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Editorial

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Steven P.C. Groot

Bioscience, Wageningen University & Research, P.O. Box 16, 6700 AA Wageningen, The Netherlands
(E-mail: steven.groot@wur.nl)

Abstract

This issue of *Seed Science and Technology* contains a number of interesting papers worth giving additional attention. There are five papers that deal with analysis and breaking of seed dormancy, an important issue, not only for commercial practice and breeding, but also for regeneration of habitats and safeguarding biodiversity. One paper in this issue describes how mucilage production by seeds can aid in overcoming drought stress during germination of seeds from a desert plant. Combatting seed borne diseases remains an important issue. One paper describes how treatment with a combination of two fungicides proved to be effective against seed transmitted seedling blight in Norway spruce. Another paper demonstrates a method to combat a seed-transmitted bacterial infection with watermelon in the seedling stage. Seed vigour tests are needed to give a better estimation of differences between seed lots regarding field emergence. A faster vigour test for tobacco seeds is proposed in a paper in this issue. Even traditional ISTA germination tests may be improved, as demonstrated in a paper for spinach seeds, where especially large seeds can be sensitive to a high moisture level in the tests. Instruments initially used in high-technology industries or in medical care sometimes find their way to seed science applications. An example described in this issue is the use of 3D X-ray computed tomography, which enables rapid non-destructive analyses of the morphology of individual seeds, which can be correlated with germination behaviour. Another paper is on the use of multispectral imaging for seed purity analysis with alfalfa seed lots.

Editorial

Bromus auleticus Trinius (ex Nees) is a perennial grass, native to southern Brazil, Uruguay and central Argentina. Its forage yield is comparable with tall fescue and it is therefore considered a valuable species for grassland restoration in these areas. Seed dormancy is a challenge in its breeding and for commercialisation. The efficiency of some frequently used dormancy breaking treatments was tested, using four seed lots representing three accessions (Gonzalez and Condón, 2020). Control seeds stored two months, showed for three out of the four seed lots, a maximum germination of less than 45%, while seeds from the other seed lot showed approximately 90% germination. Germination of the relatively dormant seed lots could be improved by 10 days pre-chilling at 5°C, more than halving the mean germination time. Application of 0.2% KNO_3 was ineffective, whereas 0.05% GA_3 had an intermediate effect on dormancy breakage. The advantage of the GA_3 treatment is that it is faster as it omits a ten days pre-treatment. Both dormancy breaking treatments are very useful in breeding and selection. For commercial grass seeds, a wet dormancy breaking treatment cannot easily be applied, due to difficulties with handling large volumes of grass seeds. Here, other methods are needed, perhaps longer after-ripening periods. The observation that one of the four seed lots had 90% germination after two months after-ripening indicates that either seed production conditions or genetic variation can also aid in reducing seed dormancy levels for commercial seed lots (Gonzalez and Condón, 2020).

Oil palm plantations are very productive in terms of the amount of oil produced per hectare. However, for some consumers, palm oil production is synonymous to clearing of virgin rain forest. Several initiatives are ongoing for certified sustainable palm oil production. If that is more successful, palm oil has a better future and can aid in replacing fossil oil in many applications. Nowadays, palm oil trees are mainly produced from hybrid seeds that are harvested before full maturation to avoid seed losses. With oil palm, dormancy is a problem for seedling production, as the seeds may take months to germinate and the germination frequency is often low. In Malaysia, total germination can be significantly improved by exposing the seeds to naturally alternating temperature instead of constant 30°C as presently used in the nurseries (figure 1; Norsazwan *et al*., 2020). An additional advantage is that expensive controlled climate chambers are not needed.

Figure 1. Oil palm D × P germination was studied by Norsazwan *et al*. (2020). (**A**) Freshly harvested fruits (before removal of the mesocarp); (**B**) seeds immediately after mesocarp removal using a mechanical de-pericarper; (**C**) germinated seeds. Photos taken by and published with permission of Mohd Norsazwan Ghazali.

