A Review of Purity Test of Soybean

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Abstract: Pure Seed testing is determining the standards of a seed lot viz., physical purity, moisture, germination and ODV and thereby enabling the farming community to get quality seeds. The Seed Testing Laboratory is the hub of seed quality control. Seed testing services are required from time to time to gain information regarding planting value of seed lots. Seed testing is possible for all those who produce, sell and use seeds. in this paper discuss how to measure purity of soybean and methods of purity test of soybean.

Keywords: pure seed testing, objective, importance, role of purity test and methods

1. Pure Seed Testing

Pure Seed testing is determining the standards of a seed lot viz., physical purity, moisture, germination and ODV and thereby enabling the farming community to get quality seeds. The Seed Testing Laboratory is the hub of seed quality control. Seed testing services are required from time to time to gain information regarding planting value of seed lots. Seed testing is possible for all those who produce, sell and use seeds.

2. Objective and Importance of Pure Seed Testing

Seed testing is required to achieve the following objectives for minimizing the risks of planting low quality seeds.

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<th>s.no</th>
<th>Objective of Pure Seed Testing</th>
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<td>1.</td>
<td>To identify the seed quality, problem and their probable cause</td>
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<td>2.</td>
<td>To determine their seed quality, that is, their suitability for planting</td>
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<td>3.</td>
<td>To determine the need for drying and processing and specific procedures that should be used</td>
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<td>4.</td>
<td>To determine if seed meets established seed quality standards or labelling specifications.</td>
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<td>5.</td>
<td>To establish seed quality and provide a basis for price and consumer discrimination among lots in the market. The primary aim of the seed testing is to obtain accurate and reproducible results regarding the quality status of the seed samples submitted to the Seed Testing Laboratories.</td>
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Importance Pure Seed Testing

The importance of seed testing was realized more than 100 years ago for assured planting values. The adulteration of vegetable seeds by stone dust which was packed in some parts of the world.

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<td>1.</td>
<td>Seed testing has been developed to aid agriculture to avoid some of the hazards of crop production by furnishing the needed information about different quality attributes viz., purity, moisture, germination, vigour and health.</td>
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<td>2.</td>
<td>These quality attributes are seed moisture content, germination and vigour, physical and genetic purity, freedom</td>
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3. Role of Seed Testing Laboratories

- Seed testing laboratories are essential organization in seed certification and seed quality control programmes. The main objective is to serve the producer, the consumer and the seed industry by providing information on seed quality. Test results may cause rejection of poor seed multiplication or low grade seed in a count of law.
- Analysis of seed in the laboratory: Seed testing is possible for all those who produce, sell and use seeds. Seed testing is highly specialized and technical job. With a view to maintain uniformity in quality control the seed analysis laboratory includes for distinct sections.

Section for purity testing: Purity analysis of seed lot is considered under two factors
- Testing the cleanliness of seed lot and
- Testing the genuineness of the cultivar
  - Section for moisture testing
  - Section for viability, germination and section for vigour testing.
s.no | Name of the test | Method of Purity test
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1. | Peroxidase test | Vegetable soybean seed coat was separated by soaking in water for two hours. The seed coats were placed in test tubes with 3 to 4 ml of 0.5 per cent guaiacol solution. After ten minutes, a drop of 0.1 per cent hydrogen peroxide solution was added by gently swirling. Change in the colour of the solution to dark red/brown indicated a positive reaction whereas, no change in colour indicated the negative reaction. The observation on colour change was taken within 10 seconds after adding H2O2. They were grouped as either reddish brown (+ve) or no change in colour (-ve).
2. | Potassium hydroxide (KOH) test | Seeds were soaked in four per cent KOH solution for three hours and thereafter change in colour of the solution was observed. Based on the intensity of the colour reaction, the genotypes were classified into four groups viz., light yellow, medium yellow, medium red and dark red colour (Papp et al., 1997).
3. | Sodium hydroxide (NaOH) test | Seeds were soaked in two per cent NaOH solution for one hour and thereafter change in colour of the solution was observed. Based on the intensity of the colour reaction, the genotypes were classified into three groups viz., light yellow, dark yellow and dark red (Papp et al., 1997).
4. | Bio-chemical markers | Electrophoretic analysis of soluble seed protein Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis of total soluble seed protein was carried out by using 12 percent polyacrylamide gel according to the method prescribed by Laemmli (1970) with slight modifications. The electrophoresis was done in vertical slab gel of 16 cm x 14 cm x 1 mm dimension. The detailed procedure was as follows:
(a) Sample preparation
Sample preparation done in vertical slab gel of 16 cm x 14 cm x 1 mm dimension. The detailed procedure was as follows:

(b) Preparation of gel for electrophoresis
i) Resolving gel (12 %) was prepared by mixing 12.4 ml of 30 percent acrylamide solution, 23.8 ml of resolving gel buffer, 13.8 ml of water, 0.4 ml of 10 percent SDS, 0.4 ml of 10 percent APS and 0.6 ml TEMED and quickly poured into the gel plates leaving a margin of 2.0 to 3.0 cm on upper side of the gel. Overlay with water and left for polymerization for about 30 minutes.

(c) Electrophoresis
The upper and lower reservoirs of electrophoretic unit were filled with electrode buffer. Then 50 to 60 µl of protein extract was loaded into the wells of stacking gel by layering them under electrode buffer using micropipette. A current of 1.5 ma per well was applied until the tracking dye crossed the stacking gel. Later the current was increased to 2 ma per well and voltage up to 120. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel, which took six to