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Completed by:
International Seed Testing Association (ISTA)
Zürichstrasse 50, 8303 Bassersdorf
Switzerland

Designed by: HeartWood Editorial
www.heartwoodeditorial.co.uk

ISBN: 978-3-906549-63-7

Edition 2019: 550 copies

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Hyderabad 2019
Preface

Dear Symposium participants,

Welcome to the 32nd ISTA Congress, and to Hyderabad, India.

The Seed Symposium this year has a theme of Seed Technology and Quality in a Changing World. Agriculture has always been extremely important in India, with records of agricultural practice in the country dating back to 6000 BC, and today over half of India’s workforce being employed in agricultural industries. Seeds are crucial to agriculture, and seed quality is vital to ensuring successful crop production. In a constantly developing and changing world it is important to ensure that new research and technologies are studied, understood and used to allow continued production of high quality seeds.

The Symposium will be made up of five sessions. Each session will be headed up by a lead speaker who is an expert in their field, and will contain further presentations selected by the Scientific Advisory Committee. Topics covered range from seed viability and seed production to seed health and new technologies in seed testing.

The Seed Symposium takes place once every three years and offers a fantastic opportunity to learn about recent research in seed science and advancements in seed quality assessment. The Symposium brings together scientists, technologists and analysts from universities, the seed trade and government, providing an opportunity to discuss recent developments in seed science and foster ideas for the future.

I hope that you are looking forward to the Symposium, and that you will enjoy the programme we have put together for you as well as the opportunity to interact with seed scientists and seed technologists from around the world.

Laura Bowden
ISTA Seed Symposium Convenor
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# Programme

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<tr>
<td>15:00–18:00</td>
<td>Congress registration desk open</td>
</tr>
<tr>
<td>18:00</td>
<td>Welcome reception</td>
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</table>

## Wednesday 26 June

**ISTA Seed Symposium day 1**

**08:00–18:00** Congress registration desk open

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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</thead>
<tbody>
<tr>
<td>09:30–11:30</td>
<td>Opening ceremony</td>
</tr>
<tr>
<td>11:30–11:50</td>
<td>2019: 150th anniversary of seed testing</td>
</tr>
<tr>
<td>11:50–12:00</td>
<td>Opening the ISTA Seed Symposium 2019: ‘Seed technology and quality in a changing world’</td>
</tr>
<tr>
<td>12:00–15:30</td>
<td>Oral Session 1: Developments in viability and vigour testing</td>
</tr>
<tr>
<td>12:00–12:30</td>
<td>Keynote presentation: Safeguarding genome integrity in germination and seed longevity</td>
</tr>
<tr>
<td>13:30–13:50</td>
<td>Quantifying seed vigour – a mathematical–statistical approach</td>
</tr>
<tr>
<td>13:50–14:10</td>
<td>New technology for estimation of germination of cereal seeds</td>
</tr>
<tr>
<td>14:10–14:30</td>
<td>An early count of radicle emergence and measurement of the leakage of electrolytes predict the percentage of normal seedlings</td>
</tr>
<tr>
<td>14:30–14:50</td>
<td>Understanding the mechanism of differential seed vigour status and field establishment in sweet, dent, flint, waxy, QPM and popcorn genotypes under sub-optimum temperatures and its improvement through seed enhancement treatments</td>
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</tbody>
</table>
### 32nd ISTA Congress – Seed Symposium
Hyderabad, India, 26–28 June 2019

<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Speakers</th>
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<tbody>
<tr>
<td>14:50–15:10</td>
<td>Development of a seed vigor test using single count of radicle emergence in chili (<em>Capsicum annuum</em>) seeds</td>
<td>Satriyas Ilyas, Dian Intan Karuniasari, Bogor Agricultural University, Indonesia</td>
</tr>
<tr>
<td>15:10–15:30</td>
<td>Multispectral imaging in sugar beet seed (<em>Beta vulgaris</em>) to determine germination</td>
<td>Kenan Genctuerk, Peter M Deplewski, Michael Kruse, University of Hohenheim, Germany</td>
</tr>
<tr>
<td>15:30–16:00</td>
<td>Coffee break</td>
<td></td>
</tr>
<tr>
<td>16:00–18:00</td>
<td>Poster session A</td>
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**Thursday 27 June**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Speakers</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00–17:30</td>
<td>Congress registration desk open</td>
<td></td>
</tr>
<tr>
<td><strong>ISTA Seed Symposium day 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08:30–11:30</td>
<td>Oral Session 2: Ensuring seed quality for future generations</td>
<td></td>
</tr>
<tr>
<td>08:30–09:00</td>
<td><strong>Keynote presentation:</strong> Setting standards for native seed in ecological restoration</td>
<td>Kingsley Dixon, Olga Kildisheva, Adam Cross, Curtin University, Australia</td>
</tr>
<tr>
<td>09:00–09:20</td>
<td>Referencing the Global Information System of Plant Genetic Resources for Food and Agriculture</td>
<td>Fiona Hay, Fransisco López, Marco Marsella, Ruaraidh Sackville Hamilton, Aarhus University, Denmark</td>
</tr>
<tr>
<td>09:20–09:40</td>
<td>First ever seed standards of <em>Lasiurus sindicus</em> for quality assurance</td>
<td>Vijay Dunna, Indian Agricultural Research Institute, India</td>
</tr>
<tr>
<td>09:40–10:00</td>
<td>The seed semi-permeable layer and its relation to seed quality assessment in grass species</td>
<td>Yanrong Wang, Qiu Jin, Xiaowen Hu, Lanzhou University, China</td>
</tr>
<tr>
<td>10:00–10:30</td>
<td>Coffee break</td>
<td></td>
</tr>
<tr>
<td>10:50–11:10</td>
<td>Quality seed production in cucumber (<em>Cucumber sativus</em>) cv. Pusa Barkha</td>
<td>Dilshad Ahmad, Sudhir Kumar Jain, Bhoopal Singh Tomar, Indian Agricultural Research Institute, India</td>
</tr>
<tr>
<td>11:10–11:30</td>
<td>New approaches to provide a rapid assessment of germination in seeds of native species</td>
<td>Maria Marin, Alison Powell, Giles Laverack, Stan Matthews, University of Aberdeen, UK</td>
</tr>
<tr>
<td>Time</td>
<td>Session/Activity</td>
<td>Presenter(s)</td>
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<tr>
<td>11:30–15:00</td>
<td>Oral Session 3: Ensuring seed health and implications of change for seed pathology</td>
<td></td>
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<tr>
<td>11:30–12:00</td>
<td>Keynote presentation: How a basic understanding of watermelon seed infection by <em>Acidovorax citrulli</em> has improved seed health testing</td>
<td><em>Ron Walcott</em>, University of Georgia, USA</td>
</tr>
<tr>
<td>12:00–12:20</td>
<td>Low-pressure plasma seed treatment as a substitute for chemical seed treatment in case of common bunt (<em>Tilletia caries</em>)</td>
<td><em>Sebastian Bopper</em>, Sophie Mühlberger, Michael Kruse, University of Hohenheim, Germany</td>
</tr>
<tr>
<td>12:20–12:40</td>
<td>Is seed a pathway? Updating the <em>List of Seed-Borne Diseases</em></td>
<td><em>Nicolas Denancé</em>, Christine Henry, <em>Terry Aveling</em>, Valérie Grimault, SNES, GEVES, France</td>
</tr>
<tr>
<td>12:40–13:40</td>
<td>Lunch break</td>
<td></td>
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<tr>
<td>13:40–14:00</td>
<td>Seed transmission of soybean yellow mottle mosaic virus in natural and experimental hosts</td>
<td><em>Sandra Nagamani</em>, Ankita Tripathi, <em>HK Dikshit</em>, <em>Bikash Mandal</em>, <em>Atul Kumar</em>, Indian Agricultural Research Institute, India</td>
</tr>
<tr>
<td>14:00–14:20</td>
<td><em>Pinus patula</em> in South Africa: effect of seed-borne mycoflora on germination, their control and first report of <em>Sydowia polyspora</em></td>
<td><em>Renaan Thompson</em>, <em>Terry Aveling</em>, <em>Mervyn Beukes</em>, <em>Guro Brodal</em>, University of Pretoria, South Africa</td>
</tr>
<tr>
<td>14:40–15:00</td>
<td>How to adapt methods to evaluate seed health of treated seeds</td>
<td><em>Isabelle Serandat</em>, <em>Geoffrey Orgeur</em>, Valérie Grimault, GEVES, France</td>
</tr>
<tr>
<td>15:00–15:30</td>
<td>Coffee break</td>
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<tr>
<td>15:30–17:30</td>
<td>Poster session B</td>
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<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00–16:00</td>
<td>Congress registration desk open</td>
</tr>
<tr>
<td><strong>ISTA Seed Symposium day 3</strong></td>
<td></td>
</tr>
<tr>
<td>08:30–11:30</td>
<td><strong>Oral Session 4: Seed production in a changing environment</strong></td>
</tr>
<tr>
<td>08:30–09:00</td>
<td><em>Keynote presentation: The changing face of the Indian seed sector: challenges and opportunities</em>&lt;br&gt;<em>Raj S Paroda, Trust for Advancement of Agriculture Sciences, India</em></td>
</tr>
<tr>
<td>09:00–09:20</td>
<td>Sunflower field establishment: germination and seedling growth related to temperature&lt;br&gt;<em>Marie-Hélène Wagner, Carolyne Dürr, Didier Demilly, Thierry Andre, Benoit Bleys, Christophe Bailly, Marion Laporte, Sylvie Ducournau GEVES, France</em></td>
</tr>
<tr>
<td>09:20–09:40</td>
<td>Rice seed production in a changing climate&lt;br&gt;<em>Duangporn Angsumalee, Punnee Thongket, Srisakul Thamdee, Watcharapong Wannawong, Wongduan Fakkam, Nimit Khamboonme, Wannamaneek Phongpaiboon, Kanuttaya Lalitchamroon, Kannika Seenuamnak Ministry of Agriculture and Cooperatives, Thailand</em></td>
</tr>
<tr>
<td>09:40–10:00</td>
<td>Impact of the maternal environment on seed physiology and biochemistry in barley&lt;br&gt;<em>Manuela Nagel, Erwann Arc, Loïc Rajjou, Oscar Lorenzo Sánchez, Andreas Börner, Ilse Kranner Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben), Germany</em></td>
</tr>
<tr>
<td>10:00–10:30</td>
<td><strong>Coffee break</strong></td>
</tr>
<tr>
<td>10:30–10:50</td>
<td>Novel breeding methods and innovations in seed production for delivering quality seed in changing climates&lt;br&gt;<em>Ashok Kumar A, Shivaji P Mehtre, Jayakumar Jaganathan, Hari Prasanna, Sunita Gorthy, Kotla Anuradha, Sharad Rao Gadakh, Uttam Chavan, Kalpande HV, Ashok Jadhav, Vilas A Tonapi International Crops Research Institute for the Semi-Arid Tropics, India</em></td>
</tr>
<tr>
<td>10:50–11:10</td>
<td>Effect of cultivars and maternal environment on seed quality in <em>Vicia sativa</em>&lt;br&gt;<em>Xiaowen Hu, Lingjie Yang, Rong Li Lanzhou University, China</em></td>
</tr>
<tr>
<td>11:10–11:30</td>
<td>Seed quality development in pepper (<em>Capsicum annuum</em>) grown in organic and conventional cultivation systems&lt;br&gt;<em>Kutay Coşkun Yıldırım, İbrahim Demir Atatürk Horticultural Central Research Institute, Turkey</em></td>
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<table>
<thead>
<tr>
<th>Time</th>
<th>Session/Activity</th>
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| 11:30–12:00   | **Keynote presentation:** Plant phenotyping – opportunities, challenges and integrated approaches for seed phenotyping and beyond  
**Ulrich Schurr**, Forschungszentrum Jülich, Germany                                                                 |
| 12:00–12:20   | Identification of morphologically similar seeds using computer vision and image analysis  
**Ruojing Wang**, Xin Yi, Mark Eramian, Jennifer Neudorf, Angela Satzl, Janine Maruschak  
Canadian Food Inspection Agency, Canada                                                                 |
| 12:20–12:40   | Multispectral imaging in seed testing  
Aarhus University, Denmark                                                                 |
| 12:40–13:40   | Lunch break                                                                                                                                     |
| 13:40–14:00   | Can DNA barcoding and Next Generation Sequencing technology detect invasive plant species in seed lots?  
**Marie-José Côté**, Steve Jones, Nicole Wurm, Adam Colville, Marc-Olivier Duceppe, Sarah Kyte, John Chmara, Marie-Claude Gagnon  
Canadian Food Inspection Agency, Canada                                                                 |
| 14:00–14:20   | Machine vision technology for establishing varietal distinctness  
**Monika Joshi**, Nachiket Kotwaliwale, Shyamal K Chakrabarty, Amitava Akuli  
Indian Agricultural Research Institute, India                                                                 |
| 14:20–14:40   | Image recognition technology on assisting paddy (*Oryza sativa*) seed germination testing  
**I-Cheng Chen**, Ting-Lin Chang, Shi-Jie Luo, An-Qin Xu, Kuo-Yi Huang  
Taiwan Seed Improvement and Propagation Station, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu |
| 14:40–15:00   | Chlorophyll fluorescence as an indicator of seed quality and longevity in soybean  
**Irfan Afzal**, Umair Usman  
University of Agriculture, Pakistan                                                                                   |
| 15:00–15:30   | Coffee break                                                                                                                                     |
| 15:30–16:00   | Symposium overview and conclusion                                                                                                                |
| 19:00         | Congress dinner                                                                                                                                  |
Oral Presentations

ORAL SESSION 1 – DEVELOPMENTS IN VIABILITY AND VIGOUR TESTING

Keynote presentation: Safeguarding genome integrity in germination and seed longevity

Wanda Waterworth
Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK
(w.m.waterworth@leeds.ac.uk)

Successful germination is important for crop yields and plant survival in natural ecosystems. Deterioration in seed quality is associated with the accumulation of cellular damage to biological macromolecules, including lipids, protein and DNA. Striking levels of DNA damage associated with seed ageing are further increased in seeds exposed to environmental stresses. Genome integrity is crucial for cellular survival and the faithful transmission of genetic information between generations, so repair of this damage in early germination is essential to minimise growth inhibition and mutation of genetic information. We have shown that maintenance of seed germination vigour and viability requires several distinct DNA repair pathways specific for particular forms of DNA damage. In particular, mutants lacking factors essential for repair of highly cytotoxic chromosomal breaks are hypersensitive to seed ageing and these studies have demonstrated that DNA double-strand breaks (DSBs) are rate-limiting for germination. The crucial link between genome integrity and progression of seed germination was further supported by our findings that the DNA damage signalling kinases ATAXIA TELANGIECTASIA MUTATED (ATM), and that ATM and RAD3-RELATED (ATR) control seed vigour and viability. In response to seed ageing, ATM delays germination, whereas atm mutant seeds germinate in the presence of DNA damage, resulting in extensive chromosomal abnormalities. Mechanistically, ATM functions through control of DNA replication in imbibing seeds, which is mediated by transcriptional control of the cell cycle inhibitor SIAMESE-RELATED 5. Collectively, our findings provide insight into the roles of genome maintenance mechanisms and DNA damage response pathways in regulating germination, a process critical for plant survival in the natural environment and crop production. Our current research is revealing how DNA damage signalling integrates repair, cell cycle activation and cell death in seeds. Understanding the mechanistic basis of seed vigour and viability will underpin the directed improvement of crop varieties with enhanced germination resilience and longevity and support preservation of genetic resources in seedbanks.
Quantifying seed vigour – a mathematical–statistical approach

Malavika Dadlani¹, Rathindra Narayan Basu² and Bina Punjabi³
¹Formerly at IARI (Indian Agricultural Research Institute), New Delhi, India
²Formerly at Calcutta University, Kolkata, India
³Formerly at GN College, Mumbai, India
(malavikadadlani.md@gmail.com)

The importance of vigour, a seed quality parameter in seed quality assurance, needs no emphasis. Though in use for several decades by seed researchers and professionals, vigour was included in ISTA’s recommended seed testing protocols only in 2002. Since vigour is a so-called qualitative attribute, which can be assessed using an array of diverse parameters, there is neither a single universal test for all species, nor a uniform method to express the correlation of such tests with the planting value of the seed lot. Some tests, such as those based on seedling growth, which are easy to perform with accuracy at different seed testing laboratories, often do not give a reliably high correlation with field emergence, whereas others, like electrical conductance (EC) of seed leachates and release of volatile aldehydes by seeds, do not differentiate between a viable and non-viable seed unless measured on a single seed. An attempt was made to address these concerns by adopting a data transforming technique prior to bivariate correlation – regression analyses. Germination factor (GF), seedling factor (SF), and germination-cum-seedling factor (GSF) were incorporated to recalculate the correlations between field emergence and several laboratory-tested vigour indicators from a number of published data sets. These not only resulted in positive correlations between field emergence and all vigour parameters, including EC of seed leachates and release of volatile aldehydes from hydrated seeds (which are normally negatively correlated), but also significantly improved the correlation coefficient values. The paper presents the methodology of transformation, recalculation and comparative analyses of data sets with appropriate examples.

New technology for estimation of germination of cereal seeds

Thomas Borjesson¹, Johannes Ravn Jorgensen², Lars Wadso³, Wilco Ligterink⁴, Elisabeth Janver⁵ and Pernilla Andersson⁶
¹Agrovast Livsmedel AB, PO Box 234, SE-532 23 Skara, Sweden
²Department of Agroecology, Aarhus University, Forsogsvej 1, DK-4200 Slagelse, Denmark
³Department of Building Materials, Lund University, PO Box 117, SE-221 00 Lund, Sweden
⁴Keygene NV, Agrobusiness Park 90, NL-6708 PW Wageningen, Netherlands
⁵Viking Malt AB, Exportgatan 1, SE-302 50 Halmstad, Sweden
⁶Seed Division, Swedish Board of Agriculture, SE-268 31 Svalöv, Sweden
(thomas.borjesson@agrovast.se)

The objective of the study was to compare three different quick methods: 1) The VideometerLab3 is equipped with 19 light-emitting diodes at wavelengths ranging from 375 to 970 nm, where the object is sequentially LED illuminated and images captured at each wavelength band. Twenty cereal seeds were placed in a petri dish on top of a blue filter paper and multispectral images were captured at intervals of 24 hours. 2) Isothermal calorimetry, a technique in which heat generation in a sample can be very precisely monitored. Heat production rate (thermal power) is measured at constant temperature. A commercial calorimeter with 125 ml vials with aeration was used. Each sample consisted of 100 grains placed on two levels on pleated germination paper. 3) Using the GERMINATOR technique, 20 cereal seeds were incubated on moistened blue filter paper in transparent rectangular incubation trays and at intervals of 12 hours the trays were photographed with a RGB camera mounted on a stand and connected to a computer. Using visual scripting, both germinated and non-germinated seeds could be detected. Altogether, 73 barley, wheat and oat samples were analysed. For reference, all samples were analysed for seed germination at the Swedish Board of Agriculture, Seed Division, using the method as described in the ISTA Rules. At 72 hours after incubation, a fair to good agreement between the results from all methods and
percentage normal sprouts were found for all grain types. The most important differences between the techniques were as follows: VideometerLab3 allows images of individual kernels to be saved. It is also easy to modify the settings depending on cereal type, etc., and it may be possible to distinguish fungal infections and early onset of germination using a combination of different wavelengths. The isothermal calorimetry potentially allows larger sample sizes than the other methods, which makes the measurement more representative and manual labour could also be minimised. It was also possible to distinguish differences in germination already after 24 hours, using the slopes of the heat generation curves. The GERMINATOR set-up has the advantage of being a non-complicated and inexpensive technology. It is also equipped with an efficient evaluation methodology. Even if samples from different cereal types were combined, a good agreement between mean values for three or four measurements and seed germination was found. It can also be scaled up using larger trays.

An early count of radicle emergence and measurement of the leakage of electrolytes predict the percentage of normal seedlings

Stan Matthews1, Kazim Mavi2, Marie-Hélène Wagner3 and Alison Powell1
1University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen, UK
2University of Mustafa Kemal, Department of Horticulture, Faculty of Agriculture, Antakya Turkey
3GEVES-SNES, 25 rue Georges Morel, CS-90024, Beaucouzé, France
(agr791@abdn.ac.uk)

There is interest in the development of more rapid methods for testing normal germination than is possible using the standard germination test. This paper reports the investigation of an early count of radicle emergence (RE) and measurement of the electrical conductivity (EC) of solute leakage to predict the percentage of normal seedlings. The percentage RE after 48 hours germination of ten commercially available radish seed lots consistently predicted the final percentage normal germination (NG) after ten days at 20°C. The coefficients of determination ($R^2$) between RE and NG were 0.90***, 0.94*** and 0.80*** in three germination tests, indicating that the early RE count was highly predictive of NG. The EC of seed soak water at 20°C was also consistently highly predictive of percentage normal seedlings. The $R^2$ for EC measurements taken after 17 and 24 hours with NG in four germination tests was consistently greater than 0.84***. EC readings taken at 1, 3 and 5 hours alongside two of the germination tests were also predictive of NG ($R^2$ 0.76*** on five occasions; 0.73** on one occasion). Similar findings were seen for seed lots of oilseed rape in a seed company in Scotland and in France (GEVES). For nine lots with NG greater than 68%, the RE after 48 hours in Scotland and 72 hours in France was highly predictive of NG after 14 days ($R^2$ 0.92*** and 0.77***). In the same seed lots, the EC of seed soak water after 24 hours was highly predictive of NG at GEVES ($R^2$ = 0.87*** and the seed company ($R^2$ = 0.79***). For both species, RE was predictive of mean germination time (MGT) which is the average lag period from the start of imbibition to RE. Evidence suggests that this lag period is needed before RE for the metabolic repair of the effects of seed ageing, which is the basis of several validated ISTA vigour tests. There is increasing evidence that ageing/repair occurs in many species and that early leakage is a feature of a species with two living cotyledons. Ten ISTA laboratories are examining the relationships between RE and NG in a number of species within routine testing, as part of a programme of work co-ordinated by the ISTA Seed Science Advisory Group. The potential for an early count of RE to provide a quick guide to the assessment of normal germination in a number of species will be discussed.
Understanding the mechanism of differential seed vigour status and field establishment in sweet, dent, flint, waxy, QPM and popcorn genotypes under sub-optimum temperatures and its improvement through seed enhancement treatments

Sudipta Basu¹, Vishwanath Sharma¹, Sandeep K Lal¹, Mohammad Athar¹, Firoz Hossain², Anjali Anand³ and Ashwani Kumar⁴

¹ICAR-IARI (Indian Agricultural Research Institute), Division of Seed Science and Technology, Pusa Campus, New Delhi 110012, India
²ICAR-IARI, Division of Genetics, Pusa Campus, New Delhi 110012, India
³ICAR-IARI, Division of Plant Physiology, Pusa Campus, New Delhi 110012, India
⁴ICAR-IARI Regional Station, Kunjpura Road, Karnal, Haryana, India
(sudipta_basu@yahoo.com)

Sub-optimal temperatures (less than 10°C) affect seed germination, seedling vigour and field establishment of speciality maize genotypes, especially in the early spring–summer and winter seasons. Among speciality maize genotypes, quality protein maize and sweet corns (sugary, shrunken, double recessive types) exhibited low field emergence with poor plant stands which affected their yield and seed quality. Rapid germination and stand establishment are critical under low temperature conditions. A comparative seed germination assessment at 15°C, 20°C (sub-optimum) and 25°C (optimum) was undertaken with seeds of different compositional groups, i.e. sweet corn (sugary, shrunken, double recessive), dent, flint, waxy, popcorn and quality protein maize, under lab conditions to study the association of seed composition with seed vigour status and field establishment under sub-optimum conditions. The results showed that all the compositional types failed to germinate at 15°C, except for waxy and popcorn types (10–20% germination). The genotypes under study exhibited moderate (10–60%) and high (80–95%) germination under 20°C and 25°C respectively. Among the compositional groups, popcorn and waxy corn showed good germination whereas sweet corns and QPM types showed poor germination and vigour, both under 20°C and 25°C. Sweet corns [sugary (51.5%) shrunken (55.5%), double recessive (20.3%)] and QPM (58.5%) showed the lowest germination and vigour under low temperature (20°C) conditions. Field emergence studies [December 2017; minimum (6.51°C) and maximum (20.7°C) temperature] showed poor field emergence and plant establishment in all the genotypes, the poorest being in sweet corns [sugary (28.2%), shrunken (25.5%) and double recessive (10.0%)] and QPM (35.5%), accompanied with low speed of germination and seedling growth. Popcorn (65.5%), flint (52.5%) and waxy (55.5%) corns exhibited moderately good field emergence and seedling growth under sub-optimum conditions. The correlation studies showed high correlation of field emergence and lab germination in all the compositional groups under study. The differential seed vigour status of the speciality maize genotypes could be attributed to their variations in seed physical properties (seed size, texture, seed coat and compactness) and compositional status (starch type, sugar and oil content) which influenced their physiological performance. To alleviate poor field emergence under sub-optimum conditions, seed enhancement treatments were used: hydro-priming (17 h / 25°C), halo-priming (KNO₃ at 3%), matrix priming (vermiculite), magnetic priming (200 Hz/h) and bio-priming (Trichoderma viridae). Among the treatments, seed hydro-priming (17 h / 25°C) could be adopted for alleviating the effect of sub-optimum temperatures on field emergence and early seedling vigour in speciality maize genotypes.
Development of a seed vigour test using single count of radicle emergence in chili (Capsicum annuum L.) seeds

Satriyas Ilyas and Dian Intan Karuniasari
Bogor Agricultural University, Department of Agronomy and Horticulture, Bogor, Indonesia (satriyas252@gmail.com)

Development of a seed vigour test is needed in order to conduct a fast, precise and simple test. A vigour test based on the rate of radicle emergence (RE) has been validated by ISTA (2014) for maize seeds. The objective of this research was to develop the RE test for chili seeds and correlate it with other vigour tests and field emergence. The experiments were conducted at the Seed Science and Technology Laboratory and at Leuwikopo field station, Department of Agronomy and Horticulture, Bogor Agricultural University, Indonesia, from February to May 2016. The experiment was arranged in a completely randomised design for the one conducted in the laboratory, and a completely randomised block design for the one conducted in the field, using one factor (seed lots) and four replications. The seed lots consisted of four lots of curly red chili seeds cv. Kencana (89–95.5% germination) and two lots of large red chili seeds cv. Anies IPB (70–90% germination). Observation of RE was conducted using the top of paper method at 25°C for 60–140 h (with 6 h-interval) after sowing. Results of the experiments showed that RE test, which was counted at 120 h, was significantly correlated and could predict mean germination time ($R^2 = 0.892$), normal germination percentage ($R^2 = 0.988$), vigour index ($R^2 = 0.659$), speed of normal germination ($R^2 = 0.864$), normal germination percentage after accelerated ageing ($R^2 = 0.685$) and after controlled deterioration ($R^2 = 0.788$), hypocotyl length ($R^2 = 0.758$), field emergence ($R^2 = 0.899$), and mean emergence time ($R^2 = 0.999$). Seedling growth parameters in the field were not correlated with RE; however, RE was able to predict variation in hypocotyl length ($R^2 = 0.697$) and root length ($R^2 = 0.651$). Therefore, a single count of radicle emergence test for chili seeds at 25°C counted at 120 h could be used as a vigour test.

