Can we fast track seed quality assessment?

by

JYOTHISH BABU E. (2018-11-156)

M.Sc. (Ag) Seed Science and Technology

Seminar report submitted in partial fulfillment of requirement of course

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DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF HORTICULTURE

KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA – 680656

THRISSUR

2019

DECLARATION

I, Jyothish Babu E (2018 -11- 156), hereby declare that the seminar report entitled ‘Can we fast track seed quality assessment?,’ has been completed by me independently after going through the reference cited herein and I have not copied from any of the fellow students or previous seminar reports.

Vellanikkara — Jyothish Babu E.

05 – 02 – 2020 — (2018 – 11 – 156)

CERTIFICATE

This is to certify that the seminar report entitled ‘Can we fast track seed quality assessment?’ has been solely prepared by Jyothish Babu E (2018 -11- 156), under my guidance and has not been copied from seminar reports of seniors, juniors or fellow students.

Vellanikkara
05/02/2020

Dr. Rose Mary Francies
(Major Advisor)
Professor and Head
Department of Seed Science and Technology
College of Horticulture, Vellanikkara

CERTIFICATE

Certified that the seminar report entitled 'Can we fast track seed quality assessment?,' is a record of seminar presented by Jyothish Babu E (2018 -11-156) on 22th November, 2019 and is submitted for the partial requirement of the course ST 591.

Dr. Anil Kuruvila
Professor
Department of Agricultural Economics
College of Horticulture, Vellanikkara

Dr. Reshmy Vijayaraghavan
Assistant Professor
Department of Plant Pathology
College of Horticulture, Vellanikkara

Dr. Sangeetha Kutty.M
Assistant Professor
Department of Vegetable Science
College of Horticulture, Vellanikkara

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1. Introduction

Seed is the vital input in agriculture. Although technology has modernized much of farming's day-to-day operations, in the absence of a steady supply of high-quality seed, crop yields and economic returns would be greatly decreased. The efficacy of other agriculture inputs in farming is dependent on seeds. It is estimated that quality seed alone accounts for 20 - 25 per cent increase in productivity. Only good quality seeds respond well to the fertilizers and other inputs. Hence, access to quality seeds is considered as an important factor for increased production, productivity and in attaining food as well as social security of the country.

2. Seed quality

Seed quality describes the potential performance of a seed lot. Trueness to variety; the presence of inert matter, seed of other crops, or weed seed; germination percentage; vigor; appearance; and freedom from disease are important aspects of seed quality. High-quality seed lots should meet minimum standards for each of these characteristics. The standards of official certification agencies are usually accepted as the minimum requirements for high-quality seed.

2.1. Importance of seed quality

- Ensures genetic and physical purity of the crops
- Gives desired plant population
- Capacity to withstand the adverse conditions
- Seedlings produced will be more vigourous, fast growing and can resist pest and disease incidence to certain extent
- Ensures uniform growth and maturity
- Development of root system will be more efficient that aids absorption of nutrients efficiently and result in higher yield.
- It will respond well to added fertilizer and other inputs.
- Good quality seeds of improved varieties ensures higher yield at least 15 – 20 %

2.2. Components of seed quality

The different components of seed quality are physical quality, genetic quality, physiological quality and pathological quality (Thomson, 1979). Quality seeds are required to meet the standards for genetic and physical purity, physiological soundness and health status.

2.2.a. Physical purity

It is the cleanliness of seed from other seeds, debris, inert matter, diseased seed and insect damaged seed. The seed with physical quality should have uniform size, weight, and colour and should be free from stones, debris, and dust, leafs, twigs, stems, flowers, fruit well without other crop seeds and inert material. It also should be devoid of shriveled, diseased mottled, moulded, discoloured, damaged and empty seeds. The seed should be easily identifiable as a species of specific category of specific species.

Lack of physical purity will thereby indirectly influence the field establishment and planting value of seed. This quality character could be obtained with seed lots by proper cleaning and grading of seed (processing) after collection and before sowing / storage.

2.2.b. Genetic purity

It is the true to type nature of the seed. *i.e.*, the seedling / plant / tree from the seed should resemble its mother in all aspects. This quality character is important for achieving the desired goal of raising the crop either yield or for resistance or for desired quality factors.

2.2.c. Physiological quality

It is the actual expression of seed in further generation / multiplication. Physiological quality characters of seed comprises of seed germination and seed vigour. The liveliness of a seed is known as viability. The extent of liveliness for production of good seedling or the ability of seed for production of seedling with normal root and shoot under favorable condition is known as germinability. Seed vigour is the energy or stamina of the seed in producing elite seedling. It is the sum total of all seed attributes that enables its regeneration of under any given conditions.

