Computer vision for monitoring seed germination from dry state to young seedlings

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Seedling establishment is a crucial stage for plant production. High throughput phenotyping of a large number of seed and seedling traits becomes a great challenge to contribute to a better understanding of the mechanisms which lead to the establishment of a vigorous crop stand. Seed vigour and seedling establishment have, in several species, been related to the time course of germination, as determined by periodic counts of radicle protrusion (Matthews and Powell, 2011). However, the production of time course curves for seed lots is difficult and time consuming. An automated system using computer vision is a more efficient alternative. In this paper we describe such a system, which is capable of generating the time courses for large numbers of seeds, and illustrate its application to compare breeding lines and seed lots of a number of species.

Germination monitoring by computer vision

The French Seed Testing Station has been involved for many years in research programmes based on image analysis. For germination testing, a prototype was developed on sunflower (Ducournau *et al.*, 2004, 2005) to provide full informative data, which can be difficult and time-consuming to record on individual seeds. Since then, this prototype has been improved in order to analyse more species and more seeds per species. Now, three vision machines (Fig. 1) have been developed with Jacobsen tables which allow seed germination with continuous watering and at accurately controlled temperatures between 10 and 30 °C (± 0.5 °C). Four calibrated
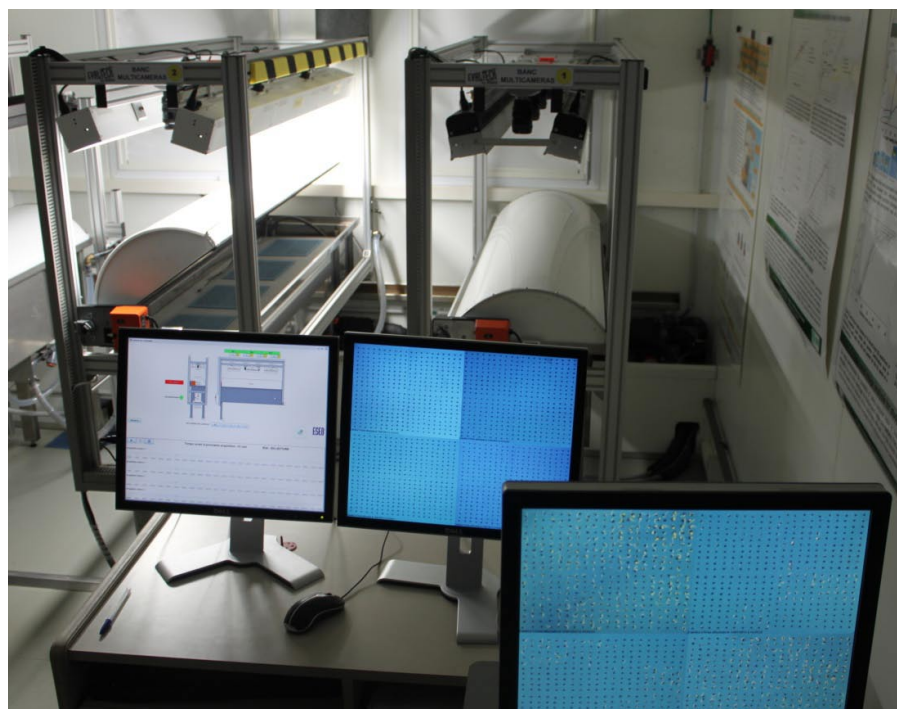

Figure 1. Automated germination tools.

cameras (PixeLINK® PL-A662 1.3 MP) are placed on each table to analyse large samples (up to 400 seeds per camera), and image analysis was improved to record germination parameters of individual seeds (4 replicates of 50 or 100 seeds per lot for one curve). The automated devices are driven by in-house developed software which is sequenced to a predetermined frequency (usually every two hours at 20 °C) as follows: switching on the light (6 × 36 W cold white light), opening the cover, taking and saving a picture of the seeds for each camera, switching off the light and closing the cover. The software also names and stores images from each camera in separate directories according to the species name, in order to ensure complete traceability of the experiments. Experiments were performed, using a range of magnifications, to test and validate the system with various species, from small seeds such as oilseed rape or tomato to large seeds such as sunflower or maize (Table 1).