Multispectral imaging in sugar beet seed (Beta vulgaris) to determine germination behaviour

Kenan Genctuerk¹, Peter M Deplewski² and Michael Kruse¹
¹University of Hohenheim, Fruwirthstr. 21, Stuttgart, Germany
²KWS Saat SE, Grimsehlstr. 31, Einbeck, Germany (kenan.genctuerk@uni-hohenheim.de)

Multispectral imaging has become an interesting non-destructive method in seed testing, as it can detect superficial seed properties that cannot be detected with the naked eye. In this study, the imaging system VideometerLab was used to find spatial and spectral parameters that correlate with inner quality parameters of sugar beet seed (Beta vulgaris). The system can illuminate objects evenly with 19 different wavelengths, ranging from 365 to 970 nm. A sensor then measures absorption and reflection and assigns a value for each pixel. By adding longpass filters with different wavelength thresholds, fluorescence values can also be quantified. Each image taken covers at least the size of a standard petri dish with 50 sugar beet seeds. The time to take an image varies between 10 and 60 seconds, depending on the number of wavelength–filter combinations and the exposure time. Afterwards, digital image analysis can be performed to extrapolate relevant data. This can be done for each object individually, thereby enabling single seed evaluation. For this experiment, KWS SE (Einbeck, Germany) provided 25 lower quality and 25 high quality seed samples, each of a different variety of sugar beet. Seed samples were polished. Standard quality tests were conducted to determine germination percentage and germination speed. From each sample, 130–200 seeds were examined with the VideometerLab and various correlation and principal component analyses were performed to evaluate the power of the multispectral imaging system for estimating germination behaviour. This analysis is based on the expectation that the
spectral properties of the fruit coat can indicate the germination properties of the embryo within the seed. Results show that there is variation of spectral properties of the fruit surface after polishing, both between samples and also between seeds within samples. Different structures of the fruit coat show different spectral properties and have different importance for estimating germination behaviour of the embryo. The relevance of these structures will be illustrated by pictures and analyses. If germination behaviour can be estimated accurately and reliably, time-consuming and labour-intensive tests could potentially be bypassed.
ORAL SESSION 2 – ENSURING SEED QUALITY FOR FUTURE GENERATIONS

Keynote presentation: Setting standards for native seed in ecological restoration

Kingsley Dixon¹, Olga Kildisheva² and Adam Cross¹
¹Curtin University, School of Molecular and Life Sciences, Kent Street, Bentley, Perth, Western Australia 6102, Australia
²Chicago Botanic Garden, USA
(kingsley.dixon@curtin.edu.au)

The global rise in ecological restoration in response to widespread loss of ecosystems and land degradation is to be applauded. However, both statutory large-scale restoration (e.g. Bonn Challenge, Africa’s Great Green Wall, etc.) and in-country funded programmes (e.g. GondwanaLink in Australia) will require quanta of native seed unprecedented in human history. Each year, the demand for native seed increases by an estimated 30%, meaning that the time is ripe to understand if, where and when native seed standards have a part to play. Just as agriculture and horticultural seed are supported by ISTA standards, the $1 trillion ecological restoration programmes should have similar standards. Here I will present the newly completed International Standards for Native Seed, compiled by an international team of restoration ecologists who are also seed specialists. The Standards speak to the unique and often confounding issues associated with native seed collection, storage and deployment in comparison to crop species. The presentation will give the opportunity for audience interaction.
Referencing the Global Information System of Plant Genetic Resources for Food and Agriculture

Fiona Hay¹, Francisco López², Marco Marsella² and Ruaraidh Sackville Hamilton²
¹Aarhus University, Forsogsvej 1, Flakkebjerg, Slagelse, Denmark
²Food and Agriculture Organisation (FAO), Viale delle Terme di Caracalla 1, Rome, Italy
(fiona.hay@agro.au.dk)

As the Editor of Seed Science and Technology, I often receive submissions in which an experiment or series of experiments have been carried out on a seed lot or a number of seed lots, the source of which is not always specified or only provided in relatively vague terms. In the case of experiments carried out on genebank material, the obvious and traditional way to refer to a particular sample is by using the accession number (which may contain letters as well as numbers), which is unique to that particular material in that genebank (though not seed lot, although that can also be specified). Conversely, countries that have provided material to genebanks complain that there is not enough information about the distribution of that material and the results of the subsequent research work carried out on it. To address this need, the Secretariat of the International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA) has created the Global Information System (GLIS) on PGRFA. Every accession (or sample derived from an accession) now has its own global unique identifier, a Digital Object Identifier (DOI) for germplasm. With this innovative mechanism, when a genebank sample is used in a study that is subsequently published, by listing the DOI(s) in the metadata of the article, the GLIS can cross-reference the article to the accession and to data records in other databases, making it possible for anyone to see information about the material, which increases its potential use and value. This presentation will describe how this is working and hopefully encourage all users of genebank material to make sure they correctly reference their material with the DOI(s) to enhance its value for plant breeders, farmers and other researchers.

First ever seed standards of Lasiurus scindicus for quality assurance

Vijay Dunna
Indian Agricultural Research Institute (ICAR), Pusa Campus, New Delhi, India
(vijaydunna@gmail.com)

Grasslands and rangelands play a vital role in the ecology of our planet. They are not only the source of fodder for livestock and wildlife, but also maintain ecological balance and biodiversity. Perennial grasses invariably play a key role in the conservation of natural resources by preventing the denudation of degraded land masses, thus preventing soil erosion, enhancing biodiversity and increasing carbon sequestration. The permanent pastures and other grazing lands sprawled over 10 million ha in India, are degraded and need revitalisation. The availability of quality seed is one of the main obstructions for achieving this target. Lasiurus scindicus, commonly known as sewan grass in India, is a predominant grass species found under desert environments in western Rajasthan. This grass is also present in the north African, west tropical African and north east tropical African countries, as well as in Saudi Arabia, Iran, Iraq, Afghanistan and Pakistan. It serves as a good forage source for livestock under harsh arid climatic conditions and helps in stabilising the sand dunes. Even though a lot of seed is being procured under different government schemes, the lack of seed standards obscures its quality. The present study was taken up to develop protocols and standards for this important grass species for the first time, as no standards are available at national and international levels. The germination protocol was developed by standardising the period of germination along with the substrate, temperature and light sources for effective viability studies. Dormancy breaking could be achieved using GA₃ treatment. The sieve sizes of 4.75 and 3.35 mm and working sample size of 20 g were standardised, based on several seed samples collected from 83 different locations of Jaisalmer, Badmer and Bikaner regions of Rajasthan. The seed standards
The seed semi-permeable layer and its relation to seed quality assessment in grass species

Yanrong Wang, Qiujin Sun, Yanyan Lv, Jing Zhou and Xiaowen Hu
Lanzhou University, No. 768 Jiayuguan West Road, Chengguan District, Lanzhou, China (yrwang@lzu.edu.cn)

The semi-permeable layer is a layer in the seeds of certain plants that restricts or impedes the exchange of the solute while allowing the permeability of internal and external water and gas, which is valuable protection to sustain the health and secure growth, development and germination. However, the knowledge of development, location, interspecies diversity of the semi-permeable layer and its relation to seed viability and vigour tests in grass species is limited. This study investigated the seed semi-permeable layer development, location, chemical component and its relationship with the seed vigour test in several grass species. The results showed that a seed semi-permeable layer was nearly formed at 10–12 days post-anthesis (dpa), whereas seed physiological maturity was 24–26 dpa at seed mature in *Elymus nutans* (Griseb.) and *Elymus sibiricus* (L.). A lanthanum nitrate tracer and X-ray dispersive energy analysis revealed that the layer was located in the seed coat adjacent to the aleurone layer in wheat, rice, maize and foxtail millet, while between the aleurone layer and the starch cell layers in sorghum, proso millet and sorghum hybrid Sudangrass. All species readily imbibed water. Rice seeds were permeable to tetrazolium chloride (TTC), whereas the embryo stained poorly with TTC in intact seeds of the other six species (strong staining was less than 16%). Strong staining ranged from 87–100% after puncturing. Electrical conductivity (EC) was negatively correlated with germination in intact rice, maize and proso millet ($R^2 = 0.8806, 0.9325$ and $0.8524$, respectively; $P < 0.01$), but there were no negative relationships among the other four species. After puncturing, EC was negatively correlated with germination for those four species ($R^2 = 0.8763, 0.9516, 0.8854$ and $0.9517$ for wheat, foxtail millet, sorghum and sorghum hybrid Sudangrass, respectively; $P < 0.01$).

The effect of a hot-drying treatment on the genetic dissection for seed longevity in rice

Jae-Sung Lee¹, Renato Reaño¹, Nora Kuroda¹, Ruaraidh Sackville Hamilton¹ and Fiona Hay²
¹TT Chang Genetic Resources Center, International Rice Research Institute (IRRI), Los Banos, College, Laguna, 4031, Philippines
²Aarhus University, Department of Agroecology, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark (js.lee@irri.org)

In our previous studies, the harvest moisture content (MC) was significantly negatively correlated with seed longevity in a diverse rice panel. High temperature seed drying (45–60°C) prior to routine cool (15°C) drying, significantly improved seed longevity compared with immediate cool drying. This was possibly due to enhanced protection mechanisms triggered by high temperature stress when seeds are moist. The effect of seed drying method on subsequent seed longevity may interfere with genetic analysis of this trait. In order to evaluate this, we have recently started comprehensive $G$ (genotype) × $E$ (environment) × $M$ (management) analysis of the variation in seed longevity. Seed
multiplication of 300 Indica rice accessions has been conducted in the dry and wet seasons of 2019 at the International Rice Research Institute. After-harvest seeds were subjected to immediate high temperature drying (45°C 24 h, then 15°C/15% RH) or control drying conditions (15°C/15% RH throughout). Comparative seed longevity experiments and analyses were performed. Using the full sequencing data, genome-wide association mapping will be conducted, and genes associated with seed longevity in each season and drying treatment will be compared.

Quality seed production in cucumber (Cucumis sativus L.) cv. Pusa Barkha

Dilshad Ahmad1, Sudhir Kumar Jain2 and Bhoopal Singh Tomar1
1Indian Agricultural Research Institute, Pusa Campus, Delhi, India
2Indian Agricultural Research Institute, Noida Sector 36, Delhi, India
(abhu7181@gmail.com)

Seed is the prime factor that determines the quantitative and qualitative characteristics of a crop that is going to be harvested. Therefore, more attention is directed towards knowing the pattern of seed development along with the physiological and harvest maturity. As the seed advances towards maturity, a decline in moisture content is associated with the accumulation of carbohydrates and proteins, leading to a temporal variation in seed germination. The present study was conducted on cucumber (Cucumis sativus L.) cv. Pusa Barkha at the Division of Seed Science and Technology ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India. For the establishment of the onset of germination, physiological maturity and harvest maturity, the selfing of flowers was done every day throughout the period from the start of anthesis. Fruits were harvested at periodic intervals from the day of pollination. Seeds were also extracted manually from fruits and used for various studies, viz. onset of germination (on the basis of first germination), physiological maturity (on the basis of highest germination) and harvest maturity (on the basis of seed dry weight). At physiological maturity, the seed attains maximum germination and vigour, but the moisture content remains very high. At harvest maturity, however, as for physiological maturity, the seed is also maintaining high germination and vigour but low moisture content. Thus, quality cucumber seed under Delhi conditions can be harvested on 38–40 DFP. Since the freshly harvested seeds are dormant, the farmers/seed producers may use these seeds by treating with GA3 appropriately.

New approaches to provide a rapid assessment of germination in seeds of native species

Maria Marin1, Alison Powell1, Giles Laverack2 and Stan Matthews1
1University of Aberdeen, Plant and Soil Science, Cruickshank Building, St. Machar Drive, Aberdeen, UK
2Scotia Seeds, Mavisbank, Farnell, Brechin, UK
(a.a.powell@abdn.ac.uk)

Seed quality of native species is important for habitat restoration projects as it determines the ability of a seed to germinate and establish a plant. However, a survey of seed lots from eight European native species has revealed a wide range in germination (radicle emergence) from 0 to 99%. This partly reflects the absence of quality testing within many suppliers of native seed, which can be attributed to the absence of regulation and test procedures, complex dormancy breaking requirements and the long periods often needed for a germination test. We will describe three alternatives to the germination test (tetrazolium staining, TZ; electrical conductivity, EC; radicle emergence, RE) that provide simple, rapid assessments of the germination potential (radicle emergence) of native species. Tetrazolium staining was applied to 113 seed lots from eight species (Centaurea nigra, Cyanus segetum, Knautia arvensis, Papaver rhoes, Prunella vulgaris,
Rhinanthus minor, Silene vulgaris, Valeriana officinalis). ISTA guidelines for a TZ test for three genera, Centaurea, Papaver and Silene, were used, with protocols being developed for the other genera. Tetrazolium staining was highly predictive of germination for all species, identifying low quality lots in 48 hours, with coefficients of determination ($R^2$) of $>0.94^{***}$ in six species; $0.81^{***}$ and $0.80^{***}$ in the other two species. Combined data from all species revealed a universal relationship across species between the TZ staining assessment and germination ($R^2 = 0.95^{***}$). Eighty-three seed lots from seven of the above species (excluding R. minor) were used to assess the potential for EC to predict germination. The EC measurements after 24 hours were significantly correlated with germination of seeds from four genera (Centaurea, Cyanus, Prunella and Valeriana). The highly significant relationships between the 24-hour EC and EC readings after 3 ($R^2 = 0.913^{***}$) and 5 hours ($R^2 = 0.943^{***}$) soaking suggested that EC could predict germination in as little as 3 or 5 hours. Seed structure may determine the potential for EC to predict germination as there was no relationship in genera where there was an endosperm present (Silene, Knautia and Papaver). In Cyanus segetum, a high EC was also associated with slow germination and a single early count of RE (after 42 hours) predicted the germination of 12 seed lots ($R^2 \geq 0.858$). Thus, TZ and EC tests, and early RE can all provide a rapid prediction of the final germination (radicle emergence) of native species within a maximum of 48 hours.
ORAL SESSION 3 – ENSURING SEED HEALTH AND IMPLICATIONS OF CHANGE FOR SEED PATHOLOGY

Keynote presentation: How a basic understanding of watermelon seed infection by Acidovorax citrulli has improved seed health testing

Ron Walcott
Department of Plant Pathology, University of Georgia, 210 S. Jackson St., Athens, GA, USA
(rwalcott@uga.edu)

With the widespread adoption of molecular tools that facilitate economical and specific nucleic acid amplification, we now have standardised platforms for highly sensitive plant pathogen detection. Despite these powerful tools, robust seed health testing is still difficult to consistently achieve because: 1) the distribution of infested seeds within contaminated seed lots is highly variable, and 2) for many diseases, there is low or zero tolerance for pathogen propagules. As a result, appropriate sampling schemes must be designed and [often] large seed samples must be tested. This creates a challenging ‘needle in a haystack’ conundrum, whereby plant and non-target nucleic acids extracted from seeds dilute target nucleic acids to undetectable levels. To improve seed health testing, we must improve our understanding of how seeds become infected, and more importantly, where pathogen propagules are located in infected/infested seeds. Using Acidovorax citrulli as a model, we observed that watermelon seeds could be infected by bacterial penetration through the fruit rind (pericarp) or via invasion of the flower pistil. Interestingly, bacterial localisation in watermelon seed tissues differed based on the pathway of invasion. More specifically, penetration of the fruit pericarp led to A. citrulli cell localisation in the inner layers of the testa, while flower (pistil) invasion led to bacterial localisation in the endosperm/embryo tissues. The ability to detect A. citrulli varied significantly based on bacterial localisation in seed tissues and the technique used to extract the bacterial cells. While seed washing followed by polymerase chain reaction (PCR) assay efficiently facilitated detection of A. citrulli in pericarp-infected watermelon seeds, this was not the case for pistil-infected seeds. Crushing seeds prior to PCR was necessary to facilitate detection of pistil-infected seeds. It is important to note that while crushing seeds improved the extraction of bacterial cells, it also released inhibitory compounds that could negatively affect detection assays like PCR. Thus, knowledge of the location of pathogen propagules in seeds is critical for facilitating effective pathogen extraction and improving seed health testing.
Low-pressure plasma seed treatment as a substitute for chemical seed treatment in case of common bunt (*Tilletia caries*)

Sebastian Bopper, Sophie Mühlberger and Michael Kruse
University of Hohenheim, Fruwirthstr. 21, Stuttgart, Germany
(sebastian.bopper@uni-hohenheim.de)

Common bunt (*Tilletia caries* (DC) Tul. & C. Tul.) is an important seed-borne disease in wheat (*Triticum aestivum* L.). The teliospores survive on the surface of seeds. Seeds contaminated by spores must be treated with synthetic chemical agents for full prevention. Non-chemical treatments, such as the thermic methods used in organic agriculture, are effective but laborious. Thus, alternative treatments are under discussion like a low-pressure plasma treatment. The most important benefit of such a plasma treatment is that no toxic agents are applied because it is a physical seed treatment. Preliminary tests have shown that a plasma treatment has the potential to reduce the germination rate of the teliospores. However, the treatment was not as effective as a common chemical seed treatment. The biggest challenge for the plasma treatment was to overcome the robust cell wall of the teliospores. Based on these findings, further experiments were carried out. The strategy was to develop a physiological pretreatment to make the teliospores more susceptible to a plasma treatment. In the first step, the focus was to identify an effective pretreatment. Therefore, a membrane filter method was developed to test the effect on the teliospores only. For the second step, seeds of wheat were inoculated and the most promising method of step one was tested on the infected seeds. For both steps, two isolates of *T. caries* were used. The germination rate of the teliospores was significantly reduced by up to 98%. Tests with spore-contaminated seed showed a significant reduction of teliospore germination rate up to 68%. Seed germination was only impaired to a small extent. The results of the present study indicate that a plasma treatment can be used successfully to reduce the infection level without negative effects on seed germination.

Is seed a pathway? Updating the *List of Seed-Borne Diseases*

Nicolas Denancé¹, Christine Henry², Terry Aveling³ and Valérie Grimault¹
¹Laboratory of Phytopathology, SNES, GEVES, 25 rue Georges Morel – CS90024 – F49071, Beaucouzé, France
²Fera Science Ltd, York, UK
³University of Pretoria, Pretoria, South Africa
(nicolas.denance@geves.fr)

Agricultural crops are cultivated in diverse geographic regions, most often from seeds that were harvested elsewhere. Seed trading offers multiple routes for pathogen dissemination worldwide. Knowing the pests for which seed is a pathway is of importance for seed trade and regulatory purposes in countries to help governments and national plant protection organisations to define the list of pests for which seeds should be tested. Phytosanitary requirements for import of seed lots are supported by the book 'An Annotated List of Seed-Borne Diseases' (first released in 1958) that needs to be updated (last released in 1990). A few years ago, a large literature survey was undertaken in the framework of a European project, to identify references on seed-associated pests. All the data, covering 375 host plant genera, were uploaded to the ISTA database, waiting for validation by international expert scientists. To avoid repetition, references related to vegetable species were transferred to the International Seed Federation (ISF). At ISTA, the Seed Health Committee (SHC) focuses on non-vegetable species. The 'Pest List Project', funded by ISTA, was initiated in 2018. It involves the analysis of the existing references and the conduct of a review by scientific experts to provide an updated list of pests. The project deals with 11 crops: alfalfa, barley, cotton, oat, rapeseed, rice, sorghum, soybean, sunflower, triticale and wheat. Altogether, more than 1900 references dealing with 259 seed pests, seed treatment or more general topics, have been scrutinised. A shortlist of almost 300 references was selected for further investigation, as they
reported on seed-borne pathogens. These are being sent to an international board of experts from academic laboratories and private seed companies to verify their scientific relevance. An extension to the project will allow the treatment of data collected for 34 other species, mainly on forest and fruit trees, as well as some legumes. Once collected and analysed, these data will contribute to define the list of pests using seed as a pathway on a given host. The information will be released on the ISTA database and will be freely accessible to the seed community from late 2018. Current advances will be presented.

Seed transmission of soybean yellow mottle mosaic virus in natural and experimental hosts

Sandra Nagamani1, Ankita Tripathi1, HK Dikshit2, Bikash Mandal3 and Atul Kumar1
1Indian Agricultural Research Institute, Division of Seed Science and Technology, ICAR-IARI, New Delhi, India
2Indian Agricultural Research Institute, Division of Genetics, ICAR-IARI, New Delhi, India
3Indian Agricultural Research Institute, Advanced Centre for Plant Virology, ICAR-IARI, New Delhi, India
(nagamani.iari@gmail.com)

Soybean yellow mottle mosaic virus (SYMMV) is a newly identified species belonging to the genus Gammacarmovirus within the family Tombusviridae. SYMMV was initially reported from soybean in South Korea, North America and later mung bean, urdbean and soybean isolates were reported from India. However, the seed transmission nature of SYMMV is still not clear. For this purpose, seed material was collected from infected mung bean (cv. Pusa Vishal) plants which showed mosaic, mottling and puckering symptoms. Infected seed material which showed discolouration and deformation was initially tested through direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA) using SYMMV polyclonal antisera, followed by reverse transcription polymerase chain reaction (RT-PCR) with coat protein (CP) specific primers, to detect the SYMMV from various parts. The results showed a high virus concentration in both seed and cotyledons but not in the seed coat and embryo portion. Similar results were observed in RT-PCR as 1065bp amplicon from the whole seed and cotyledon. To observe the seed transmission nature, a growth test was conducted with the 30 infected seeds which showed 33% germination of seedlings with leaf necrotic symptoms. Seedlings raised from the infected seed material showed SYMMV in all parts, 1065bp amplicon with CP specific primers. The seed transmission nature of SYMMV was also tested in the experimental host French bean cv. Pusa Parvati through mechanical sap inoculation. Infected plants showed mottling on the infected leaf and chlorotic blotches on the systemic leaves. DAC-ELISA and RT-PCR were conducted with replicase, movement and CP specific primers on inoculated leaf, systemic leaf, whole flower, sepals, petals, stamen and stigma, immature pod, mature pod shell, whole seed, seed coat, cotyledons and embryo. The results showed a high concentration of SYMMV in all parts, 1065bp amplicon with CP specific primers, 450bp with movement protein primers and 700bp amplicon with replicase protein specific primers. A growth test was conducted with seed material collected from the infected plants and showed 80% germination with mild mottling symptoms. Out of 80% germination, 40% of the seedlings reacted positively with CP primers in RT-PCR. Further mechanical sap inoculation was conducted on French bean cv. Pusa Parvati with sap collected from mung bean and French bean seedlings raised from the infected seed material. However, sap inoculated plants did not react positively in either DAC-ELISA or RT-PCR with CP primers, indicating the confinement of SYMMV seed transmission to the first generation only. This is the first evidence for the seed transmission nature of SYMMV mung bean isolate.
**Pinus patula** in South Africa: effect of seed-borne mycoflora on germination, their control and first report of *Sydowia polyspora*

Renaan Thompson¹, Theresa Aveling¹, Mervyn Beukes² and Guro Brodal³

¹University of Pretoria, Department Plant and Soil Sciences, Pretoria, South Africa
²University of Pretoria, Department of Biochemistry, Genetics and Microbiology, Pretoria, South Africa
³NIBIO, Division of Biotechnology and Plant Health, Department of Fungal Plant Pathology, As, Norway

(terry.aveling@up.ac.za)

Naturally occurring and introduced pathogens have been recorded in South African pine plantations which contribute 3.4% to South Africa's GDP and covered 607 815 ha during the 2015–16 season. *Pinus patula* seeds generally have a low germination percentage (25%) within South African nurseries but the cause of this is unknown. The seeds were subjected to ISTA's ‘top of paper’ germination test but were heavily infected by fungi. The role mycoflora may play in reduced germination was evaluated by screening seed lots of *P. patula* harvested in South Africa using nutrient rich (potato dextrose agar) and nutrient deprived (malachite green agar) growth media. Numerous (451) fungi isolated from the seed were purified by means of single spore and hyphal tip isolations and stored using cryopreservation and agar slants. To evaluate seed treatments for the control of the seed-borne mycoflora, seed lots were subjected to: no treatment, stratification, bubbling, imbibition and surface disinfestation using various concentrations of NaOCl and H₂O₂ for different time intervals. Seed treatments, especially NaOCl and H₂O₂ at low concentrations improved the overall germination percentage. Among the mycoflora isolated, *Sydowia polyspora* (Bref. & Tavel) E. Müll. was morphologically identified from three different *P. patula* seed lots and the identity confirmed by sequencing of the ITS, RNA polymerase II subunit (RPB2) and the β-tubulin gene region. The sequences were blasted against Genbank and subjected to phylogenetic analyses using the CLC main workbench. *S. polyspora* was also further isolated from pine needles in seed orchards showing yellowing and browning symptoms. Pathogenicity tests of *S. polyspora* on one-year-old *P. patula* seedlings showed typical symptoms on the needles of inoculated seedlings after two weeks, with a disease incidence of 100% and disease severity ranging from 5 to 25%, while control seedlings remained symptomless. Re-isolated *S. polyspora* was sequenced to confirm identification. To the best of our knowledge, this is the first report of the seed-borne pathogen isolated from *P. patula* seed within South Africa. Further studies will include prevalence, genetic diversity of the different isolates and development of control measures for the pathogen.

**Dityluz: development of tools for the detection and viability testing of stem nematode, *Ditylenchus dipsaci*, on alfalfa seeds (*Medicago sativa* L.)**

Geoffrey Orgeur, Lorine Ledare, Thomas Baldwin, Julie Brachet, Clémence Galon, Alix Marrec, Audrey Chamaille and Valérie Grimault

GEVES, 25 rue Georges Morel, CS-900024, 49070 Beaucouzé, Angers, France

(geoffrey.orgeur@geves.fr)

In France, the stem nematode, *Ditylenchus dipsaci*, is a threat to alfalfa seed production. *D. dipsaci* is also a quarantine pest on alfalfa seeds within the European Union (Directive 2000/29/EC), only nematode-free seeds can be marketed. Until 2010, contaminated seeds were exclusively disinfected by fumigation with methyl bromide. Following the removal of this product, seed companies have had to develop alternative techniques to eliminate this pathogen, either by industrial processes or thermotherapy. The viability of nematodes is not currently a factor in the certification scheme. Therefore, seed lots are considered positive even if all the nematodes present are dead and pose no risk to the crop; this constrains the application of alternative treatment techniques. The Dityluz
The Dityluz project was based on a multiple and innovative partnership involving actors from the seed sector (UFS and GNIS) and public research (ANSES, Laboratories of Pathology and Molecular Biology of GEVES) as well as scientific and technical experts (INRA and FNAMS). Using a diversity of techniques (molecular biology, morphobiometrics, staining, etc.), the Dityluz project has allowed the development and transfer of tools to detect and differentiate live/dead nematodes in seed lots to the seed companies. A detection method on seed extracts by polymerase chain reaction (SE-PCR) was developed. The method consists of pooling the population of nematodes in a small volume and analysing it by PCR. The method was validated in a ring test where performance criteria were evaluated: the detection threshold was determined at 1 *D. dipsaci*, and sensitivity, specificity, accordance and concordance were determined at 100%. In addition, a method for assessing the viability of *Ditylenchus dipsaci* was also developed and validated according to the evaluation of performance criteria. The results of this project were made available to seed companies and the Official Control Service through the organisation of a workshop. This programme is intended to implement operational measures to improve the technical regulation and certification scheme for alfalfa seed lots.