Seed vigour determines the level of performance of seed or seed lot during germination and seedling emergence. Seed which perform well at sowing are termed as quality seed and based on the degree of performance in production of elite seedling it is classified as high, medium and low vigour seed. The difference in seed vigour is the differential manifestation of

the deteriorative process occurring in the seed before the ultimate loss of ability to germinate. Difference in seed vigour will be expressed in rate of emergence, uniformity of emergence and loss of seed germination. Hence, it is understood that all viable seeds need not be germinable but all germinable seed will be viable. Similarly all vigourous seeds will be germinable but all germinable seed need not be vigourous. Physiological quality of seed could be achieved through proper selection of seed (matured seed) used for sowing and by caring for quality characters during extraction, drying and storage. Seed with good vigour is preferable for raising a good plantation as the fruits, the economic come out are to be realized after several years. Hence selection of seed based on seed vigour is important for raising perfect finalize plantation.

2.2.d. Seed Health

Health status of seed is nothing but the absence of insect infestation and fungal infection, in or on the seed. Seed should not be infected with fungi or infested with insect pests as these will reduce the physiological quality of the seed and also the physical quality of the seed in long term storage. The health status of seed also includes the deterioration status of seed which also expressed through low vigour status of seed.

The health status of seed influences the seed quality characters directly and warrants their soundness in seed for the production of elite seedlings at nursery / field. The organisms commonly associated with seed cause diseases and damages to seed, seedlings and crops are Fungi, Bacteria, Viruses, Nematodes and Insects. (Agarwal. 1977).

3. Conventional methods to assess seed quality

3.1. Physical purity assessment

The purity analysis of a seed sample in the seed testing laboratory refers to the determination of the different components of the purity *viz.*, pure seeds, other crop seeds, weed seeds and inert matter. The equipments employed to delineate the physical purity of a seed lot are detailed below.

3.1.a. Seed blower

Seed blowers are employed for the easy separation of materials which are lighter than the normal seeds.

Plate 1: Seed blower

3.1.b. Purity working board

The seed samples are placed on the purity work board after sieving / blowing operations and separation of other crop seeds and inert matter, if any are done. After separation, the kind of weed seeds, other crop seeds to their genus and species level identify. The names and number of each are recorded. The type of inert matter present should also be noted.

Plate 2: Purity working board

3.2. Genetic purity assessment

3.2.a. Grow out test

Grow-out Test is the official measure for controlling the genetic purity of the seed lot. It serves as a pre-control as well as a 'post-control' test for avoiding genetic contaminations. According to the official regulations in India, it is prerequisite for seed certification of hybrids of certain species such as cotton, castor, musk melon and brinjal.

The test is required to be conducted for checking the sellers label with respect to genetic purity status of the seed lot under the provisions of the seeds Act 1966. In addition grow-out

test can also be used as a measure to judge the efficacy of the certification agency or the inspector.

Plate 3: Grow out test

3.3. Physiological quality assessment

3.3.a. Germination and vigour

Germination is defined as the emergence and development from the seed embryo, of those essential structures, for the kind of seed in question, indicates its ability to produce a normal plant under favourable conditions. Seed vigour is the sum of those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence.

Table 1: Duration of germination and vigour test

Crops	**First count (days)**	**Second count (days)**
Paddy	5	14
Maize	5	7
Cotton	4	12
Tomato	5	14
Ground nut	5	10

Plate 4: Top paper

Plate 5: Between paper

3.4. Assessment of moisture content

The moisture content of a seed sample is the loss in weight when it is dried. It is expressed as a percentage of the weight of the original sample. It is one of the most important factors in the maintenance of seed quality.

3.4.a. Air oven method

In this method, seed moisture is removed by drying the seed sample at a specified temperature for a specified duration. Seed moisture is removed by drying the seed sample at a specified temperature for a specified duration, Most species are dried for 1 hr at 130° C, cereals for 2 hours (130°C) and maize for 4 hours (130°C). Seeds containing high percentage of oil should be dried at 103°C for 17 hours. It can be estimated using the formula,

$$MC = \frac{M2-M3}{M2-M1} \times 100$$

MC= Seed moisture content
M1 = Weight of the empty container
M2= Weight of the container and seeds before drying
M3= Weight of the container and seeds after drying

Plate 6: Hot air oven

3.5. Assessment of seed health

3.5.a. Blotter method

Blotter tests are similar to germination tests in that seeds are placed on moistened layers of blotter paper and incubated under conditions that promote fungal growth. The seed may then be allowed to germinate and fungal seed-borne infections may manifest themselves by any pertinent signs or symptoms. The manifestations of the pathogen are influenced by the environmental conditions during incubation. The blotter test gives an indication of the infection of the seed, as shown by the presence of mycelium and fruiting bodies, and, in some tests, infection of the germinated seedlings as demonstrated by symptoms on the young plants. In some tests seeds are incubated during which they are allowed to germinate and symptoms are observed.