Image analysis is performed with ImageJ software in order to separate seeds from the background, and to measure and record selected data such as area, X and Y position of the inertia centre and curve length. After calibration, the initial seed mass can be deduced from the measurement of its surface or volume (Demilly *et al.*, 2007; Casco and Dias, 2008). It was also demonstrated that the increase in seed surface and volume observed in legume seeds is correlated with seed imbibition, i.e. the increase in seed water content (Demilly *et al.*, 2007). The automated device allows separation of radicle protrusion (germination *sensu stricto*) from elongation, using inertia centre changes and axis length after each single seed germination is completed. Thus, initial seed mass, imbibition, germination *sensu stricto* and embryonic axis elongation are automatically measured for each seed by image analysis, and constitute a database within each camera's directory. Data are then pooled and analysed with

Table 1. Species frequently phenotyped for their germination traits

Species	Total images since 2005	Sample size (single seeds)
Brassica napus	9684	68150
Medicago truncatula	5170	27825
Lycopersicum esculentum	4577	18380
Brassica rapa	2403	12865
Medicago sativa	1748	11240
Helianthus annuus	3850	10810
Allium cepa	1740	9625
Zea mays	2770	9235

Table 2. Coefficient of determination between vigour tests and emergence results of five seed lots sown in the early spring, 7 April 2006 (*significant $p < 0.05$)

Vigour test	Correlation to mean emergence (R^2)
Accelerated ageing	0.543
Conductivity	0.457
Cold test	0.619
Mean just germination time at 13 °C (manual counts)	0.690*
Mean germination time at 20 °C (vision machine)	0.664*

Excel® software, which creates a file for each date, camera (i.e. replicate) and sample, using appropriate plug-ins. A second file joins the various replicates from the same sample, and a metafile summarizes the whole experiment by cumulating data for the various samples and replicates.

Vigour testing for seed technology

In a comparative vigour testing method ring test in maize, prepared for the 2nd ISTA Vigour Testing Workshop in 2006, mean germination time (MGT) was the best indicator of field emergence (Table 2). The MGT was obtained for five samples, both automatically at 20 °C and using data obtained by manual counts at 13 °C. Both were significantly correlated with field emergence at an early sowing, while the commonly used cold test was not (Wagner *et al.*, 2007). Following international collaborative tests (Matthews *et al.*, 2011), a vigour test for maize using radicle emergence has been introduced into the ISTA Rules.

Another example of the use of the automated system is the priming of vegetable seeds. The automated germination time courses obtained clearly separated the effect of priming the samples (Fig. 2), with the exception of the slowest germinating seed lot, from which the primed sample had an MGT included in the range of unprimed samples from the same variety. Automated time courses have also shown that some cultivars can germinate very rapidly and homogeneously without any priming; but seed priming can still gain one day in germination time (Wagner *et al.,* 2010).

Phenotyping for seed science

Each automated germination device can analyse up to 1600 individual seeds simultaneously. Accurate and consistent data provided by image analysis can be used for modelling plant emergence or contributing to genetic studies. Germination high-throughput phenotyping has become a great challenge for seed biologists since the automation of genetic tools (Joosen *et al.*, 2010). In collaboration with an INRA research team (Brunel *et al.*, 2009; Dias *et al.*, 2011), the germination of a model legume species, *Medicago truncatula*, was studied for 178 recombinant inbred lines (RIL) at two temperatures. The RIL populations came from crosses between Jemalong A17 (genetically mapped) and two ecotypes,

DZA 315.16 (RIL4) and F83005.5 (RIL5). The ability to obtain information on imbibition, radicle protrusion (Fig. 3) and early seedling growth on the same seeds using computer vision allowed some distinct quantitative trait loci (QTL) to be identified for the three stages of seed germination, and their location to be found on the RIL consensus genetic map (Dias *et al.*, 2011).