**How to adapt methods to evaluate seed health of treated seeds**

Isabelle Serandat, Geoffrey Orgeur and Valérie Grimault  
GEVES, 25 rue Georges Morel, CS 900024, 49070 Beaucouzé, Angers, France  
(isabelle.serandat@geves.fr)

To a large extent, seeds traded around the world have received some kind of treatment, which can be a chemical treatment, physical treatment, disinfection or organic treatment. All of these different treatments can have consequences with regard to methods of detection of pathogens in seed lots. It is important for seed trade to be able to define if pests can be detected on treated seeds and to know if the result is due to the efficiency of treatment or if the treatment affects the performance of the method for detecting the pest. With regard to most non-chemical seed treatments, pathogens may still be detectable even though they are non-viable. This fact may lead to the need of an additional requirement for measuring the viability of any pest detected after treatment. Current detection methods have mainly been developed and validated for untreated seeds. Due to the high diversity of treatments, it could be difficult to validate the methods for each type of treatment. Thus, it is necessary either to review and adapt current methods or develop and validate new methods. Examples will be given for: methods which can be affected by the treatment and where controls have been adapted to check their performance; methods which are not affected by the treatment; and new methods to determine viability of pests after treatment.
ORAL SESSION 4 – SEED PRODUCTION IN A CHANGING ENVIRONMENT

Keynote presentation: The changing face of the Indian seed sector: challenges and opportunities

Raj S Paroda
Trust for Advancement of Agricultural Sciences (TAAS), Agriculture and Cooperation Department, Telangana Secretariat, Telangana State, Hyderabad, India (raj.paroda@gmail.com)

Considering the importance of improved seed for increasing crop productivity, the Government of India established the public sector seed organisation, the National Seeds Corporation (NSC) in 1963, followed by the State Farms Corporation of India (SFCI) in 1968. Subsequently, the Terai Development Corporation (TDC) was established in 1969, which set a unique model for the state seed corporations (SSCs) in the 1970s and 80s. Under the National Seed Project (NSP), funded by the World Bank, 15 SSCs, 22 state seed certification agencies (SSCAs) and 17 seed processing plants, were established during this period. One central seed testing laboratory, 122 notified seed testing laboratories, six ISTA accredited laboratories and 19 ISTA member laboratories, were established in the following years for seed quality assurance. To ensure competent human resources, a number of state agricultural universities created Departments of Seed Technology.

An enabling policy decision by the Indian Council of Agricultural Research in the 1990s, to provide breeder seed of the parental lines of public-bred hybrids, played a key role in accelerating the growth of the seed sector in India, which increased the availability of quality seed. Simultaneously, the New Policy on Seed Development 1988, heralded much faster growth of the private seed industry, at national and multinational levels, and some in partnership.

Recognising the importance of a regulatory system for production, testing and supply of quality seeds, a Seeds Act was promulgated by Parliament in 1966. Later, for the protection of intellectual property rights (IPRs) and breeders’ rights and to support the growth of seed sector in India, laws such as: National Biodiversity Act (NBA), Intellectual Property (IP) Order, and Protection of Plant Varieties and Farmers’ Rights Act (PPV&FRA), were enacted. A direct effect of these steps was the increased availability and use of hybrid seeds in different crops (in some cases even up to 80–95%), resulting in enhanced productivity in field crops as well as vegetable crops. The most significant change was noticed with the release of Bt cotton hybrid in 2002, leading to almost 93% coverage of cotton area (about 12.0 m ha) by 2014–15. As a result, cotton productivity increased by 139% and India became the second largest exporter of cotton in the world. By 2017, the Indian seed market reached US $3.6 billion in value, exhibiting a compound growth rate of 17%. It is expected to grow by about 14% to a value of US $8.0 billion in the next five years.

Despite these impressive achievements, the Indian seed sector today is faced with challenges of productivity gaps, low replacement rates under quality seeds of high yielding varieties (HYVs) / hybrids, lack of public–private partnership, poor access to genetic resources, declining R&D investments in plant breeding and, above all, the uncertain policy environment for scaling innovations such as genetic modification (GM) technology. These need to be addressed on priority, especially to harness the opportunities such as: favourable agro-climatic regions, more irrigation potential, existing productivity gaps, new innovations for genetic enhancements and the increasing requirement for skilled manpower for the seed sector. The future strategy ensuring much-needed reforms in the Indian seed sector, including new policy changes and focus on quality assurance with the use of IT and AI, will be addressed proactively.
Sunflower field establishment: germination and seedling growth related to temperature

Marie-Hélène Wagner¹, Carolyne Dürr², Didier Demilly¹, Thierry Andre³, Benoit Bleys³, Christophe Bailly⁴, Marion Laporte⁵ and Sylvie Ducournau¹

¹GEVES, 25 rue Georges Morel, Beaucouzé, France
²INRA, 42 rue Georges Morel, Beaucouzé, France
³SOLTIS, Domaine de Sandreau, Mondonville, France
⁴Université Pierre et Marie Curie, 4 place Jussieu, Paris, France
⁵RAGT2n, Station de Recherche La Courtade Haute, Rivieres, France
(marie-helene.wagner@geves.fr)

To avoid water stress and heat during the flowering stage, early sowing has become common practice in the production of field crops in France. As summers get hotter and drier, the practice of early sowing is now being tested on sunflower production. To achieve this, breeders require tools and data that allow them to screen the parent lines they have in their genetic resources. This will allow them to obtain hybrids capable of germinating and growing at low temperatures, that are adapted to earlier sowings or to new production areas in northern Europe. It has also been demonstrated that the longer the crop cycle is, the higher the yield will be and so the more attractive sunflower production will become for farmers. A public–private research project on sunflower has highlighted the genetic diversity of germination at low temperature, present in the reference collections of the private partners. A set of 200 genotypes (70% linoleic and 30% oleic genotypes) was phenotyped over a two-year period in different field conditions. At the same time, these genotypes were tested in controlled conditions and their germination response at different temperatures was observed using image analysis. Representative genotypes were then chosen and their base temperature for germination was determined at 2.2 ± 0.9°C with no significant difference among genotypes. This base temperature was higher and varied from 5.5 to 8.3°C for seedling growth. In both cases, linoleic genotypes and oleic genotypes did not differ significantly. The process of data collection, to automatically plot a germination curve, identified 7 days at 5°C as an efficient and rapid screening protocol to test parental lines or hybrids, for their tolerance to cold conditions during the establishment stage.

Rice seed production in a changing climate

Duangporn Angsumalee¹, Punnee Thongket¹, Srisakul Thamdee², Watcharapong Wannawong³, Wongduan Fakkam⁴, Nimit Khamboonme⁵, Wannamanee Phongpaiboon⁶, Kanuttaya Lalitchamroon⁷ and Kannika Seenuanmak⁸

¹Rice Department, Ministry of Agriculture and Cooperatives, 50 Phaholyothin Road, Ladyao, Chatuchak, Bangkok, Thailand
²Chiangmai Rice Seed Center, Chiangmai, Thailand
³Kamphaengphet Rice Seed Center, Kamphaengphet, Thailand
⁴Nakhonsawan Rice Seed Center, Nakhonsawan, Thailand
⁵Chai Nat Rice Seed Center, Chainat, Thailand
⁶Sakonnakhon Rice Seed Center, Sakonnakhon, Thailand
⁷Ubonratchathani Rice Seed Center, Ubonratchathani, Thailand
⁸Ratchaburi Rice Seed Center Ratchaburi Thailand
(duangporn.a@rice.mail.go.th)

In changing climate, rice seed production needs to be adapted to ensure food security. This study was to investigate the climate effect on seed yield, yield components and quality. Non-photosensitive rice variety RD61 was planted in winter, summer and the rainy season. Planting periods were arranged to coincide the flowering stage with the cold, heat and control treatments, during
December, April and August, respectively. The two-year field trials were conducted in 2016/2017 and 2017/2018 at 12 locations in Thailand: Chiangrai, Chiangmai, Kamphaengphet, Nakhonsawan, Nongkhai, Sakonnakhon, Roi-et, Udonratichathani, Chainat, Ratchaburi, Nakhonsithammarat and Pattalung province. Harvested seeds were kept in the commercial open warehouse of the Rice Seed Centers for 12 months. Seed viability and vigour were determined at monthly intervals. During the flowering period, the minimum temperature (12.0°C) was found in the winter crop at Chiangmai while the maximum temperature (41.2°C) was recorded in the summer crop at Nakhonsawan. These minimum and maximum temperature records were -3.3°C and +3.1°C different from the 30-year mean minimum and maximum temperature, respectively. Seed yield and yield components were significantly different in each location due to the uncontrolled environment of the farmer fields. The winter crop at most locations resulted in low seed yield, low seed set, extended time to harvest and shorter plant height. The summer crop at each location resulted in various responses in seed yield and other yield components. Overall, the average seed yield was 2.331, 3.056 and 3.343 t ha⁻¹ for winter, summer and rainy crops, respectively. Compared to the rainy crop, the average seed yield in the winter crop reduced by 30% because of the 20% decrease in seed set. In the summer crop, the average seed yield reduced by 9% due to the 6% reduction in seed set. Although the average seed weight in the summer crop was 4% heavier than those in the rainy crop, the seed weight partly compensated for the reduced seed number. Seed quality at harvest did not show a significant difference among the planting periods and locations, with on average, 95.50% in viability and 92.58% in vigour. During 12 months of storage, seed quality gradually dropped in each month. The fastest seed deterioration was found in the humid open warehouse of Chiangmai Rice Seed Center, dropping to below 80% of seed viability after six months of storage. However, seeds kept in the dry open warehouse maintained quality at above 80% of viability throughout the 12 months of storage.

Impact of the maternal environment on seed physiology and biochemistry in barley

Manuela Nagel¹, Erwann Arc², Loïc Rajjou³, Oscar Lorenzo Sánchez⁴, Andreas Börner¹ and Ilse Kranner²
¹Genebank Department, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben), Seeland, Germany
²Department of Botany and Center of Molecular Biosciences (CMBI), University of Innsbruck, Innsbruck, Austria
³Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78000, Versailles cedex, France
⁴Instituto Hispano Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca, Salamanca, Spain
(nagel@ipk-gatersleben.de)

Seed quality, important for agriculture and seed industry, comprises the biochemical and genetic makeup that affect physiological characters, such as seed germination, vigour and longevity. Adverse environmental conditions during seed development alter the biochemical composition and degrade seed quality. Therefore, the aim of the study is to understand how the genetic background in conjunction with stressful environmental conditions affect seed development and consequently, seed quality. Plants of two genotypes of two-row barley (Hordeum vulgare L.), the short-lived HOR 2110 and the long-lived HOR 4710, were subjected to drought (23/15°C, 15% field capacity) and elevated temperature (28/25°C), conditions which may appear during climate change. In both genotypes, TSW was strongly reduced by drought, and to a lesser extent, also by elevated temperatures. In both genotypes, drought had pronounced effects on the metabolite profiling, whereas elevated temperatures showed significant effects only for the genotype HOR 4710. This genotype developed smaller plants and spikes, and produced smaller and fewer seeds. Seeds of HOR 4710 were also thermodynamically dormant when they were grown at 20°C but released dormancy at 10°C. Seeds produced under drought imbibed faster and, at late imbibition, 12 metabolites
especially showed a differential accumulation. In conclusion, barley is adapted to a wide range of environmental conditions and, hence, can tolerate stressful conditions. However, seed vigour is affected by maternal environment during seed development which might be an important aspect for plant breeding and seed production.

Novel breeding methods and innovations in seed production for delivering quality seed in changing climates

Ashok Kumar A, Shivaji P Mehtre, Jayakumar Jaganathan, Hari Prasanna, Sunita Gorthy, Kotla Anuradha, Sharad Rao Gadakh, Uttam Chavan, Kalpande HV, Ashok Jadhav and Vilas A Tonapi
1International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad 502324, India
2Vasantrao Naik Marathwada Krishi Vidyapeeth (VNMKV), Parbhani, Maharashtra 431401, India
3ICAR – Indian Institute of Millets Research (IIMR), Rajendranagar, Hyderabad 500030, India
4Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, Maharashtra 413705, India
(a.ashokkumar@cgiar.org)

The global population has reached 7.2 billion and counting. Though considerable progress has been made in providing food security, nutrition security remains elusive. In fact, micronutrient malnutrition, particularly among women and children, is one of the greatest global challenges of our times. National governments and international organisations are following various approaches to combat this issue. We developed a novel breeding method called ‘biofortification’ to address this. Biofortification, increasing the micronutrient density in edible plant parts by genetic means, is a cost-effective and sustainable method to address micronutrient malnutrition. Sorghum is one of the major staples globally and it meets more than 50% micronutrient requirements of low-income group populations in predominantly sorghum eating areas. We developed biofortified sorghums with elevated levels of grain Fe and Zn, combined with higher grain yield possessing farmer-preferred grain and stover quality traits. The first biofortified sorghum cultivar ‘Parbhani Shakti’ was released in India in 2018, which, besides high Fe and Zn, has a higher protein content and lower phytates content. An innovative ‘Seed Consortium’ was established to take this variety to the farmers in the shortest possible time, so benefiting the farmers and consumers. Off-season seed production areas were identified and planting time standardised to produce quality seed to meet the seed demands in changing climates. This multi-stakeholder partnership was the key in this endeavour and the Indian National Agricultural Research System (NARS), farmers, public sector seed organisations, media and the Government played a key role, along with ICRISAT.

Effect of cultivars and maternal environment on seed quality in Vicia sativa

Xiaowen Hu, Lingjie Yang and Rong Li
Lanzhou University, No. 768 Jiayuguan West Road, Chengguan District, Lanzhou, China
(huxw@lzu.edu.cn)

Production of high quality seeds is of fundamental importance for successful crop production. However, knowledge of the effects of increased temperature resulting from global warming, on seed quality of alpine species is limited. We investigated the effect of the maternal environment on seed quality of three cultivars of the leguminous forage species Vicia sativa, giving particular attention to temperature. Plants of each cultivar were grown at 1 700 and 3 000 m a.s.l., and mass, germination, electrical conductivity (EC) of leachate and longevity were determined for mature seeds for three years continuously. Seeds of all three cultivars produced at the low elevation had
a significantly lower mass and longevity but higher EC of leachate than those produced at the high
elevation, suggesting that increased temperatures decreased seed quality. However, seed viability
did not differ between elevations. The effects of the maternal environment on seed germination
strongly depended on cultivar and germination temperature. At 10 and 15°C, seeds of ‘Lanjian 3’
produced at high elevation germinated to higher percentages and rates than those produced at
low elevation, but the opposite trend was observed at 20°C. However, for seeds of ‘Lanjian 1’ and
‘Lanjian 2’, no significant effect of elevation was observed in germination percentage. Our results
indicate that the best environment for the production of high quality seeds (e.g. high seed mass,
low EC, high seed longevity) of $V.\text{ sativa}$ is one in which temperatures are relatively low during seed
development.

Seed quality development in pepper ($Capsicum\text{ annuum}\ L.$) grown in
organic and conventional cultivation systems

Kutay Coşkun Yildirim$^1$ and İbrahim Demir$^2$

$^1$Atatürk Horticultural Central Research Institute, Suleymabey mah. Araştırma sk., Yalova,
Turkey

$^2$Ankara University, Faculty of Agriculture, Department of Horticulture, Diskapi, Ankara, Turkey
(kutaycoskun.yildirim@tarimorman.gov.tr)

The aim of the work was to compare seed quality development in conventional to organic growing
systems. High quality seed production in organic conditions as well as conventional methods is
crucial. Seed production was carried out under organic and conventional cultivation conditions
with the Yalova Yaglik 28 pepper cultivar, in 2015 and 2016 in the Experimental Field of Atatürk
Horticultural Central Research Institute, Yalova, Turkey. In both cultivation systems, fruits were
harvested in three different periods (40–55, 56–70 and 71–85 days after anthesis). Then in
each harvest period, seeds were extracted and dried. Germination (25°C, 14 days, 4 replicates
of 50 seeds), emergence (20 days, 4 replicates of 50 seeds, 23°C, peat moss) and controlled
deterioration (45°C, 20% SMC, 48 h) tests were applied to dried seeds. The differences in seed
viability and vigour have not been found significant between organic and conventionally produced
seeds. Seeds of the second harvest period (56–70 DAA) in both production systems were found to
have higher viability (90.5% and 94% in organic and conventional system) and vigour (85%, 88%
in CD; 87%, 93% in emergence test in organic and conventional growing systems, respectively)
compared to the first and third harvest period in both years. Hence, cultivation systems did not
affect the occurrence of the time of seed quality of pepper seeds. For the production of high quality
pepper seeds, the interactions between cultivation systems, environment and harvesting periods
must also be taken into account.
ORAL SESSION 5 – NEW TECHNOLOGY AND NOVEL METHODS FOR SEED QUALITY ASSESSMENT

Keynote presentation: Plant phenotyping – opportunities, challenges and integrated approaches for seed phenotyping and beyond

Ulrich Schurr
Forschungszentrum Jülich, IBG-2: Plant Sciences, Wilhelm-Johnen-Straße, 52428 Jülich, Germany
(u.schurr@fz-juelich.de)

Quantitative analysis of structure and function of plants has become a major bottleneck that must be overcome, to identify the associations between phenotypic and genotypic data that is required for practical applications and basic science. A coordinated effort is required to effectively address this gap and enable a close interaction between phenotyping experts and users from different disciplines, to specifically address the demand and requirements of different disciplines.

EU-funded research infrastructure projects such as EPPN2020 or the ESFRI-listed project EMPHASIS, aim to integrate the plant phenotyping community in Europe and to address this bottleneck. While the EPPN2020 project enables practical access to plant phenotyping installations for user-driven plant phenotyping experiments in Europe, the EMPHASIS project develops a long-term sustainable operation of the infrastructure.

In this presentation, I will introduce the current state-of-the-art in the plant phenotyping community by presenting the currently available infrastructure. A specific focus will be on the diverse options for seed phenotyping, ranging from analysis of individual seed characteristics to bulk seed properties. Novel sensors, automaton and robotics, data management and data science analysis open new windows of opportunity for seed physiology and seed testing.
Identification of morphologically similar seeds using computer vision and image analysis

Ruojing Wang¹, Xin Yi², Mark Eramian², Jennifer Neudorf¹, Angela Salzl¹ and Janine Maruschak¹
¹Canadian Food Inspection Agency, 301–421 Downey Road, Saskatoon, Canada
²Department of Computer Science, University of Saskatchewan, 110 Science Place, Saskatoon, Canada
(ruojing.wang@canada.ca)

Seed identification, or identifying plant species from seed morphological features, is a routine diagnostic test in seed certification for seed trade, phytosanitary certification for import and export of agricultural commodities, as well as regulatory monitoring, surveillance and enforcement. Seed identification is currently performed by specialised professionals and experts, with limited tools to assist them. Use of digital image analysis for the identification of seeds is not yet recognised as a validated method, although computer vision for automated seed identification had been suggested more than three decades ago. In this study, computer and seed scientists collaborated and explored the potential of computer vision to automatically distinguish similarly featured or closely related species using their digital images. An image reference data set was constructed, containing seed specimens verified to represent their respective species and typical population variation. Since the output of computer classification depends heavily on feature selection, a collection of texture, colour and shape features are considered in this study. With representative specimens, the feature of seed texture proved to be an important discriminator in our experiments for the species we used. With selected computer classification, feature, texture and local image descriptors were calculated from the image at points on a regular grid. These were encoded to a representation known as the ‘bag-of-words’ model. With the determination of the optimum grid intervals and scales, computer vision achieved a high recognition rate, greater than 97% for difficult species in seed testing. Following up with the above result on computer vision algorithms for seed identification, we conducted a user comparison study to evaluate the effectiveness of automatic identification from live seed images to species that are generally difficult for seed analysts. Three dependent variables: workload, accuracy and throughput, were evaluated. We concluded that there is a significant difference in mental demand ($P = 0.00245$) using image analysis compared to manual identification for analysts. In conclusion, computer vision and image analysis can achieve high identification accuracy with less mental demand for the analyst. Future studies on computer vision and image analysis are recommended in the areas of software and hardware coordination, construction of an effective image reference database, and the improvement of computer modelling for more species with a generic solution.

Multispectral imaging in seed testing

Birte Boelt¹, Jens Michael Carstensen², Dot Vittrup Pedersen³, Kim G Jørgensen⁴ and Kim Nielsen⁵
¹Aarhus University, Forsøgsvej 1, Slagelse, Denmark
²Videometer, Denmark
³DLF, Denmark
⁴Viking Malt, Denmark
⁵Vikima Seed, Denmark
(bb@agro.au.dk)

Multispectral imaging (MSI) is a new technology being deployed to assess seed quality parameters. The combinations of the features from multispectral images captured by visual light and near-infrared (NIR) wavelengths, have been found to be useful in the separation of infected seeds from uninfected seeds in several species (Bodevin et al., 2009; Olesen et al., 2015; Vrešak et al., 2016).
For example, multispectral imaging was used to discriminate uninfected seeds from seeds infected by five fungal species with a classification rate of 80–100% (Olesen et al., 2011). Recent studies also show the potential of MSI combined with chemometric methods for the varietal identification of rice and tomato seeds (Hansen et al., 2015; Shrestha et al., 2015), and for the determination of other crop or weed seeds (Sendin et al., 2018). In a recent review, it was concluded that MSI is a fast and reliable tool with the potential to detect and characterise fungi, insect damage, varietal purity and other seed quality components associated with surface structure and chemical composition, seed colour, morphology and size (Boelt et al., 2018). During the project ‘SpectraSeed 2012–17’, the following achievements were obtained: 1) Algorithms for the detection of Fusarium spp. with red and grey signatures were improved and the feature is currently in daily operation in the malting industry. 2) A feature to identify the most difficult weed seeds and inert matter has been developed for spinach seed lots. The vegetable seed company, Vikima Seed, acquired a VideometerLab4 in April 2017 and is currently implementing and training on the system in-house. 3) A feature for radicle emergence in two species of grasses has been developed, and germination data confirms the correlation between radicle emergence and the number of normal seedlings. The correlation is currently being updated with additional data provided by DLF. The achievements from SpectraSeed will be presented.

Can DNA barcoding and Next Generation Sequencing technology detect invasive plant species in seed lots?

Marie-José Côté1, Steve Jones2, Nicole Wurm2, Adam Colville1, Marc-Olivier Ducespe1, Sarah Kyte1, John Chmara1 and Marie-Claude Gagnon1

1Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, ON, K2H 8P9, Canada
2Canadian Food Inspection Agency, 421 Downey Road, Saskatoon, SK, S7N 4L8, Canada

To import seeds into Canada, the seed lot must be free of prohibited noxious weeds. Weed seeds mixed in with imported grain or crop seeds is one of the main pathways for unintentional introduction of invasive plants into Canada. It is also necessary that imported seed lots for resale in Canada meet the minimum standards for purity (% purity and other seed determination) and germination. Therefore, marketplace monitoring samples are taken from domestic and imported seed lots for seed purity and germination testing. Determining other seeds is done by manually searching the seed sample for contaminating seeds which are then analysed morphologically for species identification. The process can be lengthy especially for small seeds or seed mixtures like wildflower seeds. Furthermore, identification of some species by seed analyst experts can be limited to the genus or family level due to the lack of distinctive morphological characteristics.

Sanger sequencing technique (DNA barcoding) and the newer technique of Next Generation Sequencing (NGS) were used to study the potential for detecting weed seeds in seed lots. The NGS technique allows simultaneous DNA sequencing of hundreds of thousands of molecules and has been used for metagenomics studies such as the detection and identification of species contaminating other species. In our work, the first step was to create DNA barcode sequence collection representing the regulated weed species listed on the Weed Seed Order (WSO, 2016) and crop species using plant herbarium reference material. The DNA barcode collection currently holds more than 100 species regulated by Canada as plant or seeds, as well as 147 close relative species and 73 crop species. The DNA barcode collection provides the sequence information needed for the development and establishment of a workflow using the NGS technique as well as bioinformatics pipelines for data analysis. The NGS technique was developed with the aim to detect and identify weed seeds as well as other crop seeds contaminating different seed samples. Proof of concept results for the application of both methods (DNA barcode and metabarcoding) and how these can be used to support the seed analyst in their work, will be presented and discussed.
Machine vision technology for establishing varietal distinctness

Monika Joshi¹, Nachiket Kotwaliwale², Shyamal K Chakrabarty¹ and Amitava Akuli³
¹ICAR-Indian Agricultural Research Institute, Division of Seed Science and Technology, New Delhi, India
²Central Institute of Agricultural Engineering, Agroproduce Processing Division, Bhopal, India
³Centre for Development of Advanced Computing (C-DAC), Kolkata, India

Use of machine vision involving imaging and scanning technologies, holds considerable promise in establishing varietal distinctness on the basis of seed parameters. The present study is the outcome of a multi-institutional, three-year research project to develop methodology for distinguishing among varieties/germplasm of rice, Indian mustard and chickpea using visible imaging; and to optimise imaging and image processing under the visible spectral domain. Acquiring high quality images is an important step in machine vision methods. An imaging set-up was fabricated that has provision for both front light and backlight. Front light is useful for illumination of samples and back light is used when an outline of the samples (e.g. leaf boundary, leaf serration, etc.) is required. A huge database of images (approximately 15000) was created for all three crops by recording visible images at different resolutions. Image Nomenclature software was developed in MS Access for generating a file name according to plant part image, which helps in making a correct file name by eliminating the human error while naming images manually. The web-based database of digital images was created on http://cropcorpus.ciae.res.in. A web application has also been developed for information storage and retrieval. A flatbed scanner was used for image acquisition; further processing of the images was done with the help of Grain Size & Shape software, which is a graphical user interface (GUI) software wherein commands are given by the user through appropriate buttons/inputs. The software also shows and reports colour names of the five nearest matching colours, according to Royal Horticultural Society colour nomenclature. Regardless of the placement or number of grains, Grain Size & Shape software can isolate all seeds and measure various morphometric parameters to an accuracy of 0.1 mm. In addition to the above, another software namely Rice Panicle Analysis software, was also developed which successfully distinguishes rice varieties on the basis of panicle traits. To optimise imaging and image processing parameters under the visible spectral domain, surface morphological features accounting for major variability have been identified. The project has also successfully identified the most important chromatic and surface textural properties for classification using discriminant analysis.

Image recognition technology on assisting paddy (Oryza sativa L.) seed germination testing

I-Cheng Chen¹, Ting-Lin Chang¹, Shi-Jie Luo¹, An-Qin Xu² and Kuo-Yi Huang²
¹Taiwan Seed Improvement and Propagation Station (TSIPS), No.6 Xingzhong St., Xinshe Dist., Taichung City 426, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu
²National Chung Hsing University, No. 145 Xingda Rd., South Dist., Taichung City 402, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu

In Taiwan, the seed germination tests of paddy (Oryza sativa L.) seeds are conducted manually by well-trained inspectors. Since the tests are time- and energy-consuming and the results may also be affected by fatigue, this research introduced the image recognition technology system to assist seed testing tasks. The neural network-based image recognition technology system is also used for seedling evaluation to analyse the seedling image by four, mainly specific, structures of the root and shoot system. The system now can capture 88.4% of seedling images grown on specific containers and the recognition results are 86.0%, equal with manual evaluation. Future improvements will be
Seedling image capture, integration of scanner and container, building the seedling image database and image recognition algorithm of ‘black rice’ functions. Expectations of this research are to reduce seed testing manpower, improve inspection efficiency and decrease manual mistakes. Therefore, this system also stabilises the supply of high-quality paddy seed.