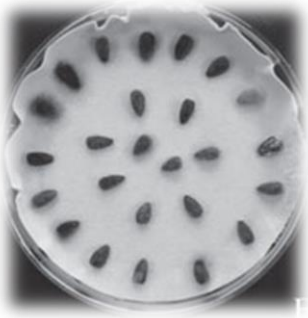


Plate 7: Blotter method

3.5.b. Agar plate methods

Agar plate is the most common method used for identification of seed borne fungi (Rao and Bramel, 2000). Incubation methods allow the detection of viable fungus material even at the preliminary phase of development of the fungus. This is done generally by placing seeds onto sterile agar media (potato dextrose or malt agars are most commonly used) to encourage the growth of seed borne fungi.

Plate 8: Agar plate method

4. Fast track technologies to assess seed quality

4.1. Assessment of physical purity

4.1.a. Ergo vision system

Technology and methods used to provide imaging-based automatic inspection and analysis of the seed sample.

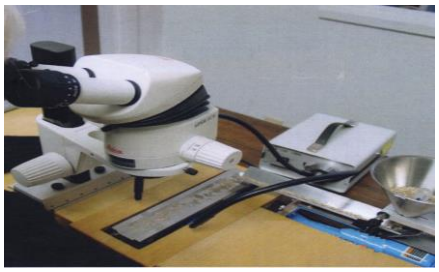
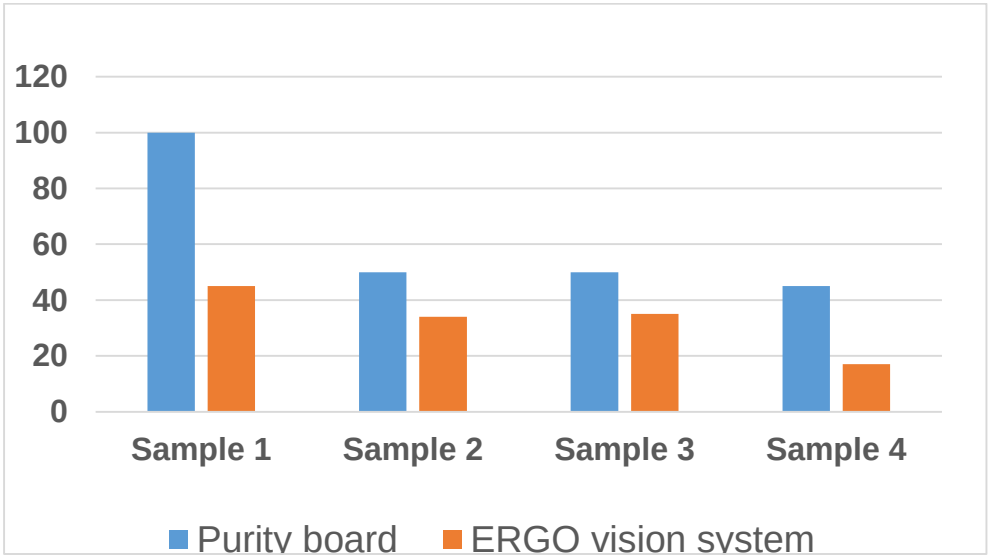


Plate 9: Ergo vision system

A study was conducted by Garey *et al.* (2009) on efficiency of purity analysis in rye. They compared the time taken for conduct of physical purity analysis using a purity working board and the Ergo-vision system on different seed samples. Results (Fig. 1) indicated that the Ergo-vision method required less time compared to the purity working board.

Fig. 1: Efficiency of purity analysis in Rye



4.2. Assessment of genetic purity

4.2.a. Molecular markers

A genetic marker is a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or trait. It can be described as a variation, which may arise due to mutation or alteration in the genomic loci that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long one, like mini & micro satellites.

Comparison of traditional grow out test and DNA based PCR assay to estimate F_1 hybrid purity in cauliflower was carried out by Pattanaik *et al.* (2018). It was evident that although the morphological variations in parents and hybrid can be identified by GOT, it would take about 70 days for its estimation. In contrast, on using molecular markers the purity can be identified within 48 hours.