The rate of extinction of animal and plant species has never been so high. With plants that produce desiccation-tolerant seeds, we at least have the option to save seeds in genebanks. However, to multiply the seeds and reintroduce these species in the wild, the seeds should germinate, which can be a challenge when the seeds show dormancy. This is the case for *Pterygopleurum neurophyllum* (Maxim.) Kitag., an endangered species indigenous to Korea and Japan (figure 2A, B; Kwon *et al*., 2020a). When seeds from this plant are shed, they have an under-developed embryo as do seeds from many other members of the *Apiaceae* family, to which it belongs. The authors tested systematically several known dormancy breaking treatments. They found that a combination of 50% sulphuric acid scarification, followed by 12 weeks cold imbibition to let the embryo grow, and one day in 0.05% GA_3 resulted in the highest germination, around 80%. In this same issue of *Seed Science and Technology* the Korean group also reports on other dormancy breaking experiments with another species, this time *Maesa japonica* (Thunb.) Moritzi & Zoll. seeds (figure 2C, D; Kwon *et al*., 2020b). The species, a member of the Primulaceae family, is also seriously endangered. The potential of the species for pharmaceutical use intensifies the need for optimising propagation methods. Testing under a wide range of temperatures from 15 to 35°C, they observed best germination performance at 30°C, with around two-thirds of the seeds germinating, while no germination occurred at 35°C. This germination could be improved slightly by pre-incubation in a KNO_3 solution, but most improvement was obtained by first soaking the seeds for a day in 0.1% GA_3, giving full germination. Combining both pre-treatments also resulted in faster germination.

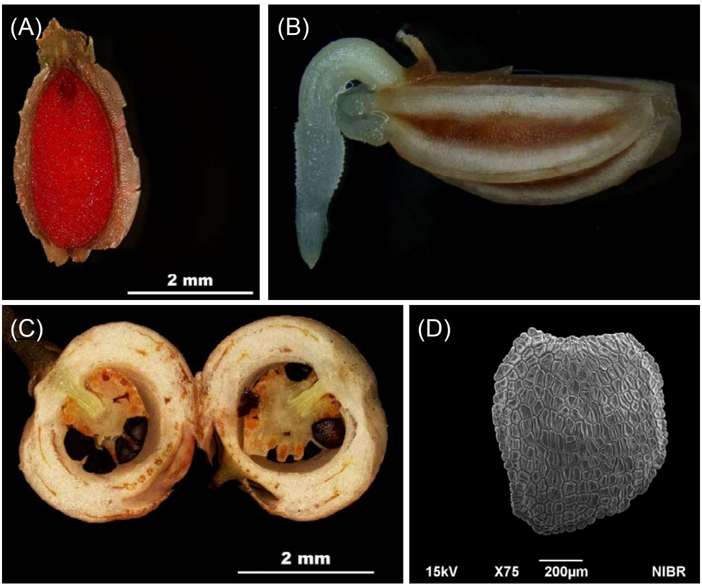

Figure 2. Images from the studies reported by Kwon *et al*. (2020a, b). (**A**) Tetrazolium-stained seed of *Pterygopleurum neurophyllum*; (**B**) germinating seed of *Pterygopleurum neurophyllum*; (**C**) *Maesa japonica* fruit; and (**D**) scanning electron microscope image of *Maesa japonica* seed. Pictures taken by and published with permission of Yu-Ri Kim (A, C, D) and Hyuk Joon Kwon (B).

Seed dormancy is also a challenge in ecological restoration programmes and reforestation. Plants in the *Fabaceae* and *Malvaceae* family frequently have seeds with a water impermeable seed coat, classified as physical dormancy (Baskin and Baskin, 2014). Heat treatments, acid or mechanical scarification are often used to break this type of dormancy. In this issue of *Seed Science and Technology*, an analysis is presented of seed dormancy and dormancy breaking treatments for 12 native tropical woody plant species with high reforestation potential in the forest-savanna ecotone in Côte d'Ivoire (figure 3; Koutouan-Kontchoi *et al*., 2020). The main emphasis of the study was seed coat water permeability. They first compared seeds mechanically scarified with a nail clipper or blade, these imbided more water compared with intact seeds, an indicator of water permeability issues. Seeds from five species (*Crossopteryx febrifuga*, *Diospyros mespiliformis*, *Ficus sur*, *Holarrhena floribunda* and *Pterocarpus erinaceus*) showed a more or less a similar imbibition capacity of intact and scarified seeds. Seeds of four of these species,