Chlorophyll fluorescence as an indicator of seed quality and longevity in soybean

Irfan Afzal and Umair Usman
Seed Physiology Lab, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan (iafzal@uaf.edu.pk)

Seed maturity is highly correlated with chlorophyll content in crops. Chlorophyll is often present in seeds during their early development. High maturity indicates high seed longevity and quality. Soybean is greatly influenced by chlorophyll content at the time of physiological maturity. During maturation, chlorophyll content of the seed generally decreases gradually. The present study was conducted to evaluate longevity of two soybean seed lots (mature and immature) by using a new method based on the detection of chlorophyll content. Chlorophyll content was determined by using a chlorophyll fluorescence (CF) mobile machine in order to evaluate immature and mature soybean seeds. Efficacy of chlorophyll content in soybean after harvest was further evaluated, using vigour and viability tests during storage in different packaging materials. Seed samples with a lesser chlorophyll fraction performed better than those with a greater chlorophyll fraction. Final germination, seedling emergence and seedling vigour were lower in seed samples with a greater chlorophyll fraction. Chemical attributes, malondialdehyde content, free fatty acid content and total soluble sugars were high in seeds with greater chlorophyll fraction. The same results were determined in hermetic bags as compared to conventional storage materials. The performance of greater and lesser chlorophyll fractions was better in hermetic bags than cloth and plastic bags. It can be concluded that there is an inverse relation between CF value and seed quality factors (viability and vigour). It is efficient to use CF analysis as a safe and non-destructive approach to determine the seed quality and maturity of soybean seeds.
**Poster Sessions**

**POSTER SESSION 1 – DEVELOPMENTS IN VIABILITY AND VIGOUR TESTING**

**P1.01** Response of rice seed to cold temperature at germination as an effective screening methodology for phenotyping and genotyping

Jhansi Rani¹, Saritha Pradhan¹, Neelima Ponnekanti¹, Keshavulu Kunusoth¹ and Damodar Raju²

¹Professor Jayashankar Telangana State Agricultural University, Seed Research and Technology Centre, Rajendranagar, Hyderabad, India
²Rice Research Centre, ARI, Rajendranagar, Hyderabad, India

Cold is the major abiotic stress affecting rice crop productivity. Low temperature stress at germination and seedling stage results in poor stand establishment and cold at reproductive stage results in spikelet sterility in indica genotypes. The genetic gain for cold tolerance under selection programmes is low due to considerable genotype × environment interaction. Thirty-nine rice genotypes were evaluated for germination and seedling cold tolerance in the laboratory based on per cent seeds with coleoptiles superior to 5 mm (PERCOL), per cent reduction in coleoptile length (REDCOL) and difference in germination percentile under normal (28°C) and cold (15°C) temperature in comparison to the tolerant checks, viz. Tellahamsa, Sheetal and JGL 3844. The genotypes Krishna, Varalu, Bhadrakali, MTU 1010, Pusa 1121, KNM 110, JGL 11118 and JGL11470, were identified as cold tolerant based on laboratory studies. The same set of 39 genotypes were evaluated for reproductive cold tolerance in the field. The genotypes, viz. MTU 1010, KNM 110, Pusa 1121 and Varalu, exhibited reproductive cold tolerance recording significantly superior spikelet fertility over checks, whereas the genotypes RNR 21582, JGL 18047, RNR 21588 recorded significantly high seed yield per plant. The confirmation of phenotypic responses of genotypes against cold tolerance was done at genetic level using linked polymorphic SSR markers. Nine genotypes, viz. WGL 32100, Swarna, RNR 15048, JGL 11470, JGL 17004, JGL 17420, KNM 110, JGL 18047 and Bhadrakali, were identified as cold resistant based on QTLs. Out of these nine genotypes, KNM 110 exhibited cold tolerance at seedling and reproductive stages whereas Bhadrakali exhibited seedling tolerance hence validated the presence of QTLs. In other genotypes, there was no correspondence between phenotype and genotype with respect to cold tolerance. Hence, large populations over locations and seasons must be evaluated for establishing correlation between lab and field parameters and for developing mapping populations and identification of more polymorphic markers linked to QTLs conferring cold tolerance.

**P1.02** Genetic diversity in sunflower and seed vigour testing: no relationship established between vigour tests and field emergence

Marie-Hélène Wagner, Charlotte Angerand and Sylvie Ducournau
GEVES, 25 rue Georges Morel, Beaucouzé, France
(marie-helene.wagner@geves.fr)

A vigour test is a complementary test to the standard germination test. Different approaches in vigour testing, which had previously been validated on other species, were tested on sunflower seeds. The electrical conductivity (EC) measurements, germination speed to reach radicle emergence (RE) test, cold test (CT) and controlled deterioration (CD) tests were carried out on 15 seed lots that achieved at least 85% of germination in the standard germination test. All vigour tests
were repeatable and differences between the samples were clearly identified, however, the EC test results varied more significantly. The variations depended more on the variety and the thickness of maternal tissues (pericarp) than on seed vigour. CD and RE tests were then evaluated on 15 new seed lots that had been produced from five parent lines and four hybrids. In 2017, field emergence was measured for hybrid cultivars in three different locations. The samples were then stored for one year in a warehouse and germination tests were carried out on four replicates of 50 seeds every three months. Seed ageing was correlated to the decrease in speed of germination during storage. However, after one year’s storage (with high temperatures experienced during summer), no significant change in standard germination was observed, and only a slight relationship ($R^2 = 0.66$) was established between the germination potential after storage and the CD test predictive results. The field emergence results depended mainly on the location, and the differences between seed lots were lower than those expected after vigour testing in the laboratory. For example, one hybrid tested had good emergence despite a delayed germination rate, while the RE test on two seed lots of another hybrid predicted the results that were observed in field. A delay in germination had already been measured on three seed lots of another variety using digital imaging. All three samples had complete and high germination, but their speed of germination was slower than that of 20 seed lots of nine other cultivars. The hypothesis of a thick pericarp limiting water absorption during imbibition could explain the delayed germination of some varieties; this genetic trait could therefore reduce the scope of application of the RE test as a seed vigour test for sunflower.

**P1.03 Dry heat treatment for removal of seed dormancy in wild and cultivated cucumber species**

Yogeesha HS and Pitchaimuttu M  
ICAR-Indian Institute of Horticultural Research, Hesserghatta Lake Post, Bangalore, India  
(yogeesha.hs@icar.gov.in)

Seed dormancy is common in fresh seeds of cucumber species that may last for two to four months in storage. This poses a problem, especially for breeders who want to speed up the breeding process. Five accessions of *Cucumis sativus*, two wild accessions of *Cucumis hardwickii* and one accession of *Cucumis metuliferus* were studied for the presence of seed dormancy. Dormancy was found in fresh seeds of all the accessions, ranging from 45% in Swarna Agethi (a cultivated line) to 99% in SM-12735 (*Cucumis hardwickii*). These seeds were subjected to various physical and chemical treatments and it was found that dry heat treatment was effective in overcoming dormancy in all three species. Further refinement of this treatment showed that dry heat treatment at 70°C for six days significantly improved the germination in all eight accessions, ranging from 20–100%, compared to untreated seeds with germination ranging from 1.3–45.3%. This treatment not only improved the seed germination percentage but also the speed of germination, as most seeds germinated within ten days of incubation at an alternating temperature of 20/30°C.
P1.04 Comparative evaluation of seed vigour tests for realistic assessment and prediction of seed quality and field performance in okra \( [Abelmoschus esculentus \text{ (L.) Moench}] \)

Sudipta Basu\(^1\), Dhanajaya P\(^1\), Vinod Kumar Pandita\(^2\) and Anjali Anand\(^3\)

\(^1\)ICAR-IARI (Indian Agricultural Research Institute), Division of Seed Science and Technology, Pusa Campus, New Delhi 110012, India

\(^2\)ICAR-IARI Regional Station, Kunjpura Road, Karnal, Haryana, India

\(^3\)ICAR-IARI, Division of Plant Physiology, Pusa Campus, New Delhi 110012, India

(sudipta_basu@yahoo.com)

Okra \( [Abelmoschus esculentus \text{ (L.) Moench}] \) is a popular fruit vegetable of India because of its high yield, wide adaptability and consumer preference. Seed germination and emergence in okra is often poor due to inadequate moisture, low temperature, soil crusting, pre-emergence damping off and a hard seed coat. The crop is highly sensitive to low temperature prevailing during the post-sowing period in the early spring–summer season which affects its field emergence and plant stand. Standard germination tests often fail to assess seed lot performance, especially under field conditions. Seed vigour tests provide a realistic assessment and prediction of seed quality. For okra, there is no recommended vigour test by ISTA; because of this, quality assessment and prediction of field emergence become a major bottleneck, especially in medium and low vigour lots of okra. The present investigation was undertaken to standardise seed vigour tests for vigour assessment in okra and identify the vigour test which could reliably predict the planting value of okra seed lots. Different vigour tests namely, accelerated ageing, controlled deterioration, saturated salt accelerated ageing and electrical conductivity tests, were standardised (temperature, duration and moisture content) using four seed lots of okra cv. A-4. Based on the simple correlations between the above vigour test combinations and field emergence, the following were the standardised tests for okra: controlled deterioration test \((24\% / 45^\circ{\text{C}} / 24\text{ h})\); accelerated ageing test \((42^\circ{\text{C}} / 72\text{ h})\); saturated salt accelerated ageing test \((42^\circ{\text{C}} / 72\text{ h})\) and electrical conductivity test \((50\text{ seeds in }100\text{ ml})\); complex stress vigour test (combination of saturated salt accelerated ageing and controlled deterioration tests). The above standardised vigour tests along with standard germination and vigour indices, were validated with 15 seed lots for seed quality assessment in okra. The correlation studies indicated that field emergence showed significantly high correlation with the standard germination test \((r = 0.736^{**})\), pot germination \((r = 0.757^{**})\), speed of germination \((r = 0.647^{**})\), vigour indices \((\text{VI I}: r = 0.612^{*} \text{ and VI II}: r = 0.602^{*})\), controlled deterioration test \((r = 0.948^{**})\), accelerated ageing test \((r = 0.776^{**})\), saturated salt accelerated ageing test \((r = 0.692^{**})\) and EC test \((r = -0.693^{**})\). The study concluded that among different vigour tests, the controlled deterioration test showed the highest correlation with field emergence in okra. A controlled deterioration test conducted at 45\(^\circ\text{C}\) for 24 h with 24\% seed moisture content could give a realistic prediction of the seed vigour potential of okra seed lots, hence could be recommended as the vigour test for okra.

P1.05 Protocols for seed germination in ashwagandha \([Withania somnifera \text{ (L.) Dunal}]\) and anise \([Pimpinella anisum \text{ L.}]\)

SK Jain\(^1\), Amrit Lamichaney\(^2\) and Menka\(^3\)

\(^1\)ICAR-Indian Agricultural Research Institute, Division of Seed Science and Technology, New Delhi 110012, India

\(^2\)ICAR-Indian Institute of Pulse Research, Kanpur 208024, India

\(^3\)Department of Agriculture, Ministry of Agriculture, Government of Uttar Pradesh, Gorakhpur 273001, India

(skjainsst@gmail.com)

Ashwagandha [Indian ginseng; \( \text{Withania somnifera \text{ (L.) Dunal}} \)], a member of Solanaceae with medicinal value, and anise [aniseed; \( \text{Pimpinella anisum \text{ L.}} \)], a member of Apiaceae with spice
value, both produce seeds that generally exhibit late, erratic and poor germination. For commercial cultivation of these, it is a prerequisite for the assessment of seed germination to evolve standard seed germination protocols. Under seed laws, it is mandatory to label seed bags or containers, and specify seed germination and other seed quality attributes. These prescriptions are based on testing protocols developed in a laboratory, which were subsequently tested for proficiency. For ashwagandha and anise seeds, information on seed germination was either fragmented or not available. In order to achieve successful commercial cultivation of the chosen seed species, development of seed germination protocols has always been necessary. In this regard, the role of ISTA is important and services rendered to resolve issues related to seed germination in prescribing seed testing protocols are fundamental. Fresh seeds of both species (15 lots of ashwagandha and 13 lots of anise) from recent harvests, were procured and used. 400 seeds (i.e. 100 seeds each in four replicates) were subjected to seed germination studies following ISTA Rules prescribed for other similar species. Optimisation of seed germination was achieved using various physical and/or chemical treatments. Based on studies at the Department of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi, it was inferred that ashwagandha seeds require top of paper (TP) substratum; 20~30°C temperature (16 h dark / 8 h) with light; first count on day 12 and final count on day 16. Anise seeds also require TP substratum; 20~30°C temperature (16 h / 8 h), irrespective of light/dark. Pre-washing of seeds in ashwagandha and anise, respectively in running water, under laboratory ambient temperature for 24 and 72 h, ranked the best, most eco-friendly and cheapest method for optimising the seed germination of dormant seeds.

P1.06 Radicle emergence test – a reliable method of assessing seedling vigour in rice genotypes

LV Subba Rao¹, S Viran Kumar², Razia Sultana², M Seshu Madhav¹ and Keshavulu Kunusoth²

¹ICAR-Indian Institute of Rice Research, Hyderabad, India
²Department of Seed Science and Technology, Seed Research and Technology Centre, Hyderabad, India
(lvsubbarao1990@gmail.com)

Thirty-six rice genotypes were evaluated for seedling vigour expression before and after accelerated ageing, to assess the association of seed and seedling traits with seed vigour and to identify the best genotypes with increased seed vigour and its related traits among different ecological systems. The study was conducted at the Department of Seed Science and Technology, Seed Research and Technology Centre, Professor Jayashankar Telangana State Agricultural University and ICAR-Indian Institute of Rice Research, Hyderabad, India. Analysis of variance indicated the existence of significant differences among the genotypes tested for the vigour and its component characters. Genotypes Aditya and Sahbagidhan recorded high values for germination percentage, radicle emergence percentage, field emergence, fresh root weight, dry root weight, root volume, root density, shoot length, fresh seedling weight, dry seedling weight, root/shoot ratio, seedling vigour index-I and vigour index-II. In relation to biochemical traits, genotype Aditya recorded the lowest EC, highest dehydrogenase and amylase enzyme activity among the 36 rice genotypes. After an accelerated ageing test, Aditya performed better in terms of minimum reduction in germination percentage, highest root length, dry seedling weight, seedling vigour index-I and seedling vigour index-II. The highest shoot length was observed in Sahbagidhan, with the lowest EC in Annada. Correlation studies revealed positive and significant association among germination traits, root traits, seedling vigour traits and biochemical traits, except for EC which was negatively correlated with the seedling vigour. Significant positive correlation was observed among the radicle emergence tests, accelerated ageing tests and field emergence test, and with seed vigour index-I and seed vigour index-II. Thus, we identified that the radicle emergence test is a more reliable vigour test when compared to other tests for assessing rice genotypes. Among the selected genotypes for different ecosystems, it is observed that germination traits, root traits, seedling vigour traits and biochemical traits recorded highest in rain-fed upland varieties followed by rain-fed shallow lowland,
irrigated medium, irrigated mid–early, irrigated early, hilly irrigated and the least in scented rice. The genotypes Aditya and Sahbagidhan in rain-fed upland, Swarna and improved Samba Mahsuri in rain-fed shallow lowland, Nidhi and ADT 37 in irrigated early, Jaya and Swetha in irrigated medium, IR-64 and JGL-3844 in irrigated mid–early, VL Dhan 85 in hilly irrigated and finally Pusa Basmati 1121 in scented rice, recorded highest for most of the seed vigour traits and were considered the best genotypes for seedling vigour character.

P1.07 The impact of light and high light on seed germination and the radicle emergence test

Laura Bowden¹ and Laurence Landais²
¹Science and Advice for Scottish Agriculture, Roddinglaw Road, Edinburgh EH12 9FJ, UK
²Groupe d’Étude et de Contrôle des Variétés et des Semences, 25 Rue Georges Morel, 49070 Beaucouzé, France
(laura.bowden@sasa.gov.scot)

Conditions must be precisely controlled when testing for seed vigour and this is especially important when test periods are short, such as for the radicle emergence (RE) test. It is well documented that light can significantly affect seed germination; depending on the species, light can be either a promoter or inhibitor of germination. However, the light requirements for the radicle emergence test are not specified. We show that light can have a significant effect on the RE result for both oilseed rape and radish. When RE tests were conducted on top of paper in Petri dishes a significant effect of light for both oilseed rape and radish was found ($P < 0.001$). RE results were higher when conducted in the dark and lower when conducted in high light, in comparison with tests conducted under standard conditions for each of the 15 oilseed rape and four radish seed lots tested. The ranking of oilseed rape lots was almost identical, irrespective of light conditions used for testing. However, in radish there were clear differences in the order in which seed lots were ranked depending on the light conditions to which seeds were exposed during the RE test. The ISTA Rules specify that oilseed rape RE tests are conducted between paper, and when tested using pleated paper in sandwich boxes we showed that there is no difference between RE tests that are conducted in light or dark conditions ($P = 0.683$). For radish the ISTA Rules specify that the RE test is conducted on top of paper, and seeds will therefore be potentially much more influenced by light conditions. Further experiments will be conducted to investigate the effect of light on a greater number of seed lots and varieties of radish, and implications for the use of RE testing to predict seed vigour will be discussed.

P1.08 Expected variance between seed germination test replicate results

Peter Deplewski¹, Hans-Peter Piepho² and Michael Kruse²
¹KWS Saat SE, Grimsehlstr. 31, 37574 Einbeck, Germany
²Institute of Crop Science, University of Hohenheim, 70593 Stuttgart, Germany
(petermichael.deplewski@kws.com)

According to ISTA, the germination percentage of a seed lot is usually determined by testing four replicates of 100 seeds. The theoretical variance of the binomial distribution is used by ISTA to define the maximum tolerated range in the germination percentages of the replicates. If the tolerated range is exceeded, the test has to be repeated. We show that the theoretical variance between the four replicates is different from the variance of the binomial distribution. The theoretical variance $s' = p \times (1 - p) / n \times N / (N - 1)$, where $p$ is the germination percentage, $n$ is the number of seeds per replicate and $N$ is the total number of seeds in all replicates. In the case of four replicates with 100 seeds each, the difference is small
but becomes bigger with decreasing sample size. We also present asymptotic and exact tests to compare the empirical variance between the four replicates with its expected variance. The exact test can lead to notably different results compared with the test based on the binomial assumption, when sample size is small and germination percentages are close to 100 or 0%. These findings have a direct impact on Table 5B in the ISTA Rules: it can be seen that in Table 5B part 1, four out of 16 values are changing; in Table 5B part 2, one out of 13 values is changing; and in Table 5B part 3, six out of 17 values are changing. Considered together, 11/46 = 23.9% of the values are changing due to the improved formula.

P1.09 Effect of seed priming with the aqueous extracts of different medicinal and aromatic plants on various seed growth parameters of farm saved seeds of wheat (Triticum aestivum L.)

Dileepkumar Masuthi, Satish D, Shivayogi Ryavalad, Laxmidevamma TN, Patil DR, Bapugouda Patil, Mulla SA and Renuka Herekurabar
University of Horticultural Sciences Bagalkot, KRC College of Horticulture, Arabhavi 591218, India
(aodileep@gmail.com)

Wheat (Triticum aestivum L.) is one of the first domesticated food crops and for 8000 years has been the basic staple food in major parts of Europe, West Asia and North Africa. It is an important cereal in many developed and developing countries of the world. Wheat is widely used for animal feed and industrial raw material in the developed countries, whereas the developing countries generally use it for feed (Rosegrant et al.). Seed germination is an essential process in plant development in order to obtain an optimal number of seedlings with rapid and uniform field emergence. This is an important factor to achieve high yield with respect to both quantity and quality in annual crops (Subedi and Ma). Growing awareness about hazardous chemicals, their impact on the environment and human health, has encouraged us to explore the effects of plant extracts, which are eco-friendly in nature and economical. Therefore, the use of eco-friendly plant-based products is the need of the day for research to enhance the germination and other seedling quality parameters over synthetic chemicals. Hence, the present investigations are to evaluate the effects of different medicinal and aromatic aqueous plant extracts on germination and seedling growth characteristics of wheat. Aqueous extracts from five medicinal and aromatic plants, viz. leaf extract of insulin (Costus igneus Nak), rosemary (Rosmarinus officinalis L.) and garden rue (Ruta graveolens L.) and root extracts of sweet flag (Acorus calamus L.) and coleus (Plectranthus scutellarioides L.), were studied for their positive effect on seed quality parameters in wheat seeds under laboratory conditions. All the plant species at 2% concentration significantly proved to enhance wheat germination, shoot length, root length, seedling dry weight and seedling vigour index-I and seedling vigour index-II, compared to the untreated control. Among the five extracts, A. calamus gave better results for germination (97.50%), seedling vigour index-I (1119) and root growth (6.36 cm), while the highest shoot growth (5.45 cm) was recorded in R. officinalis treatment. C. igneus aqueous extracts boosted the seedling dry matter content (0.168 g) and seedling vigour index-II (16.17) compared to the rest of the treatments, including the untreated control.
P1.10 Effect of chemo-priming on seed quality parameters of *Psoralea corylifolia* L.

Dileepkumar Masuthi, DR Patil, Shantappa T, Shivayogi R, BB Patil and Dhanajaya

Department of Biotechnology and Crop Improvement, KRC College of Horticulture, Arabhavi 591218, Belgaum District, Karnataka, India
(aodileep@gmail.com)

*Psoralea corylifolia* (babchi) of the Fabaceae family, is an important plant in the Indian Ayurveda and Tamil Siddha systems of medicine and in Chinese medicine. It is an endangered and rare herbaceous medicinal plant and distributed in the tropical region of the world. *P. corylifolia* seed extract contains a number of chemical compounds including flavonoids, coumarins and meroterpenes, etc. The major compounds of this plant are psoralen, angelicin, psoralone, isopsoralone, bavachin and daidzein. Psoralen is a pharmaceutical interested compound because of its photosensitising, photobiological and phototherapeutic properties, which is used for the photochemotherapy of vitiligo and skin diseases such as psoriasis, mycosis fungoides and eczema. *Psoralea* is also used in indigenous medicine as laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions. Many studies have been conducted on other crops for seed germination responses including *Orchis galilaea*, Mediterranean woody species, mustard, and *Ludwigia* species, but less attention is paid to seed germination studies in *P. corylifolia*. A laboratory experiment was conducted with different chemical treatments on the germination of this crop with various parameters, viz. final seed germination, shoot length, root length, dry weight and seedling vigour index. The treatments were potassium nitrate (KNO₃ 2%), nitric acid (HNO₃ 2%), GA₃ (50 ppm), NAA (50 ppm), untreated control for 3 hours priming. Among these, KNO₃ treatment recorded a high germination percentage, seedling vigour index-I (267) and shoot length (13.16 cm). HNO₃ showed high root length (15.26 cm). The study demonstrated that chemicals can be used to increase the germination and seed quality parameters in *P. corylifolia* crops. As this seed has a hard seed coat, the dormancy of a hard seed coat is experimented with many mechanical treatments. A different treatment of keeping seeds in a water bath at 100°C for 30 min and then keeping these seeds in a water bath for a day has given the maximum germination (55.3%) seedling vigour index-I (372) and seedling vigour index-II (1.68), compared to the control (18.67%, 122 and 0.065, respectively). In this crop, there were no experiments conducted over to fix the first and the second count. However, the two-year experiments have revealed that for the babchi seed the first count date may be by day 4 (50% seeds in my experiments given germination) and the second count day may be by day 14.

P1.11 Enhancement of seed germination in *Stylosanthes hamata*

Kanaka Durga K¹, Razia Sultana², Shashikala T³ and Keshavulu Kunusoth⁴

¹Agricultural College, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Sircilla, Rajanna Sircilla district, Telangana, India
²Department of Seed Science and Technology, PJTSAU, Seed Research and Technology Centre, Rajendranagar, Hyderabad 500030, India
³AICRP on Forages and Utilisation, PJTSAU, Rajendranagar, Hyderabad 500030, India
⁴Telangana State Seed and Organic Certification Authority, HACA Bhavan, Hyderabad, India (kanakakilaru@yahoo.com)

Establishment of a uniform and vigorous stand is of paramount importance to the successful production of many forage crops. Poor or non-uniform germination could be attributed to the presence of dormancy in forage grasses, presence of hard seeds in forage legumes, presence of appendages and poor storage conditions. Without a good stand, the effectiveness of other agronomic inputs is drastically reduced, and usually such inputs can never compensate for the negative impact of a poor stand. Keeping this in view, an experiment was formulated with an objective
to assess and enhance the germination percentage in *Stylosanthes*. A laboratory experiment was conducted at the Seed Research and Technology Centre, PJTSAU, Hyderabad during 2017–18, using freshly harvested seed of *Stylosanthes* procured from AICRP on Forages and Utilisation, ARI, Rajendranagar. Initially, the seed was tested for germination percentage (ISTA, 1989) and it was found to be 28% with fluff and 47% without fluff, indicating that presence of appendages hampered germination in *Stylosanthes*. Removal of fluff requires specially designed rotating drums and is not economically feasible. Seed enhancement techniques were used, such as seed treatment with concentrated H₂SO₄ for 1 min and hot water treatment at 80°C for 5 min, seed pelleting with clay, leaf powder of kanuga (*Pongamia pinnata*) and farmyard manure, along with a control. The experiment was conducted in four replications and the design adopted was factorial completely randomised design. The data on seed quality parameters like germination percentage, root length, shoot length, seedling length and seedling vigour indices, were collected at monthly intervals. The results indicated that seed pelleting with farmyard manure has enhanced seed germination in *Stylosanthes*, resulting in more than 46% germination (above Indian Minimum Seed Certification Standards of 40%), as compared to the control (23%) at three months after pelleting, followed by hot water treatment at 80°C for 5 min (43%). A similar trend was noticed for other seed quality characters like seedling length (6.6 cm) and seedling vigour index (261), as compared to the control (5.5 cm and 64, respectively). Therefore, from the study it may be concluded that seed pelleting with farmyard manure at three months after treatment was found effective, economical and farmer-friendly in enhancing germination percentage in *Stylosanthes*, as it enabled the slow release of nutrients and moisture required for germination.

**P1.12 Evaluation of *Vicia* (vetch) seed vigour under saline conditions**

Dušica Jovičić, Zorica Nikolić, Aleksandar Mikić, Gordana Petrović, Gordana Tamindžić, Dragana Milošević and Maja Ignjatov
Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia
(dusica.jovicic@nsseme.com)

Salinity affects almost every aspect of the physiology and biochemistry of plants, including the whole plant and at the cellular level. There is a significant variation in salt tolerance within vetch species, providing opportunities for improving salt-stress tolerance using genetic resources. Considering the significance of successful seed germination and seedling emergence for further plant establishment, plant development and achieving high crop yields, the study of the interaction between salt stress and germination is of great importance. The aim of this experiment was to evaluate the seed vigour of vetch species (*Vicia sativa*, *V. pannonica*), through vigour tests under saline conditions. Germination and vigour were assessed through the standard test, accelerated ageing test and cold test, estimating the germination percentage, root and shoot length, total fresh and dry matter accumulation, under three levels of salt stress (100 mM, 150 mM, 200 mM NaCl). The obtained results of the experiment showed significant differences between the two species in response to salinity stress. Furthermore, a species that has a higher vigour has shown noticeably higher tolerance to stress. After the accelerated ageing test, *V. pannonica* showed significantly lower values in all the tested parameters, at all concentrations of NaCl.
P1.13 Effect of priming on seed yield and quality of kabuli chickpea seeds

Axay Bhuker, Ashok Kumar, VS Mor and SS Jakhar
Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Department of Seed Science and Technology, Hisar, India
(bhuker.axay@gmail.com)

Availability of viable and vigorous seed at planting time is important for achieving targets of agricultural production. The study was conducted to assess the effect of priming on seed yield and quality of kabuli chickpea variety HK2 during Rabi 2017–18. The seed was primed with *Trichoderma harzianum* at 1.5%, Vitavax Power at 0.25%, GA₃ at 50 ppm, GA₃ at 50 ppm + seed coating with *T. harzianum* at 15 g/kg seed, sodium molybdate at 500 ppm, sodium molybdate at 500 ppm + seed coating with *T. harzianum* at 15 g/kg seed, leaf extract of *Lantana camara* at 10%, hydration for 8 h followed by dehydration, Bavistin at 3 g/kg seed. All the treatments showed a significant effect on seed quality parameters, seed yield and yield attributing characters when seed was dehydrated at 32.6°C and 42.5% relative humidity. The seeds primed with sodium molybdate at 500 ppm + seed coating with *T. harzianum* at 15 g/kg seed showed superiority over other treatments by registering maximum germination (92.00%), root length (14.93 cm), vigour index-I (2710), vigour index-II (3886), 1000 seed weight (26.67 g), number of pods per plant (105.71), number of seeds per pod (2.00), seed yield (1324.5 kg/ha) and harvest index (32.60), followed by the seeds treated with Vitavax Power at 0.25% and Bavistin at 3 g/kg seed. Minimum germination was recorded in the seed primed with GA₃ at 50 ppm (24.7%) as compared to the control (87.7%) when seed was dehydrated after priming at 85% relative humidity and 12.8°C. Priming injury was also noticed in the seeds which were completely dipped into solution throughout the priming period; no germination was observed in such seeds.