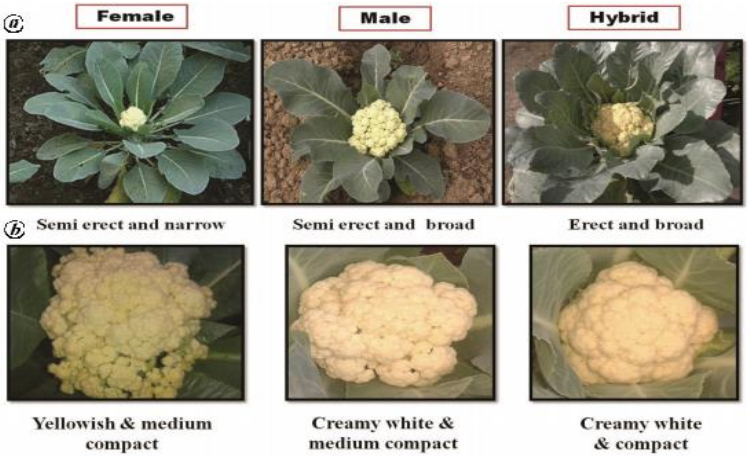


Plate 10: Variability in morphological parameters

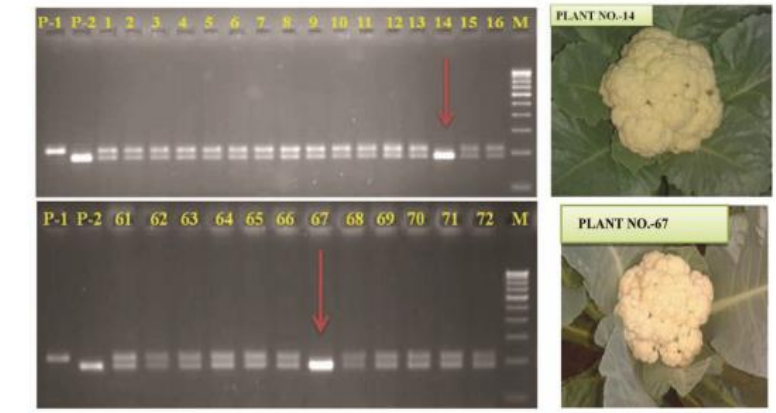


Plate 11: Phenotypically similar F1 hybrid showing genotypic off-types

4.2.b. Dip test

Lateral flow strips, also known as immunostrips, can quickly determine the presence or absence of genetically modified traits expressed in transgenic corn, cotton, and soybeans. They are relatively inexpensive and simple to use.

The technology employs qualitative colloidal gold based dipstick strip with antibody sandwich immunoassay format. It can detect specific transgenic Cry1Ac protein. The results can be visualized by naked eyes without any complex instrumentation, which provides the convenience for assay on-site. In addition, the test can be performed within 10 min without the need of using expensive equipment. Therefore, this test could be used directly in the field for the rapid qualitative screening of GM samples. Additionally, the method is economic, simple, and easy-to use.

Lateral flow strips detect protein extracted from individual leaf tissue, single seed tissue, or from a ground bulk seed sample. Once the strips are placed in the extraction solution, the liquid is wicked up the strip, carrying proteins across protein-capturing regions. Hence, it can easily detect the presence or absence genetically modified traits.

