

# Advances in seed quality evaluation techniques in soybean



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Seed is alive; and it can change over time under varying conditions. It can also vary from year to year as do planting conditions. When planting, seed vigor can be used to assist with management decisions, especially under adverse planting conditions. Seed quality is critical in the establishment of a uniform plant stand, the first step in producing a successful crop, but good planting conditions are also critical since even high quality seed can fail under too much stress. Seed quality is complex. Several factors influence seed quality including variety, purity, weather, insects, diseases, harvest moisture, handling, and storage.

The soy bean(US) or soya bean(UK) ( *Glycine max* ) is a species of leguminous native to East Asia, widely grown for its edible bean which has numerous uses. The plant is classed as an oilseed rather than a pulse by the UN Food and Agricultural Organization.

Soybean Oil and protein content account for about 60 (%) of dry soybeans by weight (protein at 40% and oil at 20 %). The remainder consists of 35(%) carbohydrate and about 5 (%) ash. Soybean cultivars comprise approximately 8% seed coat or hull, 90 (%) cotyledons and 2 (%) hypocotyl axis or germ. The U. S., Argentina, Brazil, China and India are the world's largest soybean producers and represent more than 90% of global soybean production. India produces 9.8 million metric tons against the world's total production of 249.0 million metric tons.

Furthermore, the soybean seeds when stored under ambient conditions quickly lose viability and upon planting such seeds in the next season results in very poor germination. Because of the hot and humid conditions prevailing

from March to June, the seed viability of soybeans drops by 50 (%). However in soybean, seed viability during storage was observed to be related to seed size. Thus ensuring seed quality becomes one of the important aspect of soybean production.

The seed quality evaluation can be broadly categorized under and ensured to have

- Trueness to type (often referred to as variety purity).
- Satisfactory germination and vigour.
- Freedom from other materials, including plant debris, dead or broken seeds, seeds of other crops, weed seeds, noxious and parasitic weed seeds also non-plant materials.
- Freedom from seed-borne pests and diseases.

During 2009-10 soy bean breeder seed production was 10198. 03s (q.) with a seed rate (kg/ha) of 75 kg/ha, total certified seed requirement will be 667. 5000' tonnes. Keeping these production trends “ VISION-2030” of Directorate of Seed Research, Mau, India and estimates with a target increase in SRR of 0. 5% and available SMR ratio of 16 foundation seed requirement is 20. 8594 thousand tones and breeder seed requirement of 1303. 7109 tones by 2030. This ever increasing demand for quality seed in soybean demands precise seed quality evaluation methods.

The routine seed testing methods available for seed quality evaluation of soybean are standard germination, Seedling vigour, Accelerated Ageing, Controlled Deterioration, seed leachate conductivity tests and Clorox Soak for seed coat mechanical damage as described by ISTA and AOSA. However,

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these tests are time consuming and lack reproducibility over laboratories.

Hence there is a greater need of advanced seed quality evaluation methods to overcome the aforesaid problems.

Advanced seed quality evaluation techniques:

1. Seed and seedling image analysis: Computer-aided image analysis, which are contributing to improving insight of seed morphology and biology, in terms of seed quality and germination and various aspects of seed image analysis like image acquisition and pattern recognition. Image analysis deals the means by which digital images are acquired and processed and how imaging technology is applied in seed science research in terms of varietal identification, characterization, germination, moisture, grading and sorting by analysis of seed size, shape and color parameters. Implication of new techniques for addressing a particular variety can be focused and also attention is being laid at international level for the development of suitable lab techniques like image analysis of seed or plant organs, bio chemical and molecular markers. Image analysis technique (machine vision system) is one of such systems offers the prospect that researchers will be able to study seed surface features more closely and hence increase the available character set.

a) Machine Vision System: a computerized tool for Image Analysis (IA). Its functions being similar to the human observations. 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. The term “ image analysis” has also been used in this

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context, but it more strictly refers to the extraction of numerical data from an acquired image. The colour, size, shape characteristics of plant products, and their capability to produce digital images suitable for further processing make modern image acquisition techniques highly adaptable tools. Bio-morphological seed features may be analyzed by computer-aided image analysis systems and data quickly processed.

b) Seed Analyzer based on Chlorophyll fluorescence and the maturity of seeds: Aims at Automated detection of the ripeness of the seeds and deliver the information for improving the quality of the seed lot. The maturity of the seeds can be measured with this technology. The maturity of the seeds is highly correlated with the quality of the seeds. With the Seed Analyzer the correct harvest moment can be determined, the seed quality can be improved, improved, the amount of waste can be lowered as well as been known and the priming conditions can be optimized for the seed batch.

Applications in Seed Science Research includes Distinctness, Uniformity and Stability (DUS) Testing, Varietal Identification and Characterization. Wherein, Automatic systems can be based on seed images, from which the characteristics for the classification, such as size, shape, colour and texture, can be obtained quickly. Digital image analysis offers an objective and quantitative method for estimation of morphological parameters. Besides, in routine seed testing for enhanced seed quality as

Germination: Seed germination has intrigued the human activity since the late Neolithic age, because of practical reasons becoming a milestone in the 'agriculture framework' (Evenari, 1984). The application of computational

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techniques to the study of seed germination covers three aspects: computer-assisted image analysis systems, descriptive simulation modeling, and combined relation modeling between morphological changes and biological processes. A digital image of a plant seed can be regarded as a two-dimensional object which can be measured in size, shape and color density during the development stage of germination by computer image analysis technology.