P1.14 Effect of bio-priming and concentrations on seed quality in onion

Axay Bhuker¹, Gaganpreet Singh¹, VPS Panghal² and Amit Kumar³
¹Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Department of Seed Science and Technology, Hisar, India
²CCSHAU, Department of Vegetable Science, Hisar, India
³CCSHAU Hisar, Bawal, Rewari, India
(bhuker.axay@gmail.com)

Onion seeds are considered microbiotic in nature, so the study was conducted on variety Hisar-2 during 2016–17. The results revealed that maximum germination was observed in seeds hydrated with 100 ppm GA₃ and seeds coated with aloe vera gel (89.00%) followed by seeds hydrated with 100 ppm Chitosan (87.33%), while minimum germination was observed in the control (80.33%). Similar trends were also observed for other seed quality parameters. Maximum seedling length (20.33 and 19.90 cm), seedling dry weight (30.67 and 29.87 mg), vigour index-I (1809.37 and 1771.10) and vigour index-II (2729.63 and 2658.43) were observed in seeds hydrated with 100 ppm GA₃ and seeds coated with aloe vera gel, respectively, while in field conditions, seeds coated with aloe vera gel followed by the seeds hydrated with 3% aloe vera and seeds hydrated with GA₃ 100 ppm showed superiority over the other treatments. As the concentration of Chitosan was increased from 25 ppm to 100 ppm, enhancement in germination was also increased and maximum germination was found in seeds hydrated with 100 ppm Chitosan (87.33%). Maximum seedling length (18.93 cm), seedling dry weight (26.83 mg), vigour index-I (1653.15) and vigour index-II (2343.06) were observed in seeds hydrated with 100 ppm Chitosan. Similarly, for GA₃, maximum germination (89.00%), seedling length (20.33 cm), seedling dry weight (30.67 mg), vigour index-I (1809.37) and vigour index-II (2729.63) were observed in seeds hydrated with 100 ppm GA₃ over the other concentrations. Among aloe vera, maximum germination (89.00%), seedling length
(19.90 cm), seedling dry weight (29.87 mg), vigour index-I (1771.10) and vigour index-II (2658.43) were recorded in aloe vera coated seeds over the other concentrations used. Hence, germination in onion can be maintained above Indian Minimum Seed Certification Standards (>70%) by hydrating the seeds with 100 ppm GA₃ or coating the seeds with 3% aloe vera gel, as it is cheap and easily available to farmers.

P1.15 Standardisation of seed priming duration for tomato seeds (Lycopersicon esculentum Mill.)

Kurella Sree Devi¹, Razia Sultana², Keshavulu Kunusoth³, Kamala Venkateswaran⁴ and Eruvuri Ramprasad⁵

¹Professor Jayasankar Telangana State Agricultural University (PJTSAU), Department of Agriculture, DNA Fingerprinting and Transgenic Crops Monitoring Laboratory, Andhra Pradesh, Chuttugunta, Guntur, India
²PJTSAU, College of Agriculture, Rajendranagar, Hyderabad, India
³Telangana State Seed and Organic Certification Authority, HACA Bhavan, Hyderabad, India
⁴National Bureau of Plant Genetic Resources (NBPGR), Rajendranagar, Hyderabad, India
⁵Food Corporation of India (FCI), Vellore, Tamilnadu, India (kurella_sdevi@yahoo.co.in)

The present investigation ‘Influence of seed priming on seedling quality, plant performance and yield for two seed lots (high and low vigour) of tomato cultivars (Lycopersicon esculentum Mill.)’ was conducted at the Department of Seed Science and Technology, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad during Rabi season, 2013–14. Fresh and aged seed lots of tomato cv. Arka Vikas with an initial germination of 94% (high vigour), 74% (low vigour) and initial moisture content of 8.96% and 11.08%, respectively, were used to study the effect of priming in improving the seedling quality. Priming duration was standardised for the two seed lots separately and simultaneously by imbibing water for a test period of 48 hours, taking samples at 2-hour intervals and then drying back to their original moisture content. The constant phase of water absorption observed at 20 to 26 h for the fresh seed lot and 18 to 22 h for the aged seed lot, was taken as an optimal seed priming regime as an increased performance of primed seeds imbibed for 22 h duration was observed with high germination of 96% and 93%, increased speed of germination (23.89 and 21.34) with a reduced mean germination time (2.01 days and 2.50 days), greater seedling length (11.28 cm and 10.92 cm), seedling dry weight (16.00 mg and 12.33 mg), vigour index-I (1083 and 1012) and vigour index-II (1536 and 1146) for the fresh and aged seed lots, respectively. Based on a seed priming regime, i.e. phase-II (lag phase) of seed germination and performance, the priming duration of 22 hours for both the seed lots was fixed for further investigations. The data recorded for fresh and aged seed lots for water uptake (imbibition percentage) and seedling quality parameters were analysed. The results of analysis of variance for various seed quality traits revealed that there were significant differences between the two seed lots and among the priming durations for all the characters studied, viz. imbibition percentage, germination percentage, speed of germination, mean germination time, seedling length, seedling dry weight and seed vigour indices, indicating that there is variability between the two seed lots and between different durations. The data recorded for fresh and aged seed lots for water uptake (imbibition percentage) and seedling quality parameters were analysed and the results of analysis of variance for various seed quality traits for standardisation of seed priming duration in tomato are presented. The data reveals that there were significant differences between the fresh and aged seed lots and among the priming durations for all the characters studied, indicating that there is variability for various seed quality parameters exhibited between the two seed lots and between different durations. It can be concluded that priming has a beneficial effect on achieving an improved germination rate and uniform field emergence. Based on performance, seed priming with PEG 6000 at -1.25 MPa or by moist sand matrix-priming at 80% water holding capacity for 22 hours will help in enhancing the seedling quality in tomato. It may be inferred that though osmo-priming was the best treatment method in terms of fruit yield, simple on-farm hydro-priming can be suggested for aged seed lots.
to enhance seed germination, yield and income for the farmer, irrespective of their socio-economic status under similar conditions of crop production.

**P1.16 Relationship between cell cycle, lag period, radicle emergence and field performance of alfalfa seeds**

**Mohammad Khajeh-Hosseini**, Mostafa Cheshmi and Alison Powell

1Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

2University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen, UK

(agr844@gmail.com; saleh@ferdowsi.um.ac.ir)

The purpose of this research was to investigate the relationship between alfalfa seed vigour and the cell cycle. Germination tests were conducted on 14 seed lots, produced in different locations and years in Iran, at 13, 15, 17 and 20°C in the laboratory. The number of just germinated (protrusion of radicle from the seed coat) and germinated (2 mm radicle length) seeds were counted at six-hour intervals to calculate mean just germination time (MJGT), mean germination time (MGT) and final germination. Field emergence (FE) of 14 seed lots was also investigated and emergence was counted daily. The cell cycle of the six seed lots showing a wide range of vigour was studied during germination at 20°C using flow cytometry. The overall means of MJGT and MGT of seed lots increased with decreasing temperature, although there were no significant changes in the final germination of the seed lots. MJGT and MGT of the seed lots at all temperatures were negatively correlated with FE although the relationship with FE was closer at 20°C ($P \leq 0.01$). MJGT was positively correlated ($P \leq 0.05$) with mean emergence time (MET) at all temperatures except 15°C, while MGT was positively related with MET only at 20°C. The cell cycle analyses demonstrated that firstly the high vigour seeds showed higher G2 to G1 ratio from 18 hours after the start of imbibition and secondly, they showed faster cell cycle at the first hours of imbibition during germination. The seed lots that germinated earlier (lower MJGT and MGT) also showed a greater increase in G2 to G1 ratio between 6 and 12 hours after imbibition ($P \leq 0.05$). Also, FE (%) was positively related to the rate of increase from G2 to G1 between 6 and 12 hours after imbibition ($r = 0.46^{**}$). Thus, where seed lots had a faster cell cycle, they showed a shorter MJGT and MGT in the laboratory and higher emergence in the field.

**P1.17 The relationship between cell cycle, conductivity and field emergence of chickpea seeds (desi type)**

**Mohammad Khajeh-Hosseini**, Maliheh Akbarpour and Alison Powell

1Department of Agrotechnology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

2University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen, UK

(agr844@gmail.com; saleh@ferdowsi.um.ac.ir)

The aim of this research was to investigate the relationship between chickpea seed vigour and the cell cycle. Standard germination and conductivity tests were conducted on 11 seed lots of the desi type of chickpea (*Cicer arietinum*) according to the ISTA Rules. All seed lots had standard germination $\geq 85\%$. Mean germination time (MGT) was also calculated. Field emergence was carried out in a completely randomised block design with four replications of 25 seeds from each seed lot and emerged seedlings were counted daily for 35 days to assess mean emergence time (MET) and final emergence. There was a significant negative correlation ($r = -0.791^{**}$) between conductivity and the field emergence of the 11 seed lots. However, no significant correlation between the standard seed germination and the field emergence was detected ($r = 0.326; P < 0.328$). Based on the correlation between field emergence and conductivity, six seed lots were selected with a
wide range of vigour for evaluating cell cycle progression. Cell cycle activation and DNA replication during imbibition was studied using flow cytometry in the root tip meristem at intervals of six hours until 48 hours after seeds were set to imbibite. In dry seeds, the majority of cells (≥75%) were in the 2C state (G0/G1 phase). As the imbibition period increased, more nuclei became active in the cell cycle. The starting time of the cell cycle was almost the same in all six seed lots though the speed of the cell cycle was different between the lots. In the high vigour seed lots, the speed of the cell cycle was higher than in the low vigour lots. The relationship between cell cycle, conductivity and field emergence is discussed.

P1.18 Ethephon effect on peanut seed dormancy release

Papassorn Wattanakulpakin, Kantima Thongsri, Supalak Sattayasamitsathit and Jira Suwanprasert
Phitsanulok Seed Research and Development Center, Department of Agriculture, 813 Moo. 8, Wangthong District, Phitsanulok 65130, Thailand
(kwanpapas@gmail.com)

Dormancy is an undesirable character for the large seeded peanut (Arachis hypogaea). Khon Kaen 84-7 is a popular peanut variety for food consumption in Thailand, belonging to the Virginia type. Seed of KK 84-7 have remained dormant for over two months after harvesting. According to the ISTA Rules, preheating at 40°C is recommended for breaking peanut seed dormancy but the duration is not limited depending on the variety. The germination analysis of peanut seed is normally carried out for ten days. If breaking dormancy is needed, the complete time for germination test is 17 days including the preheat time. Therefore, the most rapid and precise method to reduce the analysis time has been studied and is beneficial for the earlier issue of seed certificates. Ethephon, ethylene liberation, is plant growth regulator that has been reported to break seed dormancy. The comparison between preheating and Ethephon for breaking peanut seed dormancy were studied in this experiment. Peanut seeds were preheated at 40°C for 168 h (7 days), and another seed group was directly mixed with 0.96% Ethephon. All pretreated seeds were then planted according to the standard germination test, compared with the non-pretreated seed (control). The results showed that 0.96% Ethephon was the most beneficial for releasing dormancy in fresh seed at harvest, achieving 86% emergence with only 5% fresh seed. The normal seedlings and fresh seeds from preheating at 40°C for 168 h were 75% and 11%, respectively. Only 6% normal seedlings were observed in the control and most of the seed were still dormant (89% fresh seeds). After storage at 20°C for 28 days, improved performance of normal seedlings was observed in both the Ethephon and preheating method, which showed over 90% normal seedlings and 0–1% fresh seed. Furthermore, 42% normal seedlings and 32% fresh seeds were found in the control. The experiment suggests that 0.96% Ethephon is the best method to introduce for breaking dormancy of peanut seed KK 4-7 because it can release dormancy in fresh peanut seed after harvest. Moreover, the duration for germination test is more rapid (only 10 days) due to preheating for 168 h; this is not necessary.

P1.19 Dormancy breaking in seed lots of rose root (Rhodiola rosea L.)

Michael Kruse and Franziska A Stöhr
University of Hohenheim, Fruwirthstr. 21, Stuttgart, Germany
(michael.kruse@uni-hohenheim.de)

Rose root (Rhodiola rosea L.) is a perennial plant in the family Crassulaceae. It grows naturally in wild Arctic regions of Europe, Asia and North America. The thick rhizome of plant at least three years old, is harvested because it is rich in secondary metabolites that are used in traditional medicine. Usually, plants are harvested in their natural environment. Since the demand for the rhizomes grows, companies have started to cultivate this species and to grow plants in nearby
fields under controlled conditions. The first experience in these initiatives is that seeds show significant physiological dormancy, with germination percentages not exceeding 10%. Therefore, the aim of this study was to develop a seed pretreatment protocol to break dormancy so that seed lots with more than 80% germination are available to farmers for growing transplants and cultivation. Three different seed lots were collected in different parts of the world. The seed viability was determined in a tetrazolium test and found to be between 63 and 84%. Germination without any treatment was between 2 and 6% only. In various treatments, seed samples were imbibed for 16 h in solutions of GA₃, GA₄+7, 2,4-dichlorophenoxyacetic acid (2,4-D), KNO₃ and water, and combinations thereof, with different concentrations. After the imbibition period, seeds were dried back at room temperature and a germination test was performed two weeks later with 200 seeds at 10/25°C alternating temperature (16 h / 8 h) with light during high temperature interval. The best treatment for breaking dormancy was found to be the imbibition in a 0.035% GA₄+7 solution for 16 h in darkness at room temperature and drying back. The treated seeds can be handled as usual, as was verified by commercial farmers. Since further pretreatments according this optimum protocol were always done just in time for sowing, there is no experience about the storability of the treated seeds. The developed seed treatment was the prerequisite for the cultivation of the species and is now used regularly.
POSTER SESSION 2 – ENSURING SEED QUALITY FOR FUTURE GENERATIONS

P2.01 Indo–German cooperation on seed sector development

Ekkehard Schroeder¹, Sowmini Sunkara¹ and Keshavulu Kunusoth²
¹Indo–German Cooperation Project on Seed Sector Development, ADT Projekt GmbH, Adenauerallee 174, 53113 Bonn, Germany
²Telangana State Seed and Organic Certification Authority and Telangana State Seeds Development Corporation Ltd, Hyderabad, Telangana State, India (ekkehard.schroeder@adt.de)

The Ministry of Agriculture and Farmers’ Welfare, Government of India and the German Federal Ministry of Food and Agriculture (BMEL), have signed a joint declaration of bilateral cooperation on seed sector development, intended to strengthen the political–legal framework for the variety protection system, conservation of seed varieties and plant breeding issues including harmonisation with international standards, as well as interaction of German and Indian plant breeder associations in the first phase (07/2013–12/2016). The current and second phase of the project (1/2017–06/2019) aims to support dialog on legal framework of the seed sector, including continuation of bilateral policy dialog and exchange of experience on the regulatory framework of PVP, supporting further processes to harmonise with the International Union for the Protection of New Varieties of Plants (UPOV) convention and to facilitate India’s participation in technical meetings for exchange of ideas and the two-pronged Convention Country Agreement of mutual interest. This ‘intensification phase’ includes supplementary activities in the southern Indian state of Telangana to support the ‘seed bowl’ initiative, with more focus on legal and political frameworks to improve quality seed supply systems internally, strengthening its competitiveness globally as well as international cooperation.

The technical partners at the national level are: the Protection of Plant Varieties and Farmers’ Rights Authority, the National Bureau of Plant Genetic Resources and the National Seed Association of India. In Telangana State, partners include: the Telangana State Seed and Organic Certification Authority, Telangana State Seeds Development Corporation Ltd, Professor Jayashankar Telangana State Agricultural University and Seedsmen Association Hyderabad. The main aim of the project is to test and support concrete approaches for optimising all stages of the seed production chain, through consultation and technical training by German/international experts.

The project has created awareness of the plant variety protection system and seed conservation technologies, and has helped to strengthen the PPV&FR Act (Protection of Plant Variety and Farmers’ Rights) and the need for harmonisation with international standards. Furthermore, the project activities support the regulative political framework of the seed sector, its growth internally and internationally, and have facilitated strong seed industry development through studies, seminars, workshops, exchange of ideas, outlined practical challenges to build expertise in the seed industry and improvement in skills of seed farmers through technical publications and facilitated international competitiveness to accelerate the seed trade to address the seed bowl initiatives. Thus, the bilateral project has supported the Indian seed industry in making improvements in access to quality seed by the farming community and to accelerate seed trade.
P2.02 Evaluation of the effects of pre-planting seed conditioning on the quality of sorghum seed

Doris Kanvenaa Puozaa1, Richard Oteng-Frimpong1, Michael Teye Barnor2, Desmond Sunday Adogoba1, Abdul-Rashid Issah1, Yusif Baba Kassim3 and Marian Dorcas Quain4

1CSIR-Savanna Agricultural Research Institute (SARI), PO Box TL 52, Tamale, Ghana
2West Africa Centre for Crop Improvement (WACCI), Crop Science Department, University of Ghana, Legon, Accra, Ghana
3KNUST College of Agriculture and Natural Resources, Kumasi, Ghana
4CSIR-Crops Research Institute, Fumesua, Kumasi, Ghana

(doriskanp@gmail.com)

Poor quality seed is a major limiting factor to sorghum production and productivity in northern Ghana, resulting in farms with poor plant establishment. The seed is usually characterised by fungal infections, poor viability, vigour and uniformity in germination. Meanwhile, many household materials found within the sorghum growing communities have been proven to be effective in improving germination and uniformity in other crops. This study sought to identify affordable and eco-friendly pre-planting treatments for improved performance of sorghum seed. A seed lot with 35% germination was treated with extracts of three locally abundant organic materials, moringa (Moringa oleifera) leaves, cow dung and husk of dawadawa (Parkia biglobosa) fruit ash, at four dilution factors (0, 10, 20 and 30) and two priming times, 6 and 12 hours. Treated seeds were air dried back to their original moisture content of 9% and tested in the laboratory for germination and health. There was variable response of sorghum seed to the type of priming agent and duration of priming. Seed germination was lowest at the highest concentration (0× dilution) of the plant extracts and peaked at 20× dilution factor. At 6 hours of seed priming, cow dung at 20× and 30× dilution gave the highest germination percentage of 48%, whereas dawadawa ash at 0× dilution reduced germination from 33% to 10%. Germination ranged from 1 to 52% at 0× and 20× dilution of moringa leaf extract, respectively, when seed was primed for 12 hours. When evaluated for health, four species of fungi were identified: Phoma sorghina, Exserohilum rostratum, Fusarium miniiforme and Curvilaria lunata. P. sorghina was the most abundant species, affecting a large percentage of the seed compared to the other fungi. Seed treatment with the organic agents in this study reduced the number of seeds infected by fungal pathogens and increased germination by up to 17%.

P2.03 Influence of packaging material and location on seed storability of soybean

Razia Sultana1, Keshavulu Kunusoth2, Pallavi Mandalapu1, Bayyapu Reddy Kaipu3, Sujatha Patta1 and Jhansi Rani Kaparthi1

1Professor Jayashankar Telangana State Agricultural University, Hyderabad 500030, Telangana, India
2Telangana State Seed and Organic Certification Authority, HACA Bhawan, Nampally, Hyderabad, India
3Acharya NG Ranga Agricultural University, APGC, Lam, Guntur, Andhra Pradesh 522034, India

(raziasajid@yahoo.com)

Soybean genotypes differ in their ability to maintain seed longevity under storage. Storage of seeds until the next sowing, with appropriate packages and suitable locations, is an essential segment of the seed industry in India. Thus, the study was conducted to identify suitable locations with appropriate packaging material for safe seed storage until the next planting season, using four packaging materials (cloth bag, jute bag, HDPE bag and airtight container with drying beads) at three locations, viz. Hyderabad, Nizamabad and Guntur Districts of Andhra Pradesh, India, for ten
months under ambient conditions. The experiment was initiated with two cultivars (JS 335 and JS 93-05) with initial germination of 93% and 90%, respectively, and seed moisture content (SMC) of 9.62% and 9.16%, respectively. The experimental results revealed significant variation for the seed quality parameters studied. An overall perusal of the results at the three different locations indicated that the airtight container using drying beads (impervious) followed by HDPE bags with SMC around 6–8%, could provide better packaging material than pervious material (cloth and jute bags), to maintain seed quality irrespective of locations. Among the cultivars studied, JS 335 had higher seed quality when compared to JS 93-05 during the storage period. The Hyderabad location was found to be better for safe seed storage when compared to other locations. Thus, commercial storage of soybean seed can be carried out at below 7% SMC with moisture proof packaging material, to maintain seed germination above seed certification standards for up to ten months. In the present study, seed stored at low moisture content showed high germinability and vigour throughout the storage period.

P2.04 Impact of fungicide treatments on seed quality and storability of wheat (**Triticum aestivum** L.) under ambient conditions

VS Mor, Axay Bhuker, Pradeep Singh, Hemender Tanwar, Sushma Sharma and Anurag Malik
Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Department of Seed Science and Technology, Hisar, India
(virendermor@gmail.com)

Once the seed of food crops is treated with any pesticide to prevent infection from diseases and insect pests, the treated seed can only be used for planting and the unsown carryover seed is not recommended for food or feed purposes, to avoid health hazards to humans and animals. To use this treated seed for planting purposes in the next sowing season, an experiment was planned to study the impact of fungicide treatments on seed quality and storability of wheat during ambient storage. Six-month-old processed seed of wheat varieties (C 306, WH 1105 and WH 1124) were treated with two fungicides (Tebuconazole at 1 g/kg and Carboxin at 2 g/kg) in November 2015. The seed samples of all treatments were kept in plastic containers at ambient storage and evaluated in respect of seed quality parameters. The seed quality analysis showed that initially there was significant improvement in germination percentage and vigour index of treated wheat seed as compared to the control in all three varieties. The germination percentage of treated seed of all varieties increased at three months' storage (February 2016) but started declining after six months and untreated seed showed a continuous decline with an increase in the storage period. The vigour index of control and treated seed of all varieties showed continuous decrease with an increase in storage period. The treated seeds retained the germination percentage above Indian Minimum Seed Certification Standards (IMSCS) (85%) up to three months in C 306 for both the treatments, and up to six months in WH 1105 and WH 1124 for Tebuconazole-treated seed and nine months for Carboxin-treated seed during ambient storage. The germination percentage was retained up to 50% at 24 months (November 2017) in C 306 and WH 1105 and up to 27 months (February 2018) in the case of WH 1124. The Carboxin-treated seed showed better improvement and more longevity as compared to Tebuconazole-treated seed and untreated wheat seeds. The conclusion drawn from the study is that fungicide-treated wheat seed with Tebuconazole at 1 g/kg and Carboxin at 2 g/kg results in better seed quality enhancement and longevity in storage under ambient conditions for up to two years. The carryover treated wheat seed can be used at a higher seed rate in the next sowing season, to maintain the plant stand.
P2.05 Seed quality enhancement through Zn and Fe nanoparticles in pigeon pea

Pradeep Korishettar1 and Vasudevean SN2
1Telangana State Seed and Organic Certification Authority, 5-10-193, First Floor, HACA Bhawan, Hyderabad, India
2VC Farm, ARS Mandy, ADR, Mandy, India
(kpradeep5061@gmail.com)

Modern agriculture with its quest for technology and precision, demands that every seed should readily germinate and produce a vigorous seedling, ensuring higher yield. Zinc (Zn) and iron (Fe), being essential micronutrients required for normal plant growth and development, are important components of various enzymes that are responsible for driving many metabolic reactions. Recently, use of these elements in the form of nanoparticles (NPs) has been gaining importance especially for enhancing seed quality in a few crops, as these microelements are required in minute quantities for treating seeds. In this context, an effort was made to know the effect of seed polymer coating with Zn and Fe nanoparticles at different concentrations (10, 25, 50, 100, 250, 500, 750 and 1000 ppm) in pigeon pea, at the Department of Seed Science and Technology, College of Agriculture, Raichur, India. Among the treatments, seed polymer coating with Zn NPs at 750 ppm recorded significantly higher seed germination (96.00%), seedling length (26.63 cm), seedling dry weight (85.00 mg), speed of germination (32.95), field emergence (89.67%), seedling vigour index (2556), dehydrogenase activity (0.975 OD value) and α-amylase activity (25.67 mm), and the lowest abnormal seedlings (2.50%) over their bulk forms and control, followed by Fe and Zn NPs at 500 ppm. In contrast to the beneficial effects, these NPs also showed an inhibitory effect on germination and seedling growth at higher concentration (nano-Zn > 750 ppm and nano-Fe > 500 ppm). Hence, from the results it is concluded that Zn and Fe NPs at lower concentration can be used to enhance quality in pigeon pea seeds.

P2.06 Genotypic effects for seed characteristics and grain yield in maize (Zea mays L.)

Tulasiram Mundai1, Murali Krishna Kalahasthi1, Sunil Neelam2 and Sujatha Patta1
1Professor Jayashankar Telangana State Agricultural University, Rajender Nagar, Hyderabad, India
2Indian Institute of Maize Research, Rajender Nagar, Hyderabad, India
(mundai.tulasiram@gmail.com)

The number of kernels per row, the number of kernel rows, the 100 seed weight and seed vigour, all have a direct bearing on grain yield in maize. Hence, to determine the relative magnitude of various genotypic effects for kernels per row, number of kernel rows, 100 seed weight and seed vigour, 140 inbreds along with three checks were evaluated in an augmented block design in Kharif 2017 at the Winter Nursery Centre, Indian Institute of Maize Research, Rajendranagar, Hyderabad. Five inbreds, IC0620927-4 (10.07), IC0620983-2 (10.51), IC0620985-2 (10.18), IC0620987-5 (12.18) and IC0620994-6 (17.85), recorded significantly high genotypic values for number of kernels per row. Only two inbred lines, namely IC0621047-1 (5.22) and IC0112569-142 (6.12), recorded significant values for number of kernel rows. Inbred lines, viz. IC0620989-3 (17.17 g), IC0620994-6 (17.17 g), IC0620973-1 (15.17 g), IC0620985-2 (15.17 g) and IC0620987-6 (13.17 g), recorded significantly positive genotypic effects for 100 seed weight. Four inbred lines, viz. IC0620987-5, IC0620989-3, IC0620994-6 and IC0621018-5, exhibited significant genotypic values for seed vigour. These inbreds also exhibited significant genotypic effects for grain yield per plant. The highest grain yield of 123.83 g was recorded by IC0620994-6 followed by IC0620989-3 (109.83 g), IC0621018-2 (101.83 g) and IC0620987-5 (98.49 g). The highest yield in IC0620994-6 can be
attributed per se to a high number of kernels per row, 100 seed weight and seed vigour. Kernels per row, 100 seed weight and seed vigour contributed to a high yield in IC0620987-5.

**P2.07 Management of pulse beetle in green gram seed using a modified atmosphere with elevated CO\textsubscript{2} levels**

*Sowmya Tula\textsuperscript{1}, Kanaka Durga K\textsuperscript{2}, Razia Sultana\textsuperscript{3} and Keshavulu Kunusoth\textsuperscript{4}*

\textsuperscript{1}Office of the Assistant Director of Seed Certification, Telangana State Seed and Organic Certification Authority, Ghandhi Road, Karimnagar, India  
\textsuperscript{2}Department of Plant Breeding, Agricultural College, Siricilla, Sardapur, District of Rajanna, India  
\textsuperscript{3}Department of Seed Science and Technology, Seed Research and Technology Centre, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Rajendranagar, Hyderabad, 500030, India  
\textsuperscript{4}Telangana State Seed and Organic Certification Authority, HAKA Bhavan, Hyderabad, India  
(sowmyarao.t@gmail.com)

Management of pulse beetle through modified atmosphere using elevated levels of CO\textsubscript{2}, was conducted under laboratory conditions at the Seed Research and Technology Centre, PJTSAU, Hyderabad, using two varieties of green gram, namely LGG 460 and LGG 407, in replications of three each. Ten pairs of *Callosobruchus chinensis* adults were released into a 500 g capacity container containing 250 g of disinfested green gram seed. After 25 days of release of test insects, the weight of the seed was taken, and CO\textsubscript{2} was released at four different concentrations, namely 15, 30, 45 and 60%. An untreated control was also maintained under normal conditions without exposing the seed to CO\textsubscript{2} concentration. The results revealed that the biological parameters of the test insects, such as adult emergence, insect damage and per cent weight loss, and the seed quality parameters, such as germination and seedling vigour, were highly influenced by different levels of CO\textsubscript{2} and storage intervals and varied among the two accessions. The overall present finding on the efficacy of different concentrations of CO\textsubscript{2} on seed damage and population build-up, revealed that exposing the bruchid infested green gram seed to 45 and 60% CO\textsubscript{2} not only checked the seed damage but also checked the progeny production as compared to 15 and 30% CO\textsubscript{2} levels, even after two months of storage. The green gram seeds stored in a CO\textsubscript{2} rich atmosphere also maintained seed quality with high germination percentage and seedling vigour up to 2 MAS. Among the two accessions, LGG 460 had the least adult emergence; comparatively less insect damage with minimum weight loss besides maintaining good germination and vigour at 60% concentration of CO\textsubscript{2} at two months after storage.