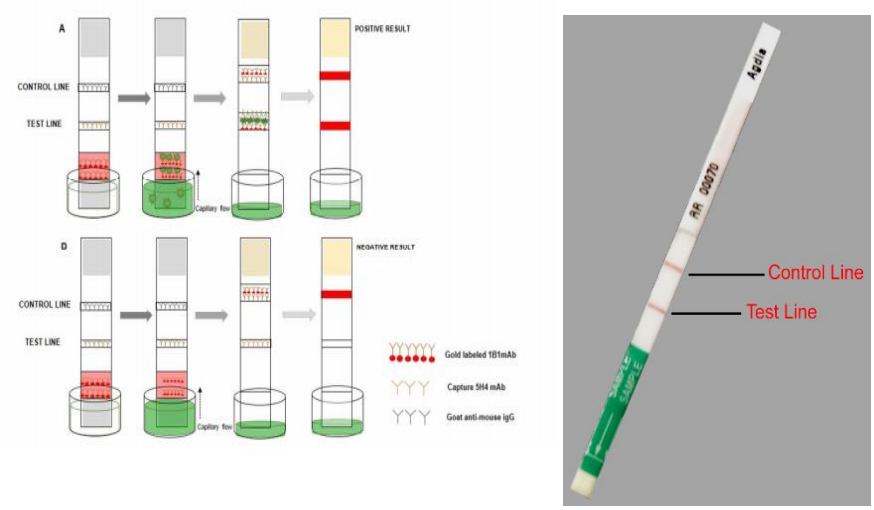


Plate 12: Lateral flow strip

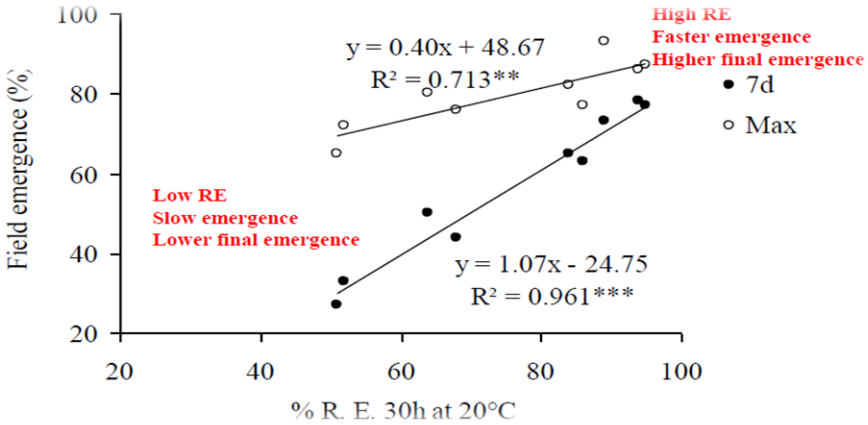
4.3. Assessment of physiological quality

4.3.a. Radicle emergence test

This is employed to predict the germination percentage of normal seedlings. Low vigour seed take long time to germinate than high seed vigour. Differences in RE rates have been attributed to the length of the delay from the start of imbibition to RE and this delay has been interpreted as dependent upon the time required for metabolic repair before RE.

Stan Mathews and Alison Powell (2012) conducted a study on radicle emergence test estimation in oil seed rape. They observed that higher the radicle emergence higher will be the final emergence, germination and vigor (Fig. 2).

Fig. 2: Radicle emergence test estimation in oil seed rape



4.3.b. Potassium leachate test

Potassium is the main inorganic ion leached by seeds during imbibition followed by sodium and calcium. This can be done by,

Miguel and Filho (2002) studied the physiological potential in maize seed by measuring the potassium leakage at different imbibition period (Table 2 and Table 3).

Table 2: Mean values from potassium leachate test in each imbibition period.

Seed lots	**Imbibition periods (min)**					
	30	60	90	120	150	180
	$\mu g\ K^{+}\ g^{-1}$ seeds					
1	66.58 a	76.90 a	85.74 a	93.24 a	99.45 a	107.51 a
2	199.13 b	216.87 b	239.33 b	246.58 b	25.27 b	264.10 b
3	215.43 b	230.51 b	238.32 b	243.08 b	247.59 b	252.00 b
4	65.14 a	73.17 a	82.05 a	90.64 a	93.64 a	102.81 a
5	188.95 b	217.72 b	230.95 b	242.35 b	252.02 b	253.62 b
C.V (%)	4.9	5.8	4.7	6.4	5.5	5.2

Table 3: Mean values from seed germination and seedling emergence

Seed lots	**Germination**	**Seedling emergence**
1	98	99
2	92	89
3	88	86
4	98	98
5	89	89
C . V (%)	3.7	5.9

They observed that lot 1 and 4 registered high seed vigour due to low value k+ leachate when compared to lot 2, 3 and 5 which is having high k+ value.

4.3.c. Moisture meter

Wet grains are good conductors of electricity while, dry grains are less conductors. Moisture content is directly proportional to the electrical conductivity of the seed. Quick and non-destructive methods of seed moisture analysis are also available.

Plate 13: Portable

Plate 14: Single seed

Plate 15: Digital

4.4 . Assessment of seed health

4.4.a. ELISA test

An enzyme-linked immunosorbent assay, also called ELISA or EIA, is a test that detects and measures antibodies in your blood. This test can be used to determine if you have antibodies related to certain infectious conditions. Antibodies are proteins that your body produces in response to harmful substances called antigens.

Sevick and Tohumcu (2011) conducted a study on The ELISA analysis result in tomato (lycopersicon esculantum mill.) seed health testing for *Tobaco mosaic virus*. They observed the absorbance value at 405 absorbance.

Table 4: Distribution of absorbance values

Range of absorbance values*	**Lot 1 (variety 1)**		**Lot 2 (variety 1)**	
	Negative	Positive	Negative	Positive
0.0 - 0.033	146	0	161	0
0.033 - 0.1	0	0	0	0
0.1 - 0.2	0	0	0	0
0.2 - 0.5	0	4	0	3
0.5 - 1.0	0	6	0	7
1.0 - 1.5	0	18	0	14
1.5 - 2.0	0	13	0	9
2.0 - 2.5	0	8	0	5
2.5 - 3.0	0	4	0	1
3.0 <	0	1	0	0
Total	146	54	161	39

When the absorbance value <0.1 shows negative result *i.e.*, sample is free from TMV and when the absorbance value >0.2 shows a positive result *i.e.*, sample is infected with TMV.