Seed phenotyping platform

The automated system for germination is part of the seed phenotyping platform located in Angers. Several species have been validated, the most important being sunflower, cabbage, tomato, oilseed rape, maize and *Medicago truncatula* (Table 1). More than 170 000 seeds have been analysed since
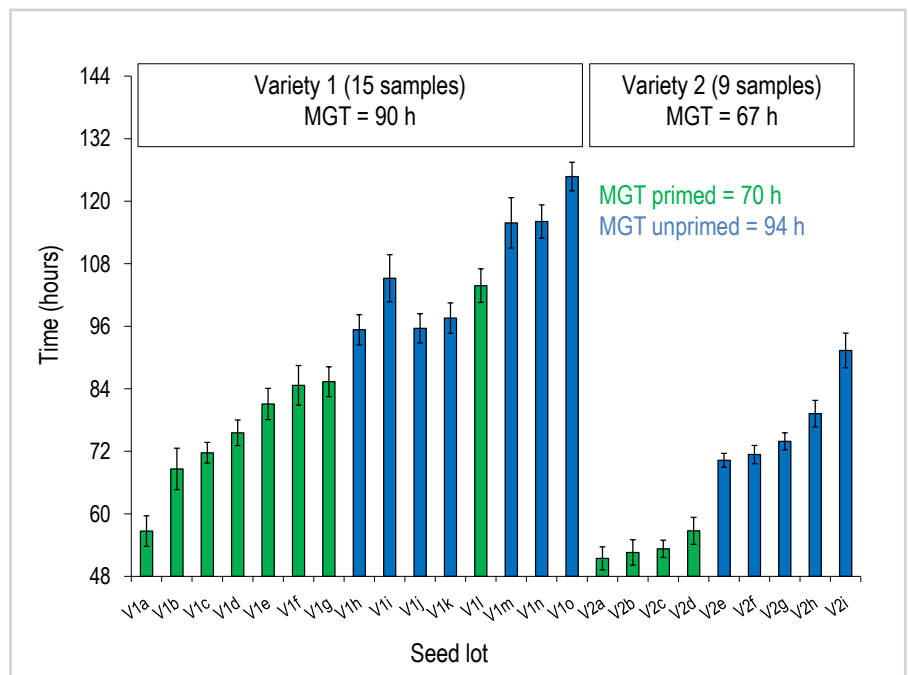

Figure 2. Mean germination times obtained at 20 °C for 24 tomato seed lots with primed samples (green bars) within two varieties (left: V1; right: V2).

2005, at first for research programmes, but during recent years, seed companies have become interested in using germination time courses for ranking seed lots.

In addition to these computer vision tools dedicated to germination traits, a series of non-invasive characteristics are currently measured using X-rays (Belin *et al.*, 2011), chlorophyll fluorescence sorting, and image analysis for morphological dimensions.

All these tools give a large range of possibilities to phenotype seeds from the dry state to young seedlings. The platform is open to seed researchers and seed companies.

Acknowledgments

Figure 3. Automated germination time courses obtained at 20 °C for 35 recombinant lines of the *Medicago truncatula* population RIL4 (dotted lines: parents A17 Jemalong and DZA 315.16).

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Wagner, M.-H., Demilly, D., Ducournau, S., Belin, E., Léchappé, J., Dürr, C. (2010). High throughput phenotyping of seeds from dry state to young seedlings. AGRO2010, The Scientific International Week around Agro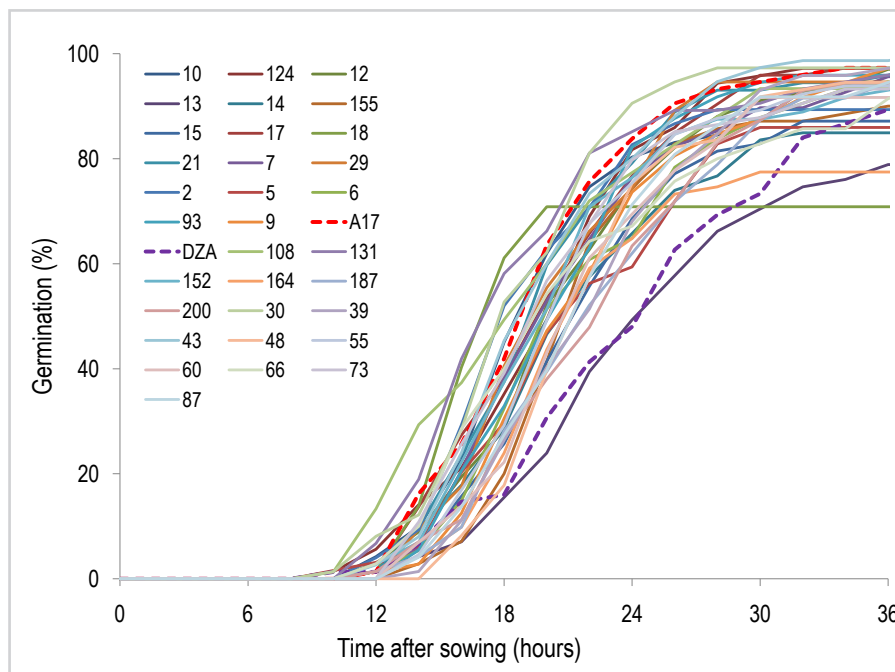nomy. 29 August–03 September 2010, Montpellier, France.