**P2.08 Desiccant drying technology for controlling pulse beetle (bruchids) in mung bean seeds**

*Razia Sultana\textsuperscript{1}, Keshavulu Kunusoth\textsuperscript{1}, Karpandi Jhansi Rani\textsuperscript{1}, Peetambar Dahal\textsuperscript{2}, Johan van Asbrouck\textsuperscript{3} and Kent J Bradford\textsuperscript{2}*

\textsuperscript{1}Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad, India  
\textsuperscript{2}Seed Biotechnology Center, UC Davis, USA  
\textsuperscript{3}Rhino Research, Phichit, Thailand  
(raziasajid@yahoo.com)

In India, mung bean seed is multiplied in the wet season, and is often stored until the next wet season for planting or even longer for carryover seeds. High temperature and humidity combine to cause rapid seed deterioration and provide a favourable environment for insect pests. Among the insect pests, bruchids (*Callosobruchus chinensis* (L.)) increase losses and adversely affect seed quality during storage. To combat these storage losses, a safe, non-toxic seed storage technology
to maintain mung bean seed quality and prevent damage from bruchids during storage, was tested at the Department of Seed Science and Technology, PJTSAU, Hyderabad, India in 2011. Freshly harvested and sterilised seeds (500 g) were artificially infested with five pairs of freshly emerged five-day-old adult bruchids and stored in cloth and hermetic containers using two types of desiccants, viz. silica gel and zeolite drying beads. Seeds were also stored in cloth bags (control) and hermetic containers with silica and zeolite desiccants, along with ten adult bruchids in each treatment. The seed moisture content (SMC) was reduced to 6.00 and 4.00% from an initial value of 10.32% using silica gel and zeolite beads, respectively. These moisture contents were maintained essentially unchanged for 30 months of storage in hermetic containers. Thus, desiccant drying beads efficiently dried the seeds to ultra-low moisture content. As a result, there was no bruchid infestation or damage, and seed and seedling quality parameters were maintained above Indian Minimum Seed Certification Standards (IMSCS), even after 30 months of storage. In contrast, 100% seed damage was seen in cloth bags after 28 months of storage, when the SMC was 10.47%. The per cent seed damage, total fungal infection percentage and mean oviposition (3.0 eggs) were also restricted in the zeolite bead treatment as compared to other treatments (control 248.0 eggs). Thus, the combination of desiccant bead drying and waterproof packaging (termed the ‘dry chain’) minimised the loss in seed germination under ambient storage and maintained the minimum seed germination standards up to 30 months, in addition to completely preventing damage due to bruchid infestation.

P2.09 Desiccant drying technology for soybean seed storage

Razia Sultana, Kaparthi Jhansi Rani, Amtul Raheem and Keshavulu Kunusoth
Department of Seed Science and Technology, Seed Research and Technology Centre, Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana State, India
(raziasajid@yahoo.com)

India has high potential for soybean cultivation, to meet its oil and protein requirements. However, there are serious obstacles in its seed production to realise the yield potential and quality seed supply. A quality seed supply system has always been one of the main challenges within the production system. Due to this importance, there has been an incessant search for effective methods of storage of seed lots within the seed supply chain. The main challenges are drying of seeds to a low moisture content and appropriate seed storage technologies to improve the seed quality system. Thus, the main aim of the study was to evaluate the effect of a desiccant drying system and storage to maintain soybean seed quality. In May 2014, freshly harvested sundried soybean seeds (variety JS 335) with an initial moisture content of 11.0%, were dried further by adding drying beads (zeolites) and stored hermetically under ambient conditions. The results indicated that the desiccant drying beads were able to lower the moisture content to 7.20% within two months and maintain low moisture of 5.16% during subsequent months. Furthermore, the seed and seedling quality was maintained for up to 12 months as compared to the control. Being an oilseed crop, seed deterioration occurred in all the treatments. However, the rate of deterioration was very low in the drying bead treatment, where the initial germination percentage of 85% was maintained at 70% at the end of 12 months storage, while there was a sharp decline to 71% within two months in cloth bags (control) with literally nil by the end of storage period. Therefore, the zeolite beads can efficiently and inexpensively remove water from seeds, helping to minimise the loss in seed germination under ambient conditions and can maintain viability of seeds for a longer time. However, this desiccant drying system is not cost effective due to the high voluminous nature of seed. Hence, desiccant drying systems can be upscaled for drying and safe storage of soybean seeds until the next planting season.
P2.10 A study on eRH threshold limits of pulse beetle [*Callosobruchus chinensis* (L.)] infestation

Razia Sultana¹, Kaparthi Jhansi Rani¹,Amtul Raheem, Keshavulu Kunusoth¹ and Kent J Bradford²

¹Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad, India
²Seed Biotechnology Center, UC Davis, USA
(raziasajid@yahoo.com)

Bruchids are a major and growing problem in the storage of pulse seeds, especially in mung bean under high temperature and relative humidity in tropical and subtropical regions. The infestation may start in the field itself and is often difficult to detect at harvest and subsequent operations. The infestation is more common in farm stored seeds in India under ambient conditions, due to an inability to dry the seeds to safe storage moisture contents, and the problem is further aggravated due to high temperature and relative humidity, thereby making storability of mung bean seed a difficult task. Ironically, there are currently no standardised equilibrium relative humidity (eRH) threshold limits for mung bean bruchids for safe storage of mung bean seeds. Within this context, an experiment was carried out in 2013 to identify the seed eRH threshold limits for bruchid infestation in mung bean, at the Department of Seed Science and Technology, PJTSAU, Hyderabad, India. Five pairs of bruchids (4–5 days old) were released into eRH treatment in airtight containers containing mung bean seeds. The data on eRH and temperature were recorded daily. Seed eRH was maintained at 20, 30, 40, 50, 60 and 70% using drying (zeolite) beads. Insect per cent mortality threshold level was recorded at 5, 7 and 11 days by destructive sampling. Insect damage was recorded by checking oviposition and number of insect emergence holes in seeds. The experiment revealed 100% bruchid mortality in the containers with below 40% eRH, in contrast to containers with above 40% eRH. The initial germination of 89% was maintained at 20% eRH, while a gradual decrease in germination percentage was observed as the eRH increased to 70%. The germination percentage was drastically reduced to 77% within one month of storage (one life cycle of bruchid) at 70% eRH and in cloth bags with above 9% seed moisture content. This indicated that the bruchid infestation could be controlled by drying to an extent of less than 8% of seed moisture content or less than 40% of eRH without use of toxic pesticides. This technology could be very useful in restricting bruchid multiplication immediately after harvest and to maintain viability of seed during post-harvest seed storage, especially in tropical and subtropical regions.

P2.11 Implementing the dry chain during storage reduces losses and maintains quality of quinoa seed

Irfan Afzal, Muhammad Bakhtavar and Shahzad Basra

Seed Physiology Laboratory, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan
(lafzal@uaf.edu.pk)

Globally, one-third of the total food produced for human consumption is lost after harvest, which adversely affects agricultural productivity and food security for the rising populations. The primary cause of such losses is poor storage and especially high seed moisture contents (SMC), which result in fungal and insect infestations and mycotoxin accumulation. Quinoa seed loses viability more rapidly than cereals because of its seed structure, which allows it to gain or lose moisture under ambient conditions. Maintenance of commodity dryness through hermetic packaging (the ‘dry chain’) may prevent these storage losses. In the present study, quinoa seeds were stored at 8, 10, 12 and 14% SMC in traditional packaging materials (paper, woven polypropylene, jute and cloth) as well as in hermetically sealed bags, under ambient storage conditions for one year. During storage, slight fluctuations were observed in the moisture contents of seeds stored in hermetic bags, while SMC increased with environmental relative humidity (RH) in all other packaging materials. Higher
storage losses (≈ 10%) were observed in traditional packaging materials compared to hermetic bags (< 1%). Seed storage in hermetic bags at 8 and 10% SMC also maintained germination, starch and crude protein contents. Malondialdehyde and aflatoxins were present at higher amounts in seeds at 14% SMC in hermetic bags and in all traditional packaging materials. In conclusion, storage losses and deterioration of seed quality with respect to germination, loss of food reserves and increased aflatoxin contamination were highly related to SMC. Maintaining the dry chain through hermetic storage of seeds at 8 and 10% SMC preserves high seed viability and vigour during storage. Thus, implementation of the dry chain concept has far-reaching consequences to improve food quality, safety and security.

P2.12 Genetic markers in seed quality control in Serbia

Zorica Nikolić, Maja Ignjatov, Dragana Miloševic, Dušica Jovičić, Gordana Petrović and Gordana Tamindžić

Institute for Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia
(zorica.nikolic@ifvcns.ns.ac.rs)

Genetic markers have been used at the Institute of Field and Vegetable Crops Novi Sad (IFVCNS) for many years, both for seed quality control and for research purposes. The first laboratory in Serbia that started applying the electrophoresis of isoenzymes was at the IFVCNS, in 1986. The distinct advantage of laboratory seed purity control is that the seed can be inspected until the next sowing at different stages, before harvesting or ripening, during or after seed processing. In addition to conventional laboratory methods in identifying pathogens, serological and molecular methods are used nowadays. The main feature of these methods is that they are fast, reliable and suitable for analysing a large number of samples. Determination of the presence and distribution of viral and bacterial diseases of paprika (Capsicum annuum L.) were performed using serological and molecular detection. In addition to determining the presence and distribution of pepper virus, identification of the most important and most frequent virus isolates was performed using the reverse transcription method followed by a polymerase chain reaction (RT-PCR). Differentiation of Xanthomonas spp., the causative agent of the bacterial pungency of pepper, was carried out using restriction fragment length polymorphism (RFLP) analysis of the hrp gene (HrpB region) and the PCR method, thus confirming the similarity of studied strains with the species X. euvesicatoria. According to the valid legislation in Serbia, commercial cultivation of genetically modified organism (GMO) crops is not allowed. The laboratories involved in GMO analyses have been accredited according to ISO / IEC 17025:2006 standard and ISTA standards, and authorised by the Ministry of Agriculture, Forestry and Water Management for analysis of seeds and product consignments of plant origin from import. All laboratories use internationally recognised methods and participate in testing the expertise for GMO analysis in inter-laboratory comparisons. Our laboratory for seed testing has conducted several studies in order to demonstrate the presence and quantity of Roundup Ready soybean in crude oil, assess DNA degradation, DNA amplification, and GMO quantification during tofu production, to report on the validity of labels on imported food samples from different countries and uptake of GM food derived from soybean into the food chain in Serbia.
P2.13 Characterisation of green gram genotypes for qualitative traits

Sowmya Tula1, Kanaka Durga K2, Razia Sultana3 and Keshavulu Kunusoth4

1Office of Assistant Director of Seed Certification, Near SRM PG College, Karimnagar, India
2Agricultural College, Professor Jayashankar Telangana State Agricultural University, Sircilla, Rajanna Sircilla District, Telangana, India
3Department of Seed Science and Technology, Professor Jayashankar Telangana State Agricultural University, Seed Research and Technology Centre, Rajendranagar, Hyderabad 500030, India
4Telangana State Seed and Organic Certification Authority (TSSOCA), HACA Bhavan, Hyderabad, India

The study on characterisation of green gram genotypes was conducted at the National Bureau of Plant Genetic Resources, Hyderabad. Seventy-three accessions of green gram collected from different sources, along with three controls (ML-267, LGG-460 and K-851), were studied for 20 qualitative characters, viz. plant growth habit, plant habit, stem colour, stem pubescence, leaf pubescence, leaflet lobes (terminal), leaf shape (terminal), leaf colour, leaf vein colour, petiole colour, leaf size (at 5th node from the base), colour of flower petal (standard), colour of premature pod, pod pubescence, pod position, pod colour, curvature of mature pod, seed colour, seed lustre and seed shape. From the above characters, some distinct morphological traits may be considered as key characters and useful for seed production programmes in varietal identification. Based on plant growth pattern, all 76 accessions significantly exhibited indeterminate growth habit. On the basis of stem pubescence, green gram accessions were categorised into three groups, i.e. sparse, moderate and dense. Out of 76 accessions, 14 accessions exhibited sparse stem pubescence, 49 accessions possessed moderate stem pubescence and 13 accessions exhibited dense pubescence. On the basis of leaf pubescence, green gram accessions were distinguished into three categories, i.e. sparse (53), moderate (16) and dense (7). As per the DUS descriptor, leaf size recorded at the 5th node from the base of the plant is grouped into three size classes, i.e. small, medium and large. In the present study, out of 76 accessions, eight exhibited small sized leaves, 43 possessed medium sized leaves and the remaining 25 had large sized leaves. Based on seed lustre, green gram accessions were grouped into dull and shiny. Out of 76 green gram accessions, 43 were dull type and 33 had the shiny type of lustre. On the basis of seed shape, green gram accessions exhibited diversity, i.e., oval and drum. Among 76 accessions, 43 exhibited oval seed shape and 33 were drum shaped. Among the distinct morphological traits, seed lustre and seed shape may serve as key characters and useful for identification of off types in seed plots of different classes of seed.

P2.14 Indigenous knowledge in sorghum and millet seed quality management among smallholder farmers in northern and eastern Uganda

Rachel Gitundu1, Ruth Njuguna2, Brenda Amulo, Helen Opie3, Dennis Nino3 and Esther Njuguna1

1International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), 30677-00100, Nairobi, Kenya
2Centre for Behaviour Change and Communication (CBCC), 5937-00100, Nairobi, Kenya
3National Agricultural Research Organization (NARO), 7084, Kampala, Uganda

In this paper, we share lessons on local seed systems management from the traditional smallholder farmers of northern and eastern Uganda. We focus on local knowledge systems applied in separation of sorghum and millet seeds from grains, preparation of the seed and grain for storage and traditional seed quality maintenance term. We attempt to document how this indigenous
knowledge is passed from generation to generation and the implications this has for delivery of new and improved varieties in the drylands. Home saving of sorghum and millet seeds from season to season by smallholder farmers in the drylands of East Africa, is an important strategy for agricultural development. Seed management between seasons requires specialised care to ensure that seed quality does not deteriorate throughout the process of post-harvest handling and long-term storage. To understand if and how indigenous knowledge is applied by farmers in sorghum and millet seed management and the impact their practices have on production, we derived in-depth life histories from 30 female and 30 male farmers in northern and eastern Uganda. The 60 farmers were identified through a process of purposive sampling, to represent sorghum and millet producers, female and male. We used a vignette as a tool to guide in-depth conversations with the community representatives. In the vignette, we introduce a young couple to the community representatives, who are making key decisions about sorghum and millet production. Through the introduction of different scenarios and using probing methodologies, we obtained insights about the actions farmers take in seed storage practices, the reasons behind them and how they maintain quality. We analysed the data thematically using content data analysis. We compared our results from results obtained through a household survey among 520 farm households in the same region. The results showed that there seems to be a difference in the pace of acceptance of new methods of seed storage between eastern and northern Uganda. This would be correlated to the number of intervention programs that have focused more on the north, in support of recovery from political instability running up to 1985. In northern Uganda, many NGOs have been in operation following the war, focusing on introduction of improved as well as quality seeds. Local seed businesses are emerging, and with them, advanced ways of managing seed from season to season. The market also plays an important role in seed exchange in northern Uganda, compared to eastern Uganda. There are several seed companies that are emerging from local seed businesses. In eastern Uganda, farmers still use traditional/indigenous methods in sorghum and millet seeds management in applying powders from Lantana camara, red pepper and dried neem tree leaves to prevent storage pest attacks. We are still analysing household survey data to find out if farmers in the north have significantly better sorghum/millet yields compared to farmers in the east, who have remained traditional in their seed management, or if the traditional seed management systems have been competitive and sustained the yield levels. Seed storage practices are context specific, depending on a community’s cultural history and the development of interventions that influence the knowledge gained by community members, as well as the infrastructure that has grown to facilitate access to improved seed storage technologies. We recommend an in-depth understanding of the local seed management context as a beginning of intervention programs.

P2.15 Evaluation of biofertiliser stages and their combinations on seed quality enhancement of chickpea (Cicer arietinum L.)

VS Mor1, SS Sindhu2, Axay Bhuker1, Ruchi Sharma2, Hemender Tanwar1, Sushma Sharma1, Anurag Malik1, VPS Sangwan1 and RC Punia1

1Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Department of Seed Science and Technology, Hisar, India
2CCSHAU, Department of Microbiology, Hisar, India
(virendermor@gmail.com)

An experiment was planned to evaluate the biofertiliser stages and their combinations on seed quality enhancement of chickpea. The seed of two chickpea varieties (HC-1 and HC-5) was treated with two biofertiliser strains (Rhizobium spp. and Bacillus spp.) of fourth- and eighth-day stages, separately and in combination. The seeds of different treatments and combinations were planted under standard conditions in the laboratory. The evaluation of seedlings was carried out to assess the germination percentage and vigour indices in different treatments and combinations. The results revealed that a significant improvement was observed in all treatments over the control. Better improvement in seed quality was observed in the Bacillus strain as compared to Rhizobium. The higher enhancement in germination and vigour was observed in the eighth-day stage of the Bacillus
strain as compared to the fourth-day stage, and in both stages of *Rhizobium* and the combination of both strains of both stages. The co-inoculation of *Rhizobium* and *Bacillus* of different stage combinations showed an improvement in the germination and vigour index of chickpea seed of both varieties over the control, but the improvement in blending application was less as compared to individual application of biofertiliser.

**P2.16 Application of Probit analysis and neuronal networks to predict seed longevity**

**Ralf Schulze Brüning, Markus Oppermann, Andreas Börner, Manuela Nagel**
Genebank Department, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben), Seeland, Germany
(nagel@ipk-gatersleben.de)

Long-term storage of orthodox, desiccation-tolerant seeds is important to conserve plant genetic resources for research, breeding and future applications. The federal *ex situ* genebank for agricultural and horticultural plants in Gatersleben, Germany, preserves more than 150,000 accessions of about 3000 different species. To assess the viability status of the different collections and the necessity of seed regeneration, more than 405,000 germination tests have been conducted since 1976. The current study aims to use the results of the germination tests to predict seed longevity and to support the management of the genebank. Therefore, results of two statistical approaches, Probit analysis and neural networks, were compared for important crop species such as barley (*Hordeum vulgare*), oilseed rape (*Brassica napus*) and sugar beet (*Beta vulgaris*). Most germination tests achieved between 60 and 100%. Therefore, the prediction error increased when germination was estimated below 60%. The best and accurate predictions were achieved for neuronal networks using a high quantity of data and additional historic data. When only germination data were used, the results of the Probit analysis were comparable with the predictions of the neuronal networks.

In conclusion, when a high quantity and quality of data is available, the application of neuronal networks on genebank data may elucidate indicators of seed longevity and support genebank management in the future.

**P2.17 Australian Grains Genebank**

**Katherine Whitehouse, Sally Norton, Giao Nguyen and Nicole Sawyer**
Agriculture Victoria, Private Bag 260, Horsham, Australia
(katherine.whitehouse@ecodev.vic.gov.au)

The Australian Grains Genebank (AGG) is the national programme for preserving grain crop genetic resources for Australia. Our mandate is to acquire, conserve and maintain viable seed, and distribute germplasm for use in Australian plant research and breeding programmes. The collection is an amalgamation of three previous genebanks in Australia: Horsham, Victoria, operated as a medium-term genebank for temperate oil seeds and pulses; and long-term genebanks at Biloela, Queensland for tropical crops and Tamworth, New South Wales for winter cereals. The AGG collection contains approximately 195,000 accessions of cultivars, breeding lines, landraces and wild relatives originating from more than 150 countries and includes approximately 850 species from over 90 genera. The AGG introduces c. 3,500 new accessions into the collection, regenerates c. 4,500 accessions and distributes c. 25,000 accessions annually. The AGG aligns with the recommendations set out by the Genebank Standards (FAO, 2013), and is in the process of reviewing protocols and genebank management practices for both regeneration and post-harvest handling. This review process will enable AGG to improve and optimise seed quality traits (ability to germinate and survive air-dry storage) while maintaining genetic diversity and integrity. Digital image analysis technologies are being evaluated to capture whole plant and seed phenotypic traits,
including RGB and hyperspectral wavelength cameras. Genomic characterisation of germplasm is being undertaken to determine taxonomic identity and will be used to evaluate genetic integrity of accessions to validate AGG regeneration and seed handling activities. This poster discusses the new technologies being used to evaluate AGG germplasm for seed quality and genetic integrity.

**P2.18 Genetic variability for seed longevity among diverse sorghum parental lines**

Kannababu Netyam, Madhusudhana Ragimasalawada, Das Indrakanta and Vilas Tonapi  
ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad 500030, Telangana, India  
(kannababu@millets.res.in)

Many high yielding sorghum hybrids, their parents and varieties, have low seed storability which is one of the major risk factors in the economics of sorghum seed trade. Often, it may not be affordable to store large quantities of various classes of sorghum seed under long-term cold storage conditions. A set of 18 diverse sorghum parental lines was evaluated for seed longevity traits (as a measure of seed storability) at the ICAR-Indian Institute of Millets Research, Hyderabad. The variations in seed quality traits of fresh, accelerated aged and stored (1 year, 2 years) seed categories among 18 parental lines, were estimated as per standard protocols. The genotypes IS10284, SSV84, IS9830, CSV 8R, N13 showed higher initial seed quality and storage potential (accelerated ageing) whereas 296B and IS2122 were poor. For seed longevity, IS9830, N13, SSV84, IS10284 and M35-1 were found superior after one year of seed storage, while N13, IS9830, IS2205, IS8525 and IS26866 after two years of storage, indicating their inherent seed longevity potential. The genotypes 296B, 27B, CS3541 (parents of some released hybrids), CSV216R and M35-1 (varieties) were inherently very poor in their seed longevity and therefore, storability. All seed physiological traits (germination, shoot length, root length, seedling dry weight, vigour index and field emergence) of fresh, accelerated aged and stored seed showed moderate to high phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV) values. The study indicated the diversity for seed longevity traits in sorghum, and the opportunity that exists to exploit the identified donors for seed longevity traits in sorghum improvement programs and to develop genetic mapping populations for trait mapping and marker-assisted breeding.

**P2.19 Fruit maturity and seed moisture content affect seed storage in papaya (Carica papaya L.)**

Wen-Ju Yang¹, Yu-Ju Ho¹, I-Cheng Chen² and Ting-Lin Chang²  
¹National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei City 10617, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu  
²Taiwan Seed Improvement and Propagation Station (TSIPS), No.6, Xingzhong St., Xinshe Dist., Taichung City 426, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu  
(wendy@ntu.edu.tw)

The storage of papaya seeds, a kind of intermediate seed, is sensitive to storage temperature and affected by seed moisture content and fruit maturity at harvest. Storing seeds of TN1 with a series of moisture contents (8.3–30.6%) at 0, 5 and 10°C, continuously for 24 months, we found that seeds with higher moisture content were more sensitive to lower temperature and intolerant to storage. Seeds of 8.3% moisture content maintained their germination throughout the experimental period at all three temperatures, indicating that the seeds were tolerant to 0°C storage; whereas, seeds of 12.3% were tolerant to 5°C and seeds of 21.7–16.4% were safe for storage at 10°C. Seeds
with 30.6% moisture content showed a rapid decrease after three months of storage at all three temperatures. Seeds (~ moisture content 10%) harvested from full yellowed fruits could maintain their germination at all three storage temperatures over the 24-month experimental period, and seeds from fruits that were not fully mature showed sensitivity to lower temperature storage (5 and 0°C). Three days of ripening of the mature green fruits may resume their ability for storage at 5 and 0°C; while three days of ripening treatment did not show the same effect on immature green fruits. Therefore, seeds from mature fruits with the seed moisture content mediated at about 10% (8.3–12.3%), was the suggested method for seed production. If the fruit colour is not yet completely yellow, three days of ripening is recommended; however, the fruit maturity should be at least mature green.

**P2.20 Alleviating dormancy in fresh seeds of barnyard millet (Echinochloa frumentacea L.) var. CO 2**

S Venkatesan¹ and K Sujatha²

¹Tamil Nadu Agricultural University, Coimbatore 625104, Tamil Nadu, India
²Tamil Nadu Agricultural University, Agricultural College and Research Institute, Madurai 625104, Tamil Nadu, India

(svengat95@gmail.com)

Seed dormancy is the resting period of seed after physiological maturity and is an adopted mechanism to overcome stress conditions. Different types of seed dormancy can be distinguished on the basis of timing of the induction of dormancy rather than the location or mechanism of dormancy. In many members of the Poaceae family, the caryopsis, the spikelet or from glumes have different levels of dormancy and in a few species, dormancy caused by chemical inhibitors present in the seed coat or glumes. The present study was conducted to alleviate dormancy in fresh seeds of barnyard millet (Echinochloa frumentacea) var. CO 2. Fresh seeds were primed with bio-priming (Pseudomonas fluorescence 2 g/kg), osmo-priming (PEG), chemo-priming (succinic acid 100 ppm), halo-priming (KH₂PO₄ 1%) hormo-priming (GA₃ 10 ppm), botanical priming (Calotropis 15%) along with hydro-priming, and seeds were soaked for about 12 h and analysed for physiological quality and biochemical parameters. The results revealed that priming the seeds with succinic acid 100 ppm for about 12 h improved the physiological quality and showed lower values for free amino acid, sugar and electrical conductivity. The percentage increase over the control was by 42%.

**P2.21 Determination of volatile composition of barnyard millet genotypes by gas chromatography–mass spectroscopy**

S Venkatesan¹ and K Sujatha²

¹Tamil Nadu Agricultural University (TNAU), Coimbatore 625104, Tamil Nadu, India
²TNAU, Agricultural College and Research Institute, Coimbatore 625104, Tamil Nadu, India

(svengat95@gmail.com)

Barnyard millet is a fair source of highly digestible protein and an excellent source of dietary fibre with a reasonable quantity of soluble and insoluble fractions. The carbohydrate content is low and slowly digestible, which makes barnyard millet a gift from nature for modern humans engaged in sedentary activities. The grains of barnyard millet are low in phytic acid and rich in iron and calcium. The present study was an effort to explore the biochemical composition of barnyard millet seeds with special reference to their volatile profiling. The seeds of barnyard millet varieties, viz. CO 2 (fresh and old) and MDU 1 (fresh and old), were used for identification of volatile compounds in hexane extract. The GC–MS analysis results revealed that 9,12-octadecadienoic acid (Z,Z) was found as a major constituent, followed by n-hexadecanoic acid, prominently in hexane extract of barnyard millet. 9,12-octadecadienoic acid (Z,Z) and linoleic acid are two essential fatty acids
that humans and other animals must ingest for good health, because the body requires them for various biological processes, but cannot synthesise them from other food components. They act as anti-cancer, anti-inflammatory, anti-androgenic and dermatitigenic. n-Hexadecanoic (palmitic acid) acts as anti-inflammatory, antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavour, haemolytic, 5-Alpha reductase inhibitor and potato mosquito larvicide.