4.4.b. Automated computer imaging systems

- **Machine vision systems (MVS)**

Machine vision refers to the acquisition of data (shape, size, etc.) *via* a video camera or similar system and the subsequent computer analysis of these data following suitable processing. Study conducted by Maheshwari (2013)

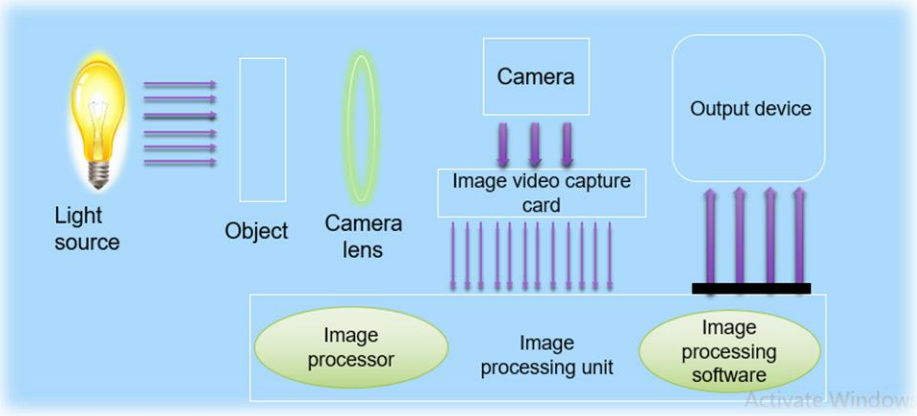


Plate 16: Working principle of MVS

- **X ray imaging**

The first to use X-rays for seed analysis was Lundstrom (1903) in coniferous tree seeds. Information on structure and condition of embryo and presence of insects, diseases or other defects can be easily analysed under X- ray radiation at very low dose (Haff & Slaughter, 2004)

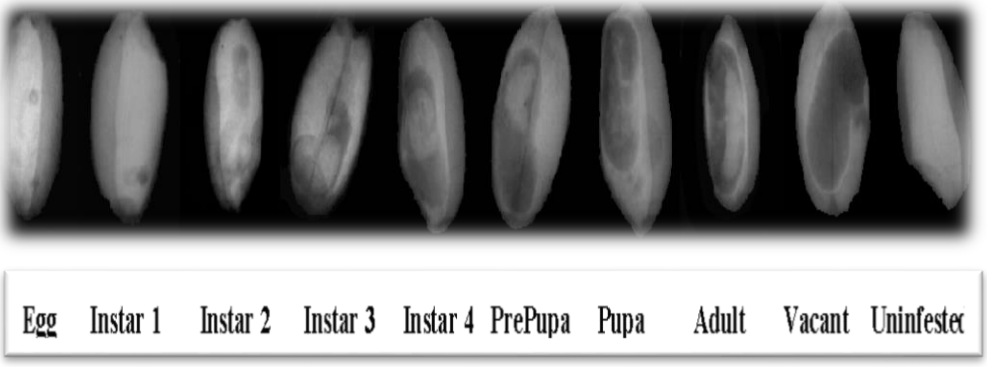


Plate 17: Digital X-ray images of different stages of insect infestation on wheat seeds

- **Hyper spectral imaging (HSI)**

HSI is an integration of digital imaging and spectroscopy it attain both spatial and spectral information from an object. It also provide physical features and chemical composition of an object. Lee *et al*. (2017) studied watermelon seeds infected with the bacteria *Acidovorax citrulli* were acquired by using the artificial inoculation method. Then samples were naturally dried under the ambient condition for 48 hours and measured In RAMAN hyper spectral imaging and they observed different images of seeds which helped in easy identification of healthy seeds and from pathogen infected seeds.

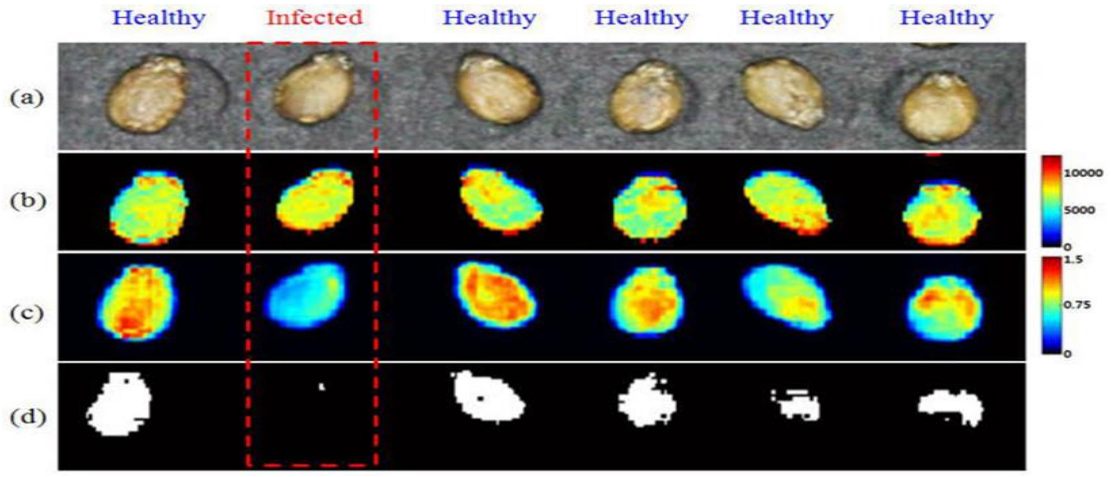


PLATE 18: Raman hyperspectral image

- **Near infrared spectroscopy (NIRS)**

The system is illuminated with monochromatic radiation. Part of the radiation is reflected by the outer surface of the sample (specular reflectance) while, a part of it penetrate deep into the inner tissues of the samples and then reflected back (diffuse reflectance). The relative amount of radiant energy absorbed at each frequency gives the amount of various organic substance present in sample.

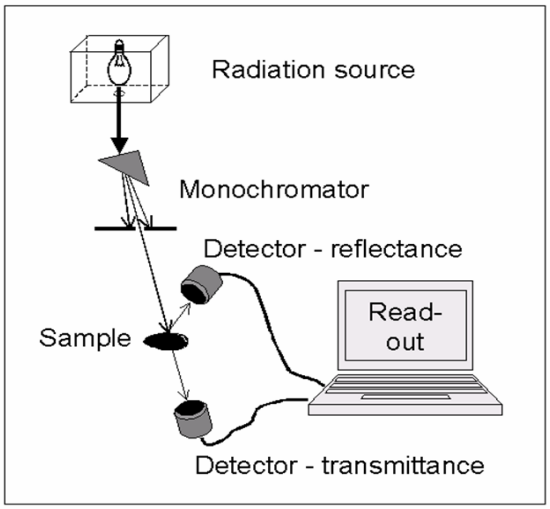


Plate 19: Working principle of NIRS

5. Application of ACIS in seed industry

- DUS testing
- Identification of cultivars
- Sorting and grading
- Automated vigor testing
- Detection of defects

6. Advantages of fast track technologies

- Fast
- High accuracy
- Automatic

7. Disadvantages of fast track technologies

- High input cost
- Scientific knowledge
- Technological skill

8. Conclusion

Although fast, accurate and mostly automatic and non-destructive, fast track technologies require high initial investment and technical expertise. The decreasing cost, increasing speed and capability of computer hardware and artificial intelligence, however, is expected to thrust this approach forward and make this technology more attractive for prospective usages in quality control and automatic inspection of seeds.

9. Discussion

Q 1. Among the seed quality parameters which quality is most important in seed technological aspect?

A. Among the different seed quality parameters genetic purity and germination are the most important

Q 2. In genetic purity assessment using molecular markers, can we use it for all crops?

A. NO. It can be used only for identified marker crops like rice, pumpkin, etc..

Q 3. What are all the different fast track technologies used in your department?

A. In our department for genetic purity assessment molecular markers are being used are for moisture estimation different moisture meters are used.

10. References

Agarwal,R. L. 1977. Seed technology. CBS publishers and distributors, New Delhi, 829p.

El-Masry, G., Mandour, N., Al-Rejaie, S., Belin, E., and Rousseau, D. 2019. Recent applications of multispectral imaging in seed phenotyping and quality monitoring- an overview. *Sensors* 19: 1090-1121.

Garay, A., Triplett, R., Hanning, S., Elias, S., and Whiting, B. 2009. Oregon State University Laboratory.Available:https://seedlab.oregonstate.edu/sites/seedlab.oregonstate.edu/file/ergovision-for-aosa-scst-2003.pdf [21 Oct. 2019].

Haff, R.P. and Slaughter, D.C. 2004. Real-time X-ray inspection of wheat for infestation by the granary weevil, *Sitophilus granarius (L.). Trans. ASAE*. 47(2): 531.

Lee, H., Kim, M., Qin, J., Park, E., Song, Y.R., Oh, C.S. and Cho, B.K. 2017. Raman hyperspectral imaging for detection of watermelon seeds infected with *acidovorax citrulli*. *Sensors*. 17(10): 2188.

Matthews, S., Wagner, M.H., Kerr, L. and Powell, A.A. 2018. Potential for early counts of radicle emergence and leakage of electrolytes as quick tests to predict the percentage of normal seedlings. *Seed Sci. Technol.* 46(1): 1-18.

Miguel, M.V.D.C. and Filho, J.M. 2002. Potassium leakage and maize seed physiological potential. *Scientia Agricola*. 59(2): 315-319.

Pattanaik, A., Reddy, D. L., Ramesh, S., and Chennareddy, A. 2018. Comparison of traditional grow-out test and DNA-based PCR assay to estimate F_1 hybrid purity in cauliflower. *Curr. Sci.* 115(11): 2095.

Sevik, M. A. and Kose-Tohumcu, E. 2011. The ELISA analysis results in tomato (*Lycopersicon esculentum Mill*.) seed health testing for tobacco mosaic virus. *Zemdirbyste Agric*. 98(3): 301-306.

Thomson, J.R. 1979. *An Introduction to Seed Technology*. Leonard Hill, New York, 252p.

KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF HORTICULTURE, VELLANIKKARA
Department of Seed Science and Technology
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Major Advisor	: Dr. Rose Mary Francies	Time	: 11.30 am

Can we fast track seed quality assessment?

Abstract

Providing high quality seeds is very important for agriculture as it is the key factor to guarantee higher yields. Germination, vigour and the progression of emerging seedlings are strongly related to resistance against biotic and abiotic stresses and the plant performance. Physical and genetic purity, seed health, moisture content, physiological attributes of seeds such as germinability and vigour define seed quality (Thomson, 1979).

Conventionally, the essential characteristics of a seed lot are determined at the Seed testing laboratories to ensure that the farming community gets high quality seeds. Although proven to be efficient, such methods are usually tedious and laborious. They also involve destructive sampling and require trained seed analysts. For example, the standard germination test which is still practiced as the official method for testing seed viability not only requires a time-period of seven to fourteen days but also warrants detecting abnormal, dead and dormant seeds to arrive upon the germinability of the seed lot. To increase the reliability, reproducibility, accuracy and speed of seed quality assessment, fast track methods with minimum human intervention are basically required to ensure availability of quality seed for production and trade purposes (El-Masry *et al*., 2019).

Fast track approaches such as Ergovision inspection system, lateral flow strip, radical emergence test, potassium leachate seed vigour test, oxygen influx Q2 scanner, portable moisture meters and the imaging techniques offer reliable alternatives to the traditional seed testing protocols. A comparative study on the efficiency of purity analysis in fodder grasses using the non-destructive Ergovision system and the conventional method of relying on seed blower and purity working board revealed that the former is more efficient and less time consuming (Garay *et al.,* 2009).

As per the International Seed Testing Association (ISTA), conduct of Grow-out test (GOT) is essential for the assessment of genetic purity of a seed lot. The major drawback of

this method is that it requires a complete cropping season to assess the genetic purity of the seed lot being tested. Pattanaik *et al.* (2018) successfully used molecular markers to assess the genetic purity of the seed lots of cauliflower. They suggested that it could reliably be used as an alternative for genetic purity testing. Lateral flow strip widely used in differentiating Bt cotton from non-GMO cotton is another successful fast track technology to assess genetic purity of a seed lot.

In addition to the use of Enzyme Linked Immuno Sorbant Assay (ELISA) test (Sevik and Kose-Tohumcu, 2011) in seed health testing, Automated Computer Imaging System (ACIS) such as X-ray imaging, machine vision imaging, hyper spectral imaging, and Near-infrared spectroscopy are gaining attention as viable non-destructive fast-track approaches to overcome the limitations of conventional methods. These have, in many instances, replaced the blotter paper method and agar plate method, traditionally used to assess the seed health status.

Although fast, accurate and mostly automatic and non-destructive, fast track technologies require high initial investment and technical expertise. The decreasing cost, increasing speed and capability of computer hardware and artificial intelligence, however, is expected to thrust this approach forward and make this technology more attractive for prospective usages in quality control and automatic inspection of seeds.

References

El-Masry, G., Mandour, N., Al-Rejaie, S., Belin, E., and Rousseau, D. 2019. Recent applications of multispectral imaging in seed phenotyping and quality monitoring- an overview. *Sensors* 19: 1090-1121.

Garay, A., Triplett, R., Hanning, S., Elias, S., and Whiting, B. 2009. Oregon State University SeedLaboratory.Available:https://seedlab.oregonstate.edu/sites/seedlab.oregonstate.edu/files/ergovision-for-aosa-scst-2003.pdf [21 Oct. 2019].

Pattanaik, A., Reddy, D. L., Ramesh, S., and Chennareddy, A. 2018. Comparison of traditional grow-out test and DNA-based PCR assay to estimate F_1 hybrid purity in cauliflower. *Curr. Sci.* 115(11): 2095.

Sevik, M. A. and Kose-Tohumcu, E. 2011. The ELISA analysis results in tomato (*Lycopersicon esculentum Mill*.) seed health testing for tobacco mosaic virus. *Zemdirbyste Agric*. 98(3): 301-306.

Thomson, J.R. 1979. *An Introduction to Seed Technology*. Leonard Hill, New York, 252p.