**Moisture:** Moisture content is the most vital factor influencing physical and mechanical properties of cereal crop seeds. For example, an increase in moisture content leads to an increase in the major, minor and intermediate diameters; increase in all linear dimensions, projected area and volume; increase in length, width, thickness, arithmetic mean diameter, geometric mean diameter, sphericity, volume and surface area. These monochromatic images acquired can be used to determine the moisture content of seeds.

**Vigor Assessment:** Vigour is the ability of a seed lot to establish normal (or usable) seedlings under diverse production environments. Use of computer-aided image analysis of seedling size overcomes many of the limitations that occur during manual vigour tests. Image analysis provides rapid measurement of an object's physical characteristics and allows quantitative, objective observation. Several commercial systems use some form of computer-aided analysis of digital images to evaluate seedling growth as a measure of seed vigour.

**Single seed oxygen measurement:** Development of automated system for scoring different seed quality parameters by detection of metabolic activity.

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This technology measures the oxygen consumption of single seeds in a closed environment. The total test is therefore performed under increasing stress conditions (oxygen stress) and gives us a deeper insight in various aspects of the seed quality.

c) Chlorophyll fluorescence of imbibing and (early) germinating seeds: The present technology focuses on chlorophyll fluorescence of imbibing and (early) germinating seeds. It claims to “ detect the metabolic activity [of seeds] during seed germination phase”. The technology enables the non-destructive evaluation of imbibing seeds on a number of characteristics, with the advantage to be able to follow the development of individual seeds in time, to be able to use or test the seeds (e. g. re-dried after priming) or the emerging pre-germinated seeds or seedlings developing from these seeds. It also potentially enables sorting.

d) Spectral imaging: Spectral imaging technology can be seen as a methodology which can add to the knowledge of seed quality aspects, the speed of testing and the reproducibility of traditional tests within and between laboratories. Seed size, shape and colour are common features that are employed as sorting parameters for improvement of seed quality. In spectral imaging the sequential exposure of the object to light of different wavelengths provides further information about topographical texture, spectral texture and gloss.

Multi- and hyperspectral imaging and analysis of the generated data are clear examples of these developments. The light sources, cameras and computers for such systems are readily available and relatively affordable.

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This opens a wide array of potential applications in seed testing at various levels, as well as research opportunities that before were only possible for a few very specialized institutions. Multispectral and hyperspectral imaging as to be part of the standard seed testing equipment in the near future.

## 2. Chemical tests:

**Peroxidase Test:** This is a test is effectively employed for cultivar separation based on high or low seed coat peroxidase activity. This information is taken from the AOSA Rules. Analysts remove and place the dry seed coat from soybean seeds into individual test tubes or suitable containers. They add 10 drops of 0.5 percent guaiacol to each test tube. After waiting 10 minutes they add one drop of 0.1 percent hydrogen peroxide to the tube. After one minute, seeds are recorded as peroxidase positive (high peroxidase activity) if there is a reddish-brown solution; or peroxidase negative (low peroxidase activity) if there is a colorless solution in the test tube.

**3. Biochemical markers for seed quality evaluation and testing:** With the advent of newer technologies to effectively quantify and detect the precedence of particular protein and isozymes. The isozymes lack repeatability owing to their specific stage and range of expression and are highly responsive for environment. However, among these Two Dimensional Protein Gel Electrophoresis (2 D PAGE) is recommended for hybrid purity testing by ISTA.

**4. DNA/Molecular markers for seed quality evaluation and testing:** Quality seeds has to meet the minimum seed certification standards and quality

attributes viz., physical purity, germination per cent, moisture content, seed  
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health and genetic purity. The genuineness of the variety is one of the most important characteristics of good quality seed. Genetic purity test is done to verify any deviation from genuineness of the variety during multiplication stages. For certification genetic purity test is compulsory for all foundation and certified hybrid seeds. Higher genetic purity is an essential requirement for the commercialization of any seed.

Importance - stable marker for genetic purity: CMS plants and its maintainer plants, which originated from female parent during multiplication, are major off-types in F1 hybrids. Maintenance of the purity of parent CMS lines is essential in achieving the purity of hybrid and thus commercial benefit. CMS purity during multiplication can only be assessed at heading stage by observing pollen fertility. The results are prone to be erratic due to different examiners and environments. So it is significant to develop a novel, simple, rapid and effective method to assess CMS seed purity during multiplication at seedling stage.

The molecular markers are more efficient in assessing genetic purity. Among markers RAPD and AFLP are dominant markers. Dominant markers unable to identify heterozygous condition (AA and aa only but not Aa).

Low reproducibility of RAPD and lengthy process of AFLP markers have made them impractical and difficult for their routine use in seed purity analysis. While SSR, SCAR, STMS are Co-dominant markers and are able to identify heterozygous condition. These markers are more popular because of their accuracy in results and are reproducible. Quick and simple processes of these markers have made them practical for their routine use in hybrid

conformity and seed purity analysis. Markers vary based on their ability to differentiate lines with the crop and hybrids and parental lines involved in developing particular hybrid.

With the advancement of science and engineering new throughout put genomics and phenomics technologies *viz.*, Nuclear Magnetic Resonance Spectroscopy, Nu PCR, rapid onsite DNA detection, Nested DNA Markers Battery, genome sampling and Genome sequencing the “ next generation seed testing” is going to transform the seed quality evaluation and testing to an elevated stature having real-time application with high degree of reliability.

New methods for seed testing are emerging with increasing technological possibilities and computer power, parallel to decreasing prices will enhance the precision and speed with which the soybean seed quality is being tested with increase in accuracy and reproducibility of results.