**P2.22 Establishment of the international standard calibration methods of moisture meters**

Yu Wen Kuo¹ and Bo Jein Kuo²

¹Taiwan Seed Improvement and Propagation Station (TSIPS), No.6, Xingzhong St., Xinshe Dist., Taichung, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu
²Department of Agronomy, National Chung Hsing University, 145 Xingda Rd., South Dist., Taichung, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu (zoe@tss.gov.tw)

In this study, we used two electronic moisture meters (resistive and capacitive) and the international standard reference method for seed moisture determination. Here, the oven method was used to determine the moisture content of Tainan No.11. The results showed that, when the oven method was used, the error values of the results measured by the electronic moisture meters exceeded the maximum tolerance of the standard values. Furthermore, the measurement results of the electronic moisture meter were corrected by using regression analysis to establish an equation for predicting the standard values. It was further found that the regression equation \( y = 1.0983x - 2.2244, \quad R^2 = 0.9854 \) obtained by the capacitance moisture meter had greater prediction ability and the result was consistent with the maximum tolerance of the error of the standard value.
P3.01 Studies on the effect and efficacy of biological seed coating against *Macrophomina phaseolina* in maize

Sujatha Patta¹, J Sandhya Rani¹, K Jhansi Rani², S Triveni² and MV Nagesh Kumar¹

¹Professor Jayashankar Telangana State Agricultural University (PJTSAU), Department of Seed Science and Technology, Seed Research and Technology Centre, Hyderabad, India
²PJTSAU, Department of Agricultural Microbiology and Bioenergy, College of Agriculture, Hyderabad, India
(patta.sujatha@gmail.com)

Biological seed coating is an ecological approach using selected fungal antagonists against soil- and seed-borne pathogens. The present investigation was aimed to take up detailed study of biological seed coating in maize. *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* showed compatibility with the insecticide Thiamethoxam and bio-friendly polymer. With fungicides, bio-agents showed some compatibility with Metalaxy and incompatibility with Sixer. All bio-agents showed inhibition of *Macrophomina phaseolina* (major causative organism of post-flowering stalk rot in maize), under *in vitro* conditions. Scanning electron micrographs (SEM) revealed the presence of biocontrol agents on seed and root surfaces of maize, even after four months of biological coating. Mycoparasitic and antagonistic activity of biocontrol agents against *M. phaseolina* was also determined through SEM. Biological seed coating showed a significant effect on maize seed quality and longevity, even after seven months of coating. Seed biologically coated with Thiamethoxam at 3 g kg⁻¹ seed + Metalaxyl at 2 g kg⁻¹ seed + bio-friendly polymer at 6 g kg⁻¹ seed + *P. fluorescens* at 10 g kg⁻¹ seed + *Bacillus subtilis* at 10 g kg⁻¹ seed, recorded good seed quality and vigour parameters. Seed yield per plot was recorded to be highest (10.08 kg) in seeds treated with Thiamethoxam at 3 g kg⁻¹ seed + Metalaxyl at 2 g kg⁻¹ seed + bio-friendly polymer at 6 g kg⁻¹ seed + *T. viride* at 5 g kg⁻¹ seed + *P. fluorescens* at 10 g kg⁻¹ seed + *B. subtilis* at 10 g kg⁻¹ seed, but lowest (9.0 kg) was observed in the untreated control. Maize seeds coated with *T. viride* + Thiamethoxam + Metalaxyl + bio-friendly polymer recorded a low disease score of 3.7 with highest efficacy, compared to the untreated control with a score of 5.9 (tooth pick method). Thus, from these findings, it is concluded that the maize seed coated with biocontrol agents, bio-friendly polymer, insecticides and compatible fungicides, retains its viability even up to seven months of treatment and with less incidence of storage pests and diseases, thereby improving the field performance of the crop.
P3.02 Studies on shelf life of bio-agents, seed longevity and field performance of biologically coated maize seed

Sujatha Patta1, K Jhansi Rani1, S Triveni2, Razia Sultana1, MV Nagesh Kumar1 and Keshavulu Kunusoth3

1Professor Jayashankar Telangana State Agricultural University (PJTSAU), Department of Seed Science and Technology, Seed Research and Technology Centre, Hyderabad, India
2PJTSAU, Department of Agricultural Microbiology and Bioenergy, College of Agriculture, Hyderabad, India
3Telangana State Seed and Organic Certification Authority (TSSOCA), HACA Bhavan, Nampalli, Hyderabad, India
(patta.sujatha@gmail.com)

Biological seed coating is a new technique of seed treatment through which a biological agent is coated over the seed surface, by mixing in a polymer that is sprayed on the seeds as they fall though a specialised machine. It is an ecological approach using selected fungal antagonists against the soil- and seed-borne pathogens, and it has the potential to provide an alternative to chemical control. The study is aimed at testing the effect of biological seed treatment with bio-friendly polymer as an adjuvant well in advance of sowing, immediately after seed processing or before packaging. The seed of maize cultivar V-23 was treated with two biocontrol agents, viz. Trichoderma viride and Bacillus megaterium, with a compatible insecticide, Thiamethoxame, against the fungicide Metalaxyl, and an untreated control at different dosages. Bio-friendly polymer was used as an adjuvant in all combinations. The seed without any treatment was used as a control. Data on viability and shelf life of bio-agents, in terms of colony forming units (cfu), and seed quality parameters like seed germination and vigour, were recorded up to 18 months of storage. The performance of treated seed was studied in the field after seven months of treatment. The results indicated the survival of Trichoderma viride at 6.2 × 10^2 cfu / 10 seeds after 14 months' treatment and that of Bacillus megaterium at 1.9 × 10^3 cfu / 10 seeds. The seed treated with either of the bio-agents in combination with insecticide and bio-friendly polymer recorded a high seed germination percentage over the untreated control at 18 months after coating, by recording less percent reduction in seed quality parameters over the initial readings. The seed treated with the above combinations, recorded a high yield compared to the control when sown. Thus, the seed coated with bio-agents along with compatible insecticide and bio-friendly polymer, resulted in better seed quality during storage and field performance in maize. Hence, the biocontrol agents can be safely applied to seed well in advance of sowing, i.e. after seed processing and seed packaging. The findings have paved a way for practical utility of biological seed treatment as a key issue in organic seed production or farming. The study needs further confirmation by testing the treated seed in different seasons and locations.

P3.03 An overview of Fusarium proliferatum on bulbs and seeds of Allium species in Italy

Ilaria Alberti1, Antonio Prodi2, Maria Teresa Senatore2, Paolo Nipoti2 and Massimo Montanari3

1CREA CI, Viale Amendola 82, Rovigo, Italy
2UNIBO, Viale Fanin, 40, Bologna, Italy
3CREA CI, Via di Corticella 133, Bologna, Italy
(ilaria.alberti@crea.gov.it)

Fusarium proliferatum is known to be a pathogen of maize, wheat, barley, sorghum, minor cereals and several other crops and it is well known to produce fumonisins. In the last decade, it has also become an emerging, globally-spreading pathogen of garlic (Allium sativum L.). In Italy, from 2011, Fusarium rot of stored garlic was detected in several production areas. Throughout the whole
crop cycle, fungal presence is not associated with any kind of symptoms, but during storage bulbs undergo a slow deterioration process: at first, brown, depressed water-soaked spots appeared and in severe infections the entire bulbs rotted, reducing garlic shelf life and causing appreciable loss of product. Similar symptoms were also observed in bulbs destined for human consumption, coming from different countries (France, Spain and China) and commercialised in Italian markets. During 2016, in seed reproduction plots in central Italy, apparently healthy plants of *Allium fistulosum* (Welsh onion or Japanese bunching onion) showed spongy consistency of basal stems and in longitudinal section, the internal tissues were rotted and appeared violet coloured. *F. proliferatum* was isolated from these plants. Seeds of *A. fistulosum* from different countries (Japan, Brazil and Korea), were checked and the same fungus was also recovered. The sanity of bulbs and seeds is crucial to obtain healthy plants, so laboratory and field trials are ongoing to evaluate the most active phytosanitary products or antagonist biocontrol agents. Moreover, nitrogen fertilisation techniques should be best implemented with other agronomic practices.

### P3.04 Molecular methods in seed health testing

**René Mathis and Thomas Baldwin**  
GEVES, 25 rue Georges Morel, CS90024, 49071 Beaucouzé, Angers, France (rene.mathis@geves.fr)

Techniques based on diverse biological principles are used for seed health testing. Some techniques have been used since the beginning of seed health testing, such as isolation of suspect pathogens and pathogenicity testing on susceptible plants (bioassays). These techniques have been complemented more recently, by serological and molecular methods. This poster presents the current state of modern techniques, with a focus on molecular methods (PCR, real-time PCR), and outlines those that are in the pipeline for future seed health testing methods (sequencing). The current use of PCR and real-time PCR methods will be reviewed and the latest changes in the use of these molecular techniques are presented. Some of the advantages and limits of molecular methods will be presented, as well as their impact on test lead-times and laboratory capacities. PCR methods are mainly used in current seed health testing for two applications, namely confirmation of symptoms in grow-out tests (for example ISTA Seed Health Method 7-030: Detection of *Acidovorax valerianellae* in corn salad seed) and the identification of bacterial isolates obtained after dilution-plating on semi-selective media (for example ISHI-Veg method v4.3.1: Detection of *Clavibacter michiganensis* subsp. *michiganensis* on tomato seed). PCR primers target regions of genomic DNA that are specific for the target pathogen; combining primers with a specific hydrolysis PCR probe (for example, TaqMan®) allows for real-time PCR isolate identification. Validated PCR identification assays can be used for distinguishing target pathogens and lookalike non-pathogenic isolates; this can reduce the number of suspect isolates that need to be tested in pathogenicity tests (bioassays). The latest application of real-time PCR has been for the detection of nucleic acid (DNA/RNA) associated with target pathogens in seed extracts, termed ‘seed-extract PCR’ (SE-PCR). Examples include SE-PCR methods for pre-screening cabbage seed lots for *Xanthomonas* (ISTA Seed Health Method 7-019, effective from January 2019) and alfalfa seed lots for *Ditylenchus* nematodes (method developed by GEVES and validated in the Dityluz project). SE-PCRs can decrease the lead-time for obtaining a negative test result, and the increased throughput can also increase laboratory capacity. However, no information is obtained about pathogen viability and there is no definitive proof of pathogenicity with these indirect tests. A negative test result with a validated SE-PCR method is sufficient for identifying negative seed lots. However, the possible presence of traces of DNA/RNA from dead pathogens means that positive SE-PCR test results need to be followed up with classical ‘direct’ techniques that confirm viability and pathogenicity.
P3.05 *Tilletia caries*: study of the most prevalent virulence of common bunt in France in order to develop a resistance test for the inscription of the varieties of common wheat in organic farming

Geoffrey Orgeur¹, Valérie Grimault¹, Valérie Cadot¹, Thomas Baldwin¹, Jean Philippe Maignel¹, Julie Gombert², Laurence Fontaine³, Philippe Du Cheyron⁴, Julien Bruyere⁵ and Jean Champion⁶

¹GEVES, 25 rue Georges Morel, CS90024, 49071 Beaucouzé, Angers, France
²FNAMS, 74, rue Jean-Jacques Rousseau, 75001 Paris, France
³ITAB, 149, rue de Bercy, 75595 Paris, France
⁴Arvalis – Institut du Végétal, 3 rue Joseph et Marie Hackin, 75016 Paris, France
⁵FREDON 39 Rue Alexandre Blanc, 84000 Avignon, France
⁶Chambre d’Agriculture Drome 145 Av. G. Brassens - CS30418, 26504 Bourg lès Valence, France

(geoffrey.orgeur@geves.fr)

Common bunt, mainly caused by *Tilletia caries* and *T. foetida*, is a re-emerging disease since 2007. Responsible for significant yield losses, this disease has an impact on seed health quality and holds an important capacity for dissemination. Currently, only 10% of varieties are considered as resistant and little work has been done on the resistance of the varieties due to a lack of tools. In conventional farming, seed treatment solves the problem, but this is not authorised in organic farming. In 2013, for variety registration to the French Catalogue, a protocol was adapted for organic farming, integrating the evaluation of resistance to common bunt for soft wheat; however, there was a lack of knowledge on the most prevalent virulences in France. The programme ABBLE was created to study variability of the population of common bunt in France and to identify the different virulences in the country. Samples were collected in the main production areas and the species of *Tilletia* were identified by GEVES. Of the 26 strains collected from 15 French departments, *Tilletia caries* was found in 100% occurrence, in association with *T. foetida*, at 15.4%. Twenty strains were sectioned to be multiplied in the field, in order to have enough inoculum to infect all the differentials. The characterisation of the virulence used a range of 15 host differentials from Metzger. After seven months of growth, symptom expression (bunt ears) was checked on each host differential for each strain. Frequency of virulence was determined in this way, revealing a predominant virulence on Bt-7, with a frequency of 48%, then on Bt-2 (24%) and Bt-15 (19%). In parallel, an early detection method using qPCR was developed on plantlets. A resistance test was carried out in controlled conditions with detection at an early stage (2 leaves) and compared with symptom expression on three susceptible varieties (Lukullus, Renan and Apache) and one resistant variety (Arezzo). Currently, the method allows the differentiation of the sensitive varieties from the resistant. The qPCR method will be validated by comparison between the number of positive plantlets detected by qPCR and symptom expression observed on ears in the field for the same varieties. Evaluation of the resistance of the varieties mainly used in organic farming, will allow better knowledge to be acquired, of the current resistance against the virulence mainly present in France. The development of an early, rapid and reliable resistance test will support breeding with a new tool for screening resistant varieties.
POSTER SESSION 4 – SEED PRODUCTION IN A CHANGING ENVIRONMENT

P4.01 Genetics of seed longevity in soybean

Jhansi Rani, Sujatha Patta, Razia Sultana and Keshavulu Kunusoth
Professor Jayashankar Telangana State Agricultural University, Seed Research and Technology Centre, Rajendranagar, Hyderabad, India
(jhansikaparthi@gmail.com)

Poor seed longevity is one of the major constraints in soybean cultivation, which is emerging as an alternative remunerative crop to cotton in certain pockets of India. The quick loss in seed viability under ambient storage often leads to a mismatch between seed demand and supply. Hence, seed longevity is equally important to that of yield traits to exploit the potential of new high yielding varieties. Seed longevity and the associated seed quality traits were shown to be under genetic control. Assessment of nature and magnitude of variability in the existing germplasm for yield components and seed quality attributes are essential, to know the inheritance pattern and to formulate an effective breeding programme. Twenty-six soybean germplasm lines were evaluated in a replicated trial for seed yield, 100 seed weight and seed quality components, viz. germination, field emergence and seedling vigour indices following accelerated ageing. The phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability ($h^2$) and genetic advance (GA) were estimated. Low PCV and GCV along with high $h^2$ and GA, were recorded for seed germination and field emergence, indicating low variability for these traits. Moderate PCV and GCV along with high $h^2$ and low GA, were recorded for seedling vigour indices. Thus, it can be inferred that the existing variability for seed quality parameters is low, hence the variability has to be created through hybridisation followed by selection in the segregating generations, as high heritability and genetic advance were recorded for these traits. The correlation between seed yield and quality parameters was weak suggesting the need for further studies with a greater number of genotypes and segregating populations for establishing the relationship between seed yield and quality for simultaneous selection of high yield and seed longevity.

P4.02 Maintenance breeding: an intrinsic pivot of the quality seed production system of IARI (Pusa)-bred basmati rice varieties

Rakesh Seth¹, AK Singh², S Gopala Krishnan², PK Bhowmick², B Haritha², Ranjith K Ellur², LV Subba Rao¹, NK Chopra¹ and VK Pandita¹
¹Indian Agricultural Research Institute-Regional Station, Karnal, Haryana 132001, India
²Division of Genetics, Indian Agricultural Research Institute, New Delhi 110012, India
³Crop Improvement, Indian Institute of Rice Research, Hyderabad 500030, India
(rsseth101@gmail.com)

Maintenance breeding is the backbone to any quality seed production system. A new improved variety is a product of consistent and time-consuming breeding efforts. These breeding gains can only be realised if genetically pure seed in adequate quantities is made available to farmers. Without proper varietal purification and maintenance, varieties become defunct, however excellent these may be in performance. Maintenance breeding a simple but key technique – for purification and stabilisation of pipeline/released varieties – with significant economic implications, as well as enhancing adaptability and longevity of these varieties. Initially, 11 varieties (six traditional and five evolved) were notified as basmati varieties under the Seeds Act (1966) and 29 varieties are currently notified as basmati varieties (source: http://apeda.gov.in). The comparative appraisal of breeder seed indents and production, over two decades, of the initial 11 basmati varieties (along with Pusa Basmati 1121), clearly demonstrates the lead role of Pusa Basmati varieties bred by the Indian Agricultural Research Institute (IARI). The rest of the [newly] notified basmati varieties
have yet to make a significant dent in the seed chain. India exported around 4.0 million MT of basmati rice in 2017–18, earning significant foreign exchange (US $4169 million; source: http://agriexchange.apeda.gov.in). A major proportion of these exports constitutes Pusa Basmati varieties. The main reason for popularity, spread and consumer acceptability of Pusa Basmati varieties is the strong varietal maintenance component underpinning the spread of genetically pure seed of these varieties. It is quite possible that if remaining basmati varieties had been given adequate maintenance breeding support by respective institutes/SAUs, these varieties could have also made a significant dent at the national level. The intrinsic and vital role played by varietal maintenance (with specific focus on traits of interest, viz. cooking quality, gene screening, etc.) in the spread of these varieties from Pusa Basmati 1 (1st evolved basmati variety) to Pusa Basmati 1121, Pusa Basmati 6, Pusa Basmati 1509, etc., is deliberated in the study. The study also delves into issues/challenges of varietal maintenance in the recently evolved disease resistant basmati varieties, bred through marker assisted backcross breeding (MABB), viz. Pusa Basmati 1637, Pusa Basmati 1728 and Pusa Basmati 1718, etc., and the likely future payoff of these integrated interventions.

P4.03 Area weighted average age of legume varieties as a measure of varietal turnover and seed systems efficiency: a critique

Chris Ojiewo1, Muricho Geoffrey1, Jean-Claude Rubyogo2, Lucky Omoigui3, Julius Manda3, Enid Katungi2, Hippolyte Affognon1, Essegbemon Akpo1 and Rajeev Varshney1

1International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)  
2International Center for Tropical Agriculture (CIAT)  
3International Institute of Tropical Agriculture (IITA)  
(c.ojiewo@cgiar.org)

Global warming has increased biotic and abiotic stresses affecting crop productivity. New and more adapted varieties are needed to combat crop productivity losses triggered by these climate change phenomena. Breeding programmes have responded by generating new varieties that have been registered and released in recent years. However, the availability of new varieties is not reflected in the genetic diversity on farmers’ fields across time and/or space. Furthermore, empirical studies have shown that this low variety turnover has significantly dampened the positive productivity gains of new varieties from the breeding programmes. Therefore, policies that can speed up varietal replacement over time and/or space will be critical in realising genetic gains of newly developed and adapted varieties. However, what is not clear is the optimal frequency of varietal replacement (turnover). Several variety turnover measurements have been developed and applied in empirical studies. In this paper, we present and discuss the merits and demerits of the area weighted average variety age (WAVA) index as a criterion. The index is based on variety age proxied by year of release and proportion of cropped area under the variety. It can be applied to compare rates of varietal replacement across time and space. Though it is an improvement on previously used measurement indices, its recent application in empirical studies has exposed it to criticisms. For example, the index is overly dependent on the accuracy of variety name as reported by the respondent and reliability of cropped area estimates. The former problem (variety name) can be addressed by DNA fingerprinting of data if samples are available. In addition, reliability of WAVA is limited because some well performing varieties are adopted much earlier, before they are formally released, due to lengthy and poorly functioning variety release systems and/or subsequent inefficient seed production, marketing and distribution systems. Computation of WAVA in such circumstances is likely to overestimate varietal turnover. On the other hand, using year of release to compute variety age in WAVA imposes a strong assumption that the variety was availed to potential adopters in the same year it was released. Yet, evidence from developing countries shows that seeds of released varieties are availed to farmers many years after official release, due to a lack of variety commercialisation efforts to facilitate adoption. Therefore, there are high chances of underestimating varietal turnover. The alternative is to use year of first active seed production, though this is subjective.
P4.04 Identification of varieties in farmers' fields through on-farm surveys and adoption studies

Chris Ojiewo¹, Essegbemon Akpo¹, Jean-Claude Rubyogo², Lucky Omoigui³, Julius Manda³, Enid Katungi², Hippolyte Affognon¹, Muricho Geoffrey¹ and Rajeev Varshney¹
¹International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)
²International Center for Tropical Agriculture (CIAT)
³International Institute of Tropical Agriculture (IITA)
(c.ojiewo@cgiar.org)

The use of quality seed of improved varieties is an imperative for enhancing genetic gain among farmers under changing and variable climatic conditions. Over the past decade, significant research and development efforts have been deployed to enhance sustainable availability and access to quality seed of tropical legumes at affordable prices in sub-Saharan Africa and South Asia through the Tropical Legumes projects (TLII and TLIII). Recent adoption survey results show that common bean variety Uyole 96, which dominated more than 60% of production in 2013 in southern Tanzania, was replaced in 2016 by Njano Uyole. In Ethiopia, Mexican 142 that controlled over 50% of the white canning bean market class and Red Wolita that controlled about 70% of the red cooking bean type in 2009, were totally replaced by new varieties promoted under the TL projects. In Nigeria, the project promoted the adoption of groundnut varieties Samnut 21, Samnut 22 and Samnut 23 released in 2000 before the release of newer varieties, Samnut 24 (2011), Samnut 25 (2013) and Samnut 26 (2013), which have replaced old varieties released between the 1960s and the early 1990s, such as Samnut 1 to Samnut 20. In Nigeria, old cowpea varieties such as Sampea 7, released in 1985, have almost been completely replaced by newer varieties such as Sampea 8, released in 2005 and promoted under the project since 2007. Better still, Sampea 8 was quickly replaced by Sampea 11 released in 2009. In Tanzania, groundnut varieties that were reigning before 2007 include Nyota, Johari, Sawia and Pendo. While Pendo is still dominant and is currently being replaced by rosette resistant Nachgwea and Mangaka, the other varieties have been largely replaced. DNA fingerprinting work to support adoption data is underway in collaboration with Intertek and DArT Pty Ltd in Australia for chickpea, groundnut and cowpea, and at CIAT headquarters in Columbia for common bean.

P4.05 Seed needs assessment in selected states in north-eastern Nigeria

Hakeem A Ajeigbe¹, Ahmadu Kamara², Abubakar H Inuwa¹, Polycarp O Odoyo³, Michael B Vabi¹ and Ignatius I Angarawai¹
¹International Crop Research Institute for the Semi-Arid Tropics, Kano Station, Nigeria
²Obafemi Awolowo University Ile-Ife, Nigeria
³Food and Agriculture Organization, Nigeria
(h.ajeigbe@cgiar.org)

A seed needs assessment was conducted in three north-eastern states of Nigeria during 2018. The objectives of the assessment were: to analyse the existing seed systems and assess the extent of community-based seed production initiatives across the three states in order to identify practical ways of improving seed security. Purposive, random sampling and rapid appraisal techniques were used to collect data from a total of 453 households, 89 seed growers, 37 agro-input dealers, and 13 seed aids organisations. Supplementary information was also obtained from 88 focus group discussions, 41 market surveys and nine key informants. Results showed that seed availability, which is the most crucial indicator of seed quantity available for next season, is low: less than 10% of respondents have enough seeds for the next season. It was noted that the majority of farmers (71%) rely on informal seed sources for most crops. Farmers rely mostly on their own production
for maize (72%) and rice (41%) seeds, with only 6% of farmers relying on seed companies. In Nigeria, maize and rice are the seed most traded by seed companies, however, their availability is still low in the three states covered, implying low coverage by seed companies. The majority (84%) of farmers have their seed sources located within their Local Government Area. A large percentage of farmers (65%) are able to afford the seed. Most farmers are self-reliant as they either use their own production (51%) or purchase seeds (40%) with cash. The majority of the farmers (86%) gave a good germination rating for all their seed sources. Own seed scored relatively low with 78% of farmers scoring it good and 21% scoring it as fair. Although the majority of farmers are able to afford seeds, approximately 40% of them are at risk because they do not find the seeds affordable. The assessment recommends that: 1) Community-based seed production should be the way forward to ensure that farmers can access quality seeds, at the right time and place; 2) Awareness raising events and demonstrations should be held to promote improved crop varieties and accompanying management practices; 3) Commercial-based activities such as seed fairs should be introduced to link community-based seed producers, seed companies and breeders; 4) Support should be given to local market seed dealers by developing their skills and knowledge on producing quality seeds, seed viability in storage and storage needs, packages/packaging materials.

P4.06 Sustainable seed systems for dryland cereals in sub-Saharan Africa

Henry Ojulong¹, E Letayo², N Wanyera³, J Ebiyau³, C Oduori⁴ and E Manyasa¹
¹ICRISAT-Nairobi, Kenya
²TARI, Tanzania
³NaSARRI, Uganda
⁴KALRO, Kenya

(hojulong@cgiar.org)

Formal seed systems in Africa have not been able to meet farmers' needs. Parastatal seed enterprises do not provide seed of many of the staple crops; the commercial sector has shown little interest in non-hybrid crops. An effective solution will probably require a combination of public-, commercial-, and local-level participation, but the precise strategies need to be identified. The seed industry has several economic characteristics that justify selective government involvement. First, developing new seed varieties involves large fixed costs in terms of fields, equipment, and scientific expertise. The development process takes a long time and only a large private company or a subsidised public entity can afford to invest in an activity with such uncertain, long-term payoffs. Secondly, seed quality varies widely, depending on the genetically determined performance potential, varietal purity, physical cleanliness and viability. However, quality is difficult for farmers to observe until after the seed has been purchased and planted. Public intervention is needed to provide farmers with information about seed quality. Thirdly, most seed consumers in sub-Saharan Africa are poor and risk averse. Cash constraints often limit their ability to make investments. The fact that small-scale agriculture is the main source of income for the majority of the poor in most African countries, suggests that there is a strong equity argument for public investments to strengthen the system of developing new crop varieties and delivering them to farmers. In many countries, the procedures for releasing new crop varieties need to be clarified, simplified and accelerated. Excessive regulations slow the diffusion of new technology to farmers without providing offsetting benefits in terms of protecting farmers from underperforming varieties. Whereas early programmes ignored the informal seed sector, there is now greater interest in understanding and learning from it, including attempts to combine the strengths of the formal and informal seed sectors. Promotion of the development of a strong seed sector in sub-Saharan Africa requires a coordinated effort between the public and private sectors, where the roles may differ across activities (seed development, production, and marketing), across crops and across countries. The public sector needs to invest more in plant breeding and the development of new varieties, particularly for open-pollinated varieties of staple food crops, while the private sector is facilitated with an enabling policy environment. This is shown to work in a number of countries.
P4.07 Opportunities and challenges to improve agriculture and seed sector value chain governance in Telangana region, India

Raghu Chaliganti¹, Keshavulu Kunusoth² and Ekkehard Schröder³
¹Consultant and Policy Advisor, Germany
²Telangana State Seed and Organic Certification Authority (TSSOCA), India
³ADT Project Consulting, Germany
(raghuchaliganti@hotmail.com)

Every agricultural season starts with stereotypical news in the media such as seed germination failure, low crop stands, no flowering, lower yields, etc. Despite comprehensive seed production laws and regulatory mechanisms, assuring a supply of quality seeds to small and marginal farmers remains a major issue. The present study aims to provide an overview of key factors of agriculture and seed sector value chain governance in Telangana region. For this purpose, present seed production models, schemes targeting small and marginal farmers and stakeholders' perceptions were examined. The study employed a combination of methods such as SWOT, seed value chain analysis and institutional analysis. The results reveal major opportunities and challenges to strengthen agriculture and seed sector value chain governance. In addition, several policy recommendations are proposed to (re-)institutionalise key components of the seed bowl initiative in Telangana region, which could also be transferred to other federal states.