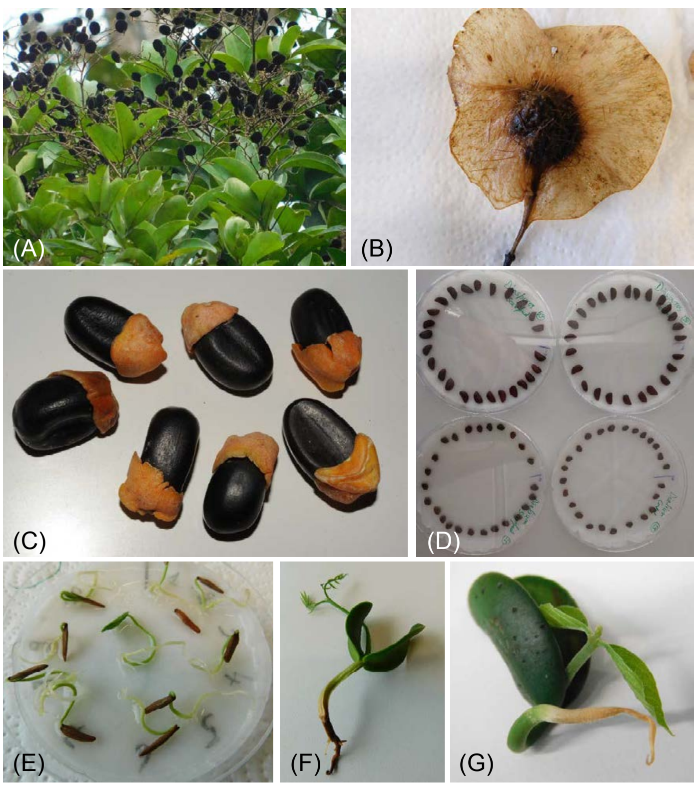

Figure 3. Fruits, seeds and seedlings of (**A**) *Dialium guineense*, (**B**) *Pterocarpus erinaceus*, (**C**) *Afzelia africana*, (**D**) *Dialium guineense* and *Diospyros mespiliformis*, (**E**) *Holarrhena floribunda*, (**F**) *Erythrophleum suaveolens* and (**G**) *Lonchocarpus sericeus*, some of the woody species from West Africa studied by Koutouan-Kontchoi *et al*. (2020). Photos taken by and published with permission of Milène Koutaouan-Kontchoi (A, B, E-G), Peter Poschlod (C) and Shyam Phartyal (D).

C. febrifuga, *F. sur*, *H. floribunda* and *P. erinaceus*, germinated to a high frequency without any additional treatments, indicating low levels of dormancy. With seeds from the other seven species (*Afzelia africana*, *Albizia ferruginea*, *Bauhinia thonningii, Ceiba pentandra*, *Dialium guineense*, *Erythrophleum suaveolens* and *Lonchocarpus sericeus*), the weight of the mechanically scarified seeds increased 3 to 26-times during five days imbibition, indicating water restrictions in water uptake through the seed coat. Response of the seeds to acid scarification, using different duration of soaking in concentrated 95-97% sulphuric acid, varied between species. Germination of *Afzelia africana* seeds was not or hardly improved by the acid scarification and even declined upon treatment duration of 40 minutes or more. Seeds from *Erythrophleum suaveolens*, on the other hand, needed at least 50 minutes acid scarification to get a significant improvement in germination.

