influence on seed germination. According to Dobrynin *et al*.,(2009) the interaction of cells with plasma might improve the activities of seedling germination enzymes and accelerate the decomposition of the seed's inner nutrients which might contribute to the increased seed reserve utilization and seedling growth. Zhou *et al.* (2012) also reported that tomato seedling growth was improved by atmospheric pressure plasma treatment9. The present study demonstrated that a cold plasma treatment promoted soybean seedling growth, especially the T2 treatment. However, if the cold plasma treatment used a lower or higher energy level then no significant effect was observed on seedling growth. Effect of cold plasma was more dramatic on root growth than shoot growth.

A novel technique to monitor real-time oxygen consumption during early phases of seed germination

A novel technique allows long-term monitoring of real time oxygen consumption during seed germination in an open system. Most current techniques used to detect oxygen consumption by seeds measure the decrease in oxygen concentration in a closed chamber. Oxygen electrodes are used to measure the steady-state concentration of oxygen in the solution, which is a function of both the rate of oxygen consumption by the seed and the rate of aeration from the atmosphere. The rate of aeration is directly dependent on the oxygen concentration of the bathing solution; therefore, previous calibration of the system allows the direct conversion of steady-state oxygen concentrations into oxygen consumption rates.

Monitoring real-time oxygen consumption in an open system

This is an adaptation of a technique and concept first developed for monitoring oxygen consumption by plant cell suspensions in an open system (Baker *et al*., 1997). Routinely, 4 g of seeds were placed in 50 ml plastic beakers fitted with No. 10 rubber stoppers. The stoppers contained three 1 cm holes, one for attachment of the oxygen electrode described below and two to ensure good air exchange. Air-saturated buffer, 1 mM MES [2-(*N*-morpholino) ethanesulphonic acid], pH 6, and supplements, such as *E. cloacae* or antibiotics, were added to a level of about half the beaker volume (20 ml for a 50 ml beaker) to allow sufficient movement and aeration of the liquid when shaken on the water bath at 27° C at speeds of 180 (routine) to 230 rpm. All components of the apparatus that were in contact with the seeds were rinsed with 10% Clorox prior to use. Rifampicin, 40 µg ml_1, was added routinely to the bathing buffer, except where mentioned, to minimize the effects of bacterial contamination.

This technique utilizes a principle that allows oxygen consumption to be estimated in an open system (Baker *et al*., 1997). The principle is based on Fick's first law of diffusion (Piper and Scheid, 1981), which states that the rate of diffusion of a gas into a liquid will increase as the partial pressure or concentration of the gas in the solution decreases. In this technique, as the rate of oxygen consumption by the submerged germinating seeds increases, the oxygen concentration of the surrounding bathing solution decreases. Consequently, this decrease in

Oxygen concentration causes an increase in the rate of diffusion of oxygen from the atmosphere. These two processes, oxygen consumption by the seed and aeration from

the atmosphere, establish a new steady state concentration of oxygen in the bathing solution, which is monitored by an oxygen electrode. Once the system has been calibrated, the net oxygen consumption by the seeds can be calculated directly from the oxygen concentration of the bathing solution. The decrease in the steady-state concentration of oxygen did not substantially limit the oxygen consumption by the seeds. Mustard seeds, 2 g in 20 ml, shaken at two different speeds, 180 rpm and 230 rpm, had steady-state concentrations of O2 of about 75% and 85% saturation, respectively, both of which corresponded to a rate of about 12 µM O2 min-1.

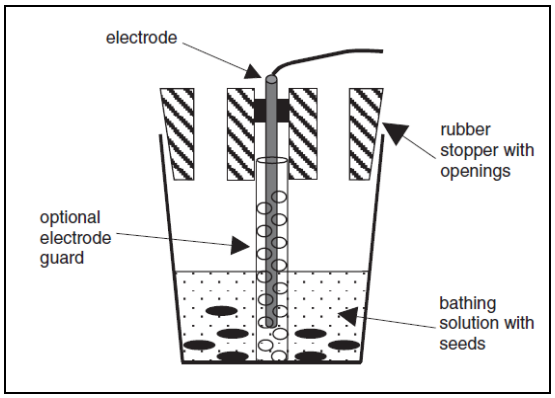


Diagram of the measurement vessel used to monitor oxygen concentration during seed germination Baker *et al*., 1997

A 50 or 100 ml beaker is fitted with a rubber stopper containing several large holes that are used to affix the electrode and allow air exchange. The optional electrode guard was made from a 5 ml plastic pipette tip with numerous holes melted into the sides. The seeds are submerged in bathing buffer, 1 mM MES, pH 6. As many as 16 of these vessels can be monitored simultaneously in a water bath shaker at 25 °C. A computer records the mVoutput from each electrode every minute.

They stated that that real-time monitoring of oxygen consumption can provide useful information about seed germination that is not easily obtained by other techniques. The technique is able to detect and quantify changes in metabolic activity that occur as seeds progress through different phases of germination. The ability to measure multiple samples simultaneously on a minute-by-minute basis allows subtle and quantifiable comparisons to be made. Therefore, potentially, the effect of various additives, hormones or treatments on seed metabolism can be quantified, as well as associated with the phase of germination affected. This technique also has potential to provide insight into interactions of seeds with microorganisms, as shown here by studies with a bacterial biocontrol agent. In addition, it offers the possibility to monitor oxygen consumption beyond germination *sensu stricto* and through embryo emergence, with small seeds that germinate rapidly, as shown here with mustard.

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