P4.08 Climate-smart rice seed production systems

Prudhvi Raj Vennela¹, Varsha Gayatonde¹, Shravan Kumar Singh² and Dhirendra Kumar Singh²
¹Banaras Hindu University, H.no. 6-65/A, Mahalakshmipuram Colony, Narapally, Ghatkesar, Hyderabad, India
²Banaras Hindu University, Department of Genetics and Plant Breeding, I.Ag.Sc., Varanasi, India
(vprudhviraj.2@gmail.com)

Rice is a major staple food crop and provides the main livelihood source for millions of households globally. However, climate change is exposing global rice production systems to a range of different shocks and stresses including a more variable water supply, increased spread of pests and diseases, and heightened vulnerability to damage from more frequent extreme weather events. These impacts are estimated to have the effect of increasing average prices by 32–37% and reducing yields by 10–15% by 2050. Climate-smart varieties and production techniques: local research institutions and government are helping to provide access and training to farmers in new seed varieties and production techniques to help adapt to climate change and reduce emissions. Particular emphasis is given to promoting gender equality and opportunities for youth employment. In rice, more than 30–50% seed is farmer-saved seed. The relative importance of seed sources varies during normal and stress periods, with farmers shifting to off-farm sources during stress periods. Farmer characteristics and ecological factors play a role in defining the type of seed used, though their significance varies by crop. Climate stresses diminish levels and quality of yield, affecting farmer seed saving, grain prices and subsequently seed access. Seed systems are important for enhancing such resilience as seed security has direct links to food security and resilient livelihoods in general; formal seed enterprises are important in delivering improved seed, especially for rice, though their importance during stress periods diminishes. Farmer characteristics and ecological factors play a role in defining the type of seed used, though their significance varies by crop. This is a recommend approach that integrates farmer seed systems with the formal system in general, but specifically focuses on strengthening social networks, promoting farmer seed enterprises and crop adaptation practices at the farm scale. Boosting the farming community in germplasm conservation is key to developing the hybrid combinations suitable for changing weather in specific regions.
Aerobic rice seed production, as well as popularising the system of rice intensification (SRI), helps to reduce water stress. Green super rice is an emerging rice type with all the characters to address stress-related plus nutrient-related issues. Therefore, giving high technical knowledge to seed producers at an organisational level, as well as farmers, certainly helps to overcome production quality issues and yield losses due to climate change.

**P4.09 Effect of different stages of seed maturation on seed physical, physiological and biochemical traits in foxtail millet [Setaria italica (L.) Beauv.]**

Kannababu Netyam, Venkateswarlu Ronda, Hariprasanna Krishna, Sooganna and Vilas Tonapi
ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad 500030, Telangana, India
(kannababu@millets.res.in)

The seed quality of foxtail millet depends on the maturity stage and agro-ecological conditions. To achieve good seed quality, it needs to be harvested at the stage of favourable seed maturity. A field experiment was conducted following the rainy season in 2017, at the Indian Institute of Millets Research, Hyderabad, India to identify appropriate stage of physiological maturity of seeds in a foxtail millet (cv. Suryanandi) population. After the completion of anthesis, the seeds were harvested at five maturity stages at weekly intervals (7, 14, 21, 28 and 35 days after anthesis; DAA) and assessed for seed quality traits. A steady and significant increase in seed hardness and test weight was recorded with seed maturation advancement from 7 to 35 DAA. However, there was a significant decline in seed yield with the delay in harvest from 28 to 35 DAA. A gradual and significant decline in seed moisture content was observed during the seed maturation stages from 7 to 35 DAA. A significant increase in seed germination, field emergence, seedling vigour and seedling dry weight values were observed with the advancement of seed maturation from 7 to 28 DAA. The estimation of alpha-amylase enzyme activity (mg of maltose release in 3 min/100 seeds) at different phases (0, 48, 96, and 240 h) of seed germination, indicated a gradual increase in activity at 48, 96 and 240 h with the advance in seed maturity from 7 to 28 DAA, whereas further advancement to 35 DAA registered a slight decline in enzyme activity at 96 and 240 h of seed germination. The results revealed that there was a significant reduction in seed yield with the delay in harvest from 28 to 35 DAA, a non-significant increase in physiological seed quality and a slight decline in alpha-amylase activity. Therefore, it can be inferred that the seeds attain physiological maturity at 28 days after anthesis in foxtail millet.

**P4.10 Seed development and maturation studies in proso millet (Panicum miliaceum L.)**

Ragupathri KP, Sujatha K and Paramasivam V
Tamil Nadu Agricultural University, Department of Seed Science and Technology, Coimbatore, India
(seedragu@gmail.com)

Minor millets are considered to be ‘future foods’: they are hardy, can be substituted for other cereals, and can better adapt to prevailing unpredicted, threatening agro-climatic and other stress conditions. Proso millet is one of the most widely cultivated minor millets owing to its high nutritional value, earliness and better yielding characters. Timely harvest of the seed crop is critical to achieving maximum seed viability and vigour in agricultural production. However, little information exists concerning when and how to harvest and to produce high quality seeds of proso millet. Changes in seed quality were investigated during seed development and maturation in proso millet.
at Department of Seed Science and Technology, Tamil Nadu Agricultural University, India. The bulk proso millet crop was raised in the field and individual flowers were tagged at the time of flowering. The seeds were collected at five-day intervals at eight reproductive growth stages, viz. 5 days after anthesis (DAA) to 40 DAA and subjected to seed quality parameter assessments. The results revealed that all seed quality traits were significantly affected by harvest time and seeds had attained physiological maturity by 35 DAA, as evidenced by the maximum panicle length (31.4 cm), width (5.3 mm), fresh weight (4.18 g), dry weight (2.15 g) and 1000 seed weight (5.85 g). The change of colour of the panicle and seed from green to lemon yellow could be considered as a visible index of physiological maturity. The seed quality parameters, viz. germination (42%), shoot length (11.3 cm), root length (14.2 cm) and dry matter (0.036 g/10 seedlings) and vigour index (489), were also found to be maximum at 35 DAA. The physiological maturity stage of proso millet was found to be 35 days after anthesis, which is the optimum stage to obtain superior quality seeds.
P5.01 Varietal identification of marigold (Tagetes spp.) using plant phenomics

Murali CN, SK Jain and Monika AJ
ICAR-Indian Agricultural Research Institute, Division of Seed Science and Technology, Pusa Campus, New Delhi, India
(muralicnagri@gmail.com)

Varietal identification in marigold is generally done using morphological descriptors and to some extent with the help of biochemical or molecular markers. However, these methods are labour-intensive, time-consuming and destructive to characterise all the available cultivars, hence some additional methods needed to be developed in addition to the DUS descriptors. Plant phenomics seems to be the best alternative, non-destructive and high-throughput phenotyping approach to be employed to overcome the problems associated with varietal identification in the field. The need for automated, fast, non-destructive and high-throughput alternative technology like plant phenomics has become a major approach in modern plant evaluation. Plant phenomics using different sensors (NIR, IR and VIS) with different views (side views at 0°, 120° and 240° and a top view) at two different intervals (85 DFS and 95 DFS) has clearly distinguished the studied African and French marigold genotypes, based on 11 morphology related phenomes. Hence, plant phenomics in combination with non-destructive evaluation of plant morphology related phenomes helps in the identification of marigold genotypes, even at early the stages of plant growth.

P5.02 Potential use of micro-morphological markers versus molecular markers in the identification of tomato (Solanum lycopersicum L.) hybrids and their parental lines

SK Jain¹, C Kiran Kumar Reddy², SR Bhat³ and Zakir Hussain⁴
¹ICAR-Indian Agricultural Research Institute, Division of Seed Science and Technology, New Delhi 110012, India
²Acharya NG Ranga Agricultural University, Agricultural Research Station, Andhra Pradesh, Kadapa 516003, India
³National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110012, India
⁴ICAR-Indian Agricultural Research Institute, Division of Vegetable Science, New Delhi 110012, India
(skjainsst@gmail.com)

The seed market of tomato (Solanum lycopersicum L.), an important vegetable crop, is dominated by its hybrids. Genetic purity problems are found to be common, especially with hybrids. It is mandatory to test the genetic purity for certified hybrid seeds in India. The conventional grow-out test method recommended for genetic purity testing has innate disadvantages, viz. it is time-consuming, labour-intensive and seasonally dependent, etc. The present study is an attempt to bridge the gap. Four commercial hybrids of tomato namely, PH-1, PH-2, PH-4 and PH-8, developed and released by the Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi, were undertaken for distinguishing all four tomato hybrids and their parental lines, using molecular, morphological and micro-morphological markers as identification keys, at seed and seedling stage. Among the various molecular markers, five SSR markers, viz. SLM-7, SLM-53, TGS-0162, SSR-63 and SSR-212 were found to be polymorphic. A combination of more than one marker could generate distinct DNA fingerprints for more than three hybrids, i.e. PH-1, -2, -4 and -8. Multiplexing was demonstrated using two markers in one hybrid for its unambiguous identification. A total of 39 morphological traits were recorded; of these, 27 were qualitative. Among
the various morphological markers, anthocyanin pigmentation on the upper ½ portion of stem, stem pubescence, leaf serration, attitude of leaflet petiole in relation to main axis, flower stigma lobes, fruit green shoulder, fruit shape in longitudinal section and fruit shape at blossom end, were found to be prominent in testing hybridity of tomato. Conversely, among the micro-morphological markers, anthocyanin pigmentation pattern on midrib of cotyledonary leaf, shape of cotyledonary leaf in cross-section, shape of first leaf blade, blistering pattern and positioning of veins on lamina of first leaf, shape of cotyledon and anthocyanin pigmentation on lower surface of first leaf, were identified as first of their kind in testing tomato hybridity and their parental lines distinctly. Thus, micro-morphological markers have the potential to distinctly identify all four tomato hybrids and their respective parental lines during seed and seedling stages, and to spot off-types and/or selfed plants, as a fast track, cost-effective (labour saving) and easy alternative to conventional grow-out tests, as well recent molecular techniques involving trained manpower.

P5.03 Use of microsatellite markers for genetic purity testing of maize (Zea mays L.) F1 hybrid

N Nethra¹, V Satya Shrii¹, HC Lohithashwa², TM Ramanappa¹ and PJ Devaraju¹
¹All India Coordinated Research Project (AICRP) on Seed Technology Research, University of Agricultural Sciences (UAS), GKVK, Bengaluru 560065, India
²Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru 560065, India
³Department of Seed Science and Technology, UAS, GKVK, Bengaluru 560065, India
(nethraharsha@gmail.com)

Genetic purity of parental lines and hybrids is of crucial importance, as it helps in successful hybrid seed production. Maize being an allogamous crop, the maximum exploitation of hybrid potential is possible only with a supply of genetically pure seeds. Application of molecular markers for genetic purity testing is used widely, owing to the time-consuming and irreproducible results of conventional methods. In this study, 30 SSR markers were used for screening maize hybrid MAH-14-5 and its parental inbreds CAL-1443 and CML-451. Of the 30 SSR markers studied, seven pairs of primers showed polymorphism for the F1 hybrid and two markers (Bnlg1520 and Bnlg1185) showed unique polymorphism to MAH-14-5 compared with two other hybrids namely, Hema and MAH-14-138. Also, the morphologically identified (plant height, silk pigmentation, leaf serration, leaf blade pigmentation, etc.) off-types in field were tested with the identified markers and the difference in band size was observed. Thus, the identified polymorphic markers, i.e. Bnlg1520 and Bnlg1185, can be used for commercial hybrid seed lot testing in future.

P5.04 Magnetic resonance imaging applied in the analysis of soybean and corn seeds

Francisco Guilhien Gomes-Junior¹, Agide Gimenez Marassi², Silvio Moure Cicero¹, Fernando Fernandes Paiva² and Alberto Tannús²
¹University of São Paulo – Luiz de Queiroz College of Agriculture, Avenida Pádua Dias, 11 – PO Box 09 – CEP 13418-900, Piracicaba, Brazil
²University of São Paulo – São Carlos Institute of Physics, Brazil (francisco1@usp.br)

Magnetic resonance imaging (MRI) is a non-invasive and non-destructive technique that provides morphological, physiological and histological information in living organisms. It is based on the acquisition of either sequential cross-sectional or 3D images of the spatial distribution of 1H nuclei in biological structures. In spite of the efficiency of MRI to evaluate metabolic activity and identify internal injuries and abnormalities in living tissues, there is not enough information about protocols for soybean and corn seed assessment. Thus, this research aimed to evaluate two different
gradient echo sequences: FLASH-3D (Fast Low Angle Shot 3D) and ZET (Zero Echo Time) for MRI of soybean and corn seeds. MRIs were obtained in a 2 T/30 cm horizontal superconducting magnet 85310HR (Oxford Instruments, Abingdon, UK) interfaced to a Bruker Avance AVIII console (Bruker-Biospin, Ettlingen, Germany) running Paravision 5.1 software (Bruker, Ettlingen, Germany). A solenoidal radiofrequency coil with 8 mm diameter (fitted for soybean) and 10 mm diameter (fitted for corn), made of AWG 36 copper wire with five turns and two variable capacitors for matching and tuning calibration, was used to evaluate each seed individually (hydrated to 16% water content on wet basis). The gradient echo FLASH-3D sequence allowed suitable image acquisition with short echo and repetition time to obtain images weighted mainly by the contrast in T1. This sequence produced reliable evaluation for soybean seed, especially in those regions where there are differentiated vascular tissues responsible for transporting nutrients from the cotyledons to the embryonic axis. On the other hand, using the ZET sequence, it was possible to observe the signal coming from the endosperm region of the corn seed, even in conditions of low water content (10 to 12% w.b.), which was not achieved with fast traditional sequences such as FLASH-3D. This imaging technique may be very useful for different applications in seed science and technology, such as monitoring of changes in internal tissues caused by mechanical damage or microorganism action, and studies of seed physiology during maturation, germination or deterioration.

P5.05 ProSeedling®: a new computerised system for seed vigour assessment of lettuce and tomato

Francisco Guilhien Gomes-Junior¹, Andréia Chudrik Jóia¹, Julio Marcos-Filho¹, Fernando Fernandes Paiva² and Alberto Tannús²
¹University of São Paulo – Luiz de Queiroz College of Agriculture, Avenida Pádua Dias, 11 – PO Box 09 – CEP 13418-900, Piracicaba, Brazil
²University of São Paulo – São Carlos Institute of Physics, Brazil (francisco1@usp.br)

The development of new computational resources to accurately evaluate the physiological potential of seed lots has shown increased interest in seed research and promising results have been obtained to compose quality control programmes adopted by seed companies. Here, an automated system based on seedling development (ProSeedling®) is presented for evaluating the vigour of lettuce and tomato seeds. The system analysis is performed in two steps: the first is the germination (on blotting paper) of lettuce (20°C for 3 days) and tomato (25°C for 4 days) followed by seedling image acquisition; the second is the analysis by software developed for each species. The acquisition of seedling images is performed by equipment containing a constant and uniform lighting device, an image capture mechanism, which provides the appropriate adjustment with reproducible positioning in three dimensions (X, Y, Z), and an image acquisition device, connected to a computer. Based on segmentation algorithms for cotyledon separation and identification of the hypocotyl and the primary root of the seedlings, numerical indexes and mean length of the seedlings are calculated. These parameters are used to identify differences between seed lots with the lowest and highest vigour. The results obtained for lettuce and tomato seedlings were equivalent to other tests used to evaluate the seed vigour of these two species. However, computerised analysis by the ProSeedling® system has the advantage regarding the accuracy, speed and possibility of exporting and storing data in spreadsheets. The system allows the acquisition of seedling images directly on the germination papers, regardless of their colouration, reducing the time required for the analysis and avoiding possible damage to the seedling structures. Furthermore, with the reproducible positioning device implanted in the equipment, it is possible to capture seedling images of almost all existing seed species, since it is possible to approach, distance and centralise the capture device according to the size of the seedlings, without loss of image quality, ensuring the proper functioning of the software subsequently used for analysis and vigour determination of the seed sample.
P5.06 Identification of invasive plant and weed species using DNA barcode sequencing

Marie-Jose Cote¹, Steve Jones², Sarah Kyte¹ and Marie-Claude Gagnon¹
¹Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, Canada
²Canadian Food Inspection Agency, 421 Downey Road, Saskatoon, Canada
(marie-jose.cote@canada.ca)

Imports of seed lots, plants and plant products into Canada must be absolutely free of prohibited noxious weed seed and regulated pest plants. Weed seeds mixed in with imported products or crop seeds is one of the main pathways for unintentional introduction of invasive plants into Canada. To prevent the introduction of new weed species through seed, the Canadian Food Inspection Agency (CFIA) regulates the import, export but also the sale of seeds in Canada. CFIA has designated certain plant species as weed seeds and is currently regulating them under the Weed Seeds Order (WSO 2016) of the Canadian Seeds Act. Any contaminating weed seeds are currently identified morphologically. However, identification by seed analyst experts can be limited to the genus or family level due to the lack of distinctive morphological characteristics. Therefore, DNA barcode sequences representing the regulated weed species listed on the WSO (2016) have been collected from a series of herbarium plant material as well as from crop species in which invasive plants and/ or noxious weed seeds may be found. The collection currently holds barcodes for 764 specimens representing the species listed on the WSO (2016) as well as another 726 specimens representing 147 close relative species. The collection also includes barcodes of 615 specimens representing 73 crop species. The purpose of this collection is to use DNA barcodes as reference material for the identification of plant pest species by sequence comparison. The DNA barcode collection also provides the sequence information needed for the development and establishment of rapid molecular screening methods to allow high-throughput identification to support seed analysts in their work.

P5.07 Detection and identification of invasive plant and weed seeds in seed lots using Next Generation Sequencing

Marie-Jose Cote¹, Steve Jones², Emilie Tremblay¹, Adam Colville¹, Nicole Wurm², Sebastien Belanger¹ and Marc-Olivier Duceppe¹
¹Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, Canada
²Canadian Food Inspection Agency, 421 Downey Road, Saskatoon, Canada
(marie-jose.cote@canada.ca)

To import seeds into Canada, the seed lot must be free of prohibited noxious weeds. Weed seeds mixed in with imported grain or crop seeds is one of the main pathways for unintentional introduction of invasive plants into Canada. It is also necessary that imported seed lots for resale in Canada meet the minimum standards for purity (% purity and other seed determination) and germination. Therefore, marketplace monitoring samples are taken and tested, along with the non-compliant import samples, for seed purity and germination. Determining other seeds is done by manually searching the seed sample for contaminating seeds which are then analysed morphologically for species identification. The process can be lengthy due to the number of seeds in the samples but also for samples containing seeds of irregular shapes, small seeds or mixed seeds like wild flower seeds. Furthermore, identification by seed analyst experts can be limited to the genus or family level due to the lack of distinctive morphological characteristics. Next Generation Sequencing (NGS) is an innovative technique allowing simultaneous DNA sequencing of hundreds of thousands of molecules and the tracking of the sequences produced all in one experiment. NGS can be used for metagenomics studies such as the detection and identification of species contaminating other species. The species identification is done using a collection of reference sequences of common genomic area, namely DNA barcodes. Therefore, a workflow using the NGS technique as well
as bioinformatics pipelines for data analysis, was developed to detect and identify weed seeds as well as other crop seeds contaminating different seed samples using barcode markers. Data resulting from the NGS analysis of different assemblages of mixed seed material will be reported and discussed.

P5.08 Methods to prevent analysts unconsciously adjusting results in germination tests

Bo-Jein Kuo¹, Si-Ting Chen¹, Ciao-Yun Syu² and Yu-Wen Kuo³
¹Department of Agronomy, National Chung-Hsing University, 145 Xingda Rd., South Dist., Taichung City, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu
²Taiwan Seed Improvement and Propagation Station (TSIPS), No.6, Xingzhong St., Xinshe Dist., Taichung City, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu (bjkuo@nchu.edu.tw)

Recently, many studies have discussed under dispersion in germination tests. Under dispersion, the empirical variance between replicates, is less than the binomial sampling variance. Most of the studies concluded that analysts unconsciously adjust the results in germination tests, resulting in an under dispersion problem. The reasons for unconscious adjustment by analysts were believed to be based on the fact that the seeds of the replicates were from the same seed lot. To confirm whether analysts in the National Seed Testing Laboratory would unconsciously adjust the results due to this belief, routine and blind tests were modified and performed in the germination tests for pepper (Capsicum annuum L.) and rice (Oryza sativa L.). Comparing the extent of under dispersion between routine and blind tests, we found that the analysts in the laboratory might unconsciously adjust the results in germination tests, resulting in the exacerbation of under dispersion. On the basis of this finding, modified multi-analysts and multi-investigation tests were separately performed in the germination tests for pepper and rice to reduce under dispersion. The data of the blind test were used to simulate the results of the modified multi-analysts test for pepper. We found that the modified multi-analysts tests could reduce the under dispersion problem. The multi-investigation test was also designed to reduce under dispersion by increasing the independency between each investigation and replicate. According to the results of the multi-investigation test for rice, we found that more frequent investigation under a fixed test period of time would cause less extent of under dispersion. It was confirmed that the multi-investigation test also could effectively reduce under dispersion. Based on our results, if analysts would unconsciously adjust the results in germination tests, the modified multi-analysts and multi-investigation tests could reduce the under dispersion which was caused by the unconscious adjustment.
P5.09 Study on discrimination system for seed characters of rice with image processing

Ciao-Yun Syu¹, Chung-Li Chen², Kuo-Yi Huang³, Yu-Wen Kuo¹, Mao-Chang Huang³ and Ting-Lin Chang¹

¹Taiwan Seed and Seedling Improvement and Propagation Station (TSIPS), No.6, Xingzhong St., Xinshe Dist., Taichung City 426, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu
²National Chung-Hsing University, 145 Xingda Rd., South Dist., Taichung City 402, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu
³Agriculture and Food Agency, No.8, Kuanghwa Rd., Nantou County, TW-Separate Customs Territory of Taiwan, Penghu, Kinmen and Matsu
(languest@tss.gov.tw)

This study aimed to establish a rapid and accurate discrimination system for rice seeds with image processing in order to maintain the quality and purity of rice seeds under a seed propagation system, as well as reducing the time and manpower for seed purity analysis. The functions of the system included auto-input, counting, image capture, inspecting rice seed, auto-output and building an image database. The inspection system focused on the foundation of a seed image database of five rice varieties in 2014, including Tainan 11, Taikeng 14, Taichung 192, Taikeng 9, and Taichung sen 10. To discriminate these varieties, ten traits including length, width, apiculus length, apiculus height, apiculus lemma depth, apiculus palea depth, apiculus angle, lemma and palea angle, upper edge of lemma and apiculus colour, were used in this study. Consequently, the valid image capture rate was about 96.9%, and the average rate for accurate discrimination for variety was about 88.8%. For individual variety, it was 96.6% for Tainan 11, 83.3% for Taikeng 14, 84.1% for Taichung 192, 82.6% for Taikeng 9, and 97.5% for Taichung sen 10.

P5.10 Identification of diagnostic molecular markers for genetic purity testing in cowpea [Vigna unguiculata (L.) Walp.]

N Nethra¹, BS Chandana¹, PJ Devaraju¹, RL Ravi Kumar² and P Rudraswamy

¹Department of Seed Science and Technology, University of Agricultural Sciences, GKVK, Bengaluru 560065, India
²Department of Plant Biotechnology and AICRP on Arid Legumes, University of Agricultural Sciences, GKVK, Bengaluru 560065, India
(nethraharsha@gmail.com)

Genetic purity is one of the important quality criteria required for successful seed production and seed certification. Hence, the experiment was set to evaluate the usefulness of SSR markers to determine genetic purity of commercial seed lots of cowpea, which are present in the seed chain of Karnataka. Out of 55 SSR markers studied, ten showed polymorphism for each of the seven varieties, which revealed 25 alleles at ten loci with an average of 2.5 alleles per locus. The SSR 6292 showed the highest number of alleles with the PIC value of 0.71. PKB-4 cultivar was found to be the most divergent, from the dendrogram constructed by UPGMA clustering. The genetic purity of the seed lots was tested by following the 20 × 20 matrix. Purity levels (%) were recorded for all the seed lots except for KBC-2, IT-38956-1 and C-152 varieties, for which the purity levels were 98, 96 and 97%, respectively. This analysis revealed that the SSR marker is a potential tool for fingerprinting of varieties as well as for the genetic purity testing of seed lots. Thus, it is helpful for delivering pure seed lots to the farmers.
P5.11 Influence of bacterial infection to physical characteristics and sowing qualities of *Zea mays* seeds

NS Priyatkin¹, MV Arkhipov¹, LP Gusakova¹, VN Pishchik¹, TA Kuznetsova², MA Kuznetsova², NN Potrakhov³, NE Staroverov³ and LE Kolesnikov⁴

¹Agrophysical Research Institute, St. Petersburg, Russia
²Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
³The First Electrotechnical University 'LETI', St. Petersburg, Russia
⁴Saint-Petersburg State Agrarian University, Pushkin, St. Petersburg, Russia

The goal of the research was the study of optical, X-ray and gas discharge characteristics of corn seed samples with the help of instrumental physical and standard methods to evaluate their sowing qualities and degree of phytosanitary risks. Four samples of corn seeds were provided by the All-Russian institute of plant protection (FSBSI VIZR St. Petersburg-Pushkin, Russia) and used as the objects of research: 1) Hybrid ROSS 272 AMV (originally from Volgograd region); 2) Hybrid of ROSS 272 AMV (originally from Saratov region); 3) Grade Krasnodar (originally from Altai region); and 4) F1 Hybrid the Leader 165 (originally from Astrakhan region). Digital optical imaging of corn seeds was obtained in the laboratory with a digital Sony Alpha NEX5 camera which captured images with a resolution of 4 592 × 3 056 pixels, and formatting of the JPG files was carried out. Macroshooting of samples of grains was carried out in two different modes: under an artificial light source with four LED lights (colour temperature 6 500 K), and under an artificial light source with one ultra-violet light. Image analysis of the seeds was carried out with the help of ARGUS-BIO software.

Digital X-ray imaging of corn seeds was obtained by the microfocus X-ray radiography technique. The hardware is presented through a mobile X-ray diagnostic PRDU-02 device (ELTEH-Med, Closed Joint Stock Company, St. Petersburg, Russia), with the digital system of visualisation of the X-ray image. Image analysis of X-ray images of seeds was carried out with the help of ARGUS-BIO software. Gas discharge images of corn seeds were obtained by the gas discharge visualisation (electrophotography) technique with the help of a GDV Camera device (Biotechprogress Ltd, St. Petersburg, Russia).

It was established that the cultivar sample Krasnodarskaya differed significantly from the three other samples in the following characteristics: average brightness (visible range of lighting), hue and colour characteristics on the RGB model (visible and UV lighting ranges). It was shown that the cultivar sample Krasnodarskaya has the minimum averaged brightness of digital X-ray images and minimum averaged intensity of gas discharge images. The minimum values of emergence rate, germination and additional growth indicators for cultivar sample grains Krasnodarskaya were revealed. We concluded that it is possible to execute a screening assessment of seed deficiency and conclude on the decrease in their growth indicators during germination with the help of non-destructive testing of the seed state, both on characteristics of the surface, and on internal characteristics of digital X-ray and gas discharge images. The phytopathogenic bacteria were identified as *Pantoea ananatis* s1 by 16 s r RNA sequencing and BLAST program.
Telangana State Seed and Organic Certification Authority
#5-10-193, 1st Floor, HACA Bhavan, Opp. Public Gardens, Hyderabad 500004, India
Phone: +91 40 23235939
Email: keshava_72@yahoo.com
www.tssoca.telangana.gov.in

International Seed Testing Association
Zürichstrasse 50, 8303 Basserdorf, Switzerland
Phone: +41 44 838 6000
Email: meetings@ista.ch
www.seedtest.org