In nature, conditions for seedling establishment are not always as ideal as in our nurseries. During evolution, plants have adapted methods to overcome stresses during germination and seedling establishment. An example is described for *Nepeta micrantha* Bunge, an ephemeral plant of the Gurbantungut Desert in China (figure 4; Zhao *et al*., 2020). *Nepeta micrantha* plants disperse seeds in the form of nutlets, which produce mucilage upon imbibition. The authors performed germination tests in different PEG concentrations with intact, mucilage-producing nutlets in comparison with nutlets where the mucilage had been removed by rubbing after five minutes imbibition. Under mild osmotic stress, they noticed a retardation of germination, but observed no difference in total germination between intact and demucilaged nutlets. At the stronger osmotic stress of 15% PEG, germination of demucilaged nutlets was higher, but when transferred to water, the recovery by the nutlets that had not germinated was better for the intact ones. These results indicate a role of the mucilage in survival of osmotic stress during imbibition.

Figure 4. Mucilage inhibits the germination of seeds of the desert ephemeral plant *Nepeta micrantha* according to Zhao *et al*. (2020). Picture taken by and published with permission of Yiguo Hou.

A more or less opposite situation is described for spinach (*Spinacia oleracea* L.) (figure 5; Magnée *et al*., 2020). High moisture levels inhibited the germination of the relative large seeds compared with smaller seeds from the same spinach seed lot. To study this phenomenon, a system was developed to control the moisture levels in filter paper germination tests. In this system the distance between the germination filter paper (blotter) and water table, connected by a water transporting filter paper wick, is regulated through the height of a layer of Styrofoam plates floating on the water. The higher this

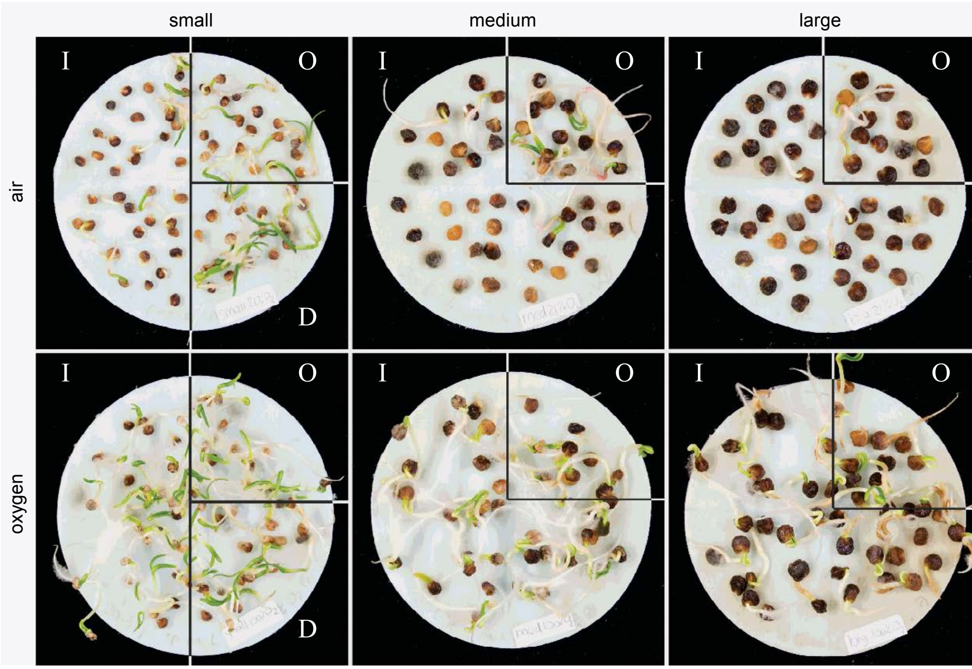

Figure 5. Differently sized spinach seeds from the same original seed lot were tested for germination on paper disks at a high moisture level, under either air or a high oxygen atmosphere. Spinach seeds with an open (**O**) pericarp, lacking the operculum, or with a damaged (D) pericarp were placed separately from intact (**I**) seeds (Magnée *et al*., 2020). Picture taken by and published with permission of Kim J.H. Magneé.

layer, the dryer the germination blotters and the better the larger spinach seeds germinate, although germination drops again when the filters get too dry causing water stress to the seeds. The inhibition of germination with the larger spinach seeds on relative wet filters can be alleviated by germinating the seeds in an environment with a higher oxygen concentration, showing that under too-moist conditions, these larger seeds, with a thick pericarp, experience oxygen shortage. The authors recommend that the moisture level of the germination blotters should be taken into account in the ISTA rules on spinach seed germination testing. The system developed by Magnée *et al.* (2020) can be used to determine the optimal distance to the water table when for instance a Jacobsen apparatus is used for germination testing.

Germination tests on paper often under estimate field emergence due to less optimal conditions upon sowing in soil. Seed lots may show variation in the difference between field emergence and paper germination, related to variation in seed vigour. A good estimation of seed vigour is important for expected field emergence and selection of seed lots. For some crops, the controlled deterioration (CD) and electrical conductivity tests have become official tests in the ISTA rules (ISTA, 2020). The reduction in final germination after CD is used to score the sensitivity of seed lots to the CD treatment and hence to indicate relative vigour. For slow germinating crops such as tobacco, the germination test after CD can take 16 days. Ma *et al*. (2020) showed that for tobacco seeds, highly correlative results can be obtained, within two days after the CD treatment, by measuring the relative EC of the treated seeds. This relative EC was calculated relative to the EC measured after boiling the seeds in a water bath.

Changes to more efficient seedling production methods can also have negative side effects, for which solutions are needed. Production of Norway spruce seedlings is now mostly done in microplant trays. However, this method requires more frequent watering, creating an ideal climate for spreading of spores of *Sirococcus conigenus* (DC.) P. Cannon and Minter. This seed borne fungus can subsequently cause high levels of seedling blight (figure 6), even when the initial seed infection is as low as 2% (Brodal *et al*., 2020). The authors tested several fungicides presently allowed in Norway for agricultural seed treatments. Of these, a combination of fludioxonil and difenoconazole proved to be rather effective and there are no sign of a phytotoxic effect on Norway spruce seed germination.

Figure 6. Norway spruce seedling infected by *Sirococcus conigenus* (Brodal *et al*., 2020). Photo taken by and published with permission of Venche Talgø.

For seed borne bacterial disease, chemical antibiotics have never been allowed and physical or other treatments have been studied as alternative. One such approach is reported in this issue (Zhao and Walcott, 2020). Bacterial fruit blotch, a seed-transmitted disease of cucurbit crop species, is caused by *Acidovorax citrulli*. During seed-to-seedling transmission, *A. citrulli* initially grows as a saprophyte on germinating seeds but subsequently switches to a pathogenic mode in the seedling stage. Presently available seed treatments, including thermotherapy and peroxyacetic acid, HCl or CaOCl have not been successful in controlling internal watermelon seed contamination by *A. citrulli*. The authors showed that switching the temperature at three days after sowing from 28 to 40°C, for a period of 11 days gives a very significant reduction in the frequency of watermelon seed/seedling colonisation by *A. citrulli*, compared with constant 28°C or only two days at 40°C.

Seed lots show inherent variation in characteristics and performance, enabling the population to survive under variations in environmental conditions. Agriculture, however, is better served with fast and uniformly germinating seeds. Techniques that can non-destructively characterise individual seeds and subsequently their germination performance will aid in understanding causal relationships. Non-destructive imaging and spectroscopic analyses of seeds and linking properties to seed quality can aid the seed industry in upgrading of seed lots or in breeding for specific properties (Xia *et al*., 2019). Two decades ago, chlorophyll fluorescence analysis was introduced as a marker for seed maturity and used to sort individual seeds (Jalink *et al*., 1998). Since then, this principle has been used by the industry to determine optimal harvest time, to characterise seed lots and for upgrading of seed lots with too many immature seeds. In this decade, with increasing computing power enabling analysis of big data and innovations in imaging technologies, new tools have become available for non-destructive analyses of seed characteristics and to link this to their performance. Hu *et al.* (2020) report in this issue of *Seed Science and Technology* on the use of multispectral imaging to discriminate between alfalfa and sweet clover seeds (figure 7). Alfalfa (*Medicago sativa* L.) is a perennial legume crop for pastures, aiding in increasing nitrogen availability. Alfalfa seed production is frequently contaminated with seeds from sweet clover species *Melilotus officinalis* (L.) Lam. or *M. albus* Medik., which is undesirable because coumarin produced by sweet clover plants can cause, when the plants are dried, 'sweet clover disease' in livestock. Alfalfa seed purity is therefore an important issue. Traditional visual analysis of alfalfa seed lot purity is difficult due to similarities in seed morphology of the contaminating sweet clover species.

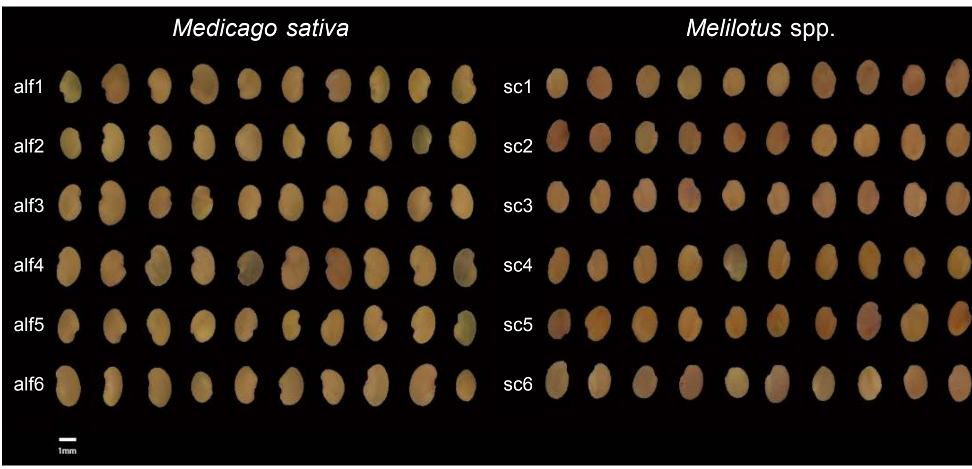

Figure 7. Images taken with a Videometer Lab4 of the six seed lots of each of *Medicago sativa* and *Melilotus* spp. studied by Hu *et al.* (2020). Images published with permission of Lingjie Yang.

The authors tested the application of multispectral imaging using the VideometerLab instrument. This instrument can analyse the reflectance of the seeds at 19 different wavelengths and also provides information on size and shape. The software provided with the apparatus enables multivariate data analysis of the acquired data to discriminate seed classes (Boelt *et al*., 2018). Morphological parameters (area, width, length, width-to-length ratio, compactness circle or compactness ellipse) failed to separate seeds of alfalfa and the two sweet clover species into two distinct groups, due to large variation between individual seeds within seed lots and an overlap between the three species. However, seeds of *Medicago sativa* and *Melilotus* spp. had a significantly different light reflectance that enabled good discrimination. Sweet clover seeds from both species showed a higher reflectance in the range from 405 to 430 nm and 660 to 880 nm compared with alfalfa seeds. Multispectral or hyperspectral analysis equipment is becoming more available for seed testing stations. Once the parameters are established, the analysis is much faster compared with traditional visual analysis. Moreover, the digital images and data can be stored for future purposes.

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SST Editorial Board

In this issue of *Seed Science and Technology* another example of a promising new technology for non-destructive characterisation of individual seeds is published. Porsch (2020) reports on the potential 3D X-ray computed tomography, also known as CT scan. The machine has a spatial resolution of 50 μm and can analyse for instance 60 individual sugar beet seeds non-destructive within three minutes, without much time needed for sample preparation. Data are provided on the size and volume of the different structures within the seed, next to their digital 3-D images. This allows correlation studies of these parameters with for instance germination performance. At present, the instrument is still rather expensive for most individual seed companies, but maybe a service provider or renting analysis time at a university can provide seed companies already with valuable information of their seed lots and potential correlations with seed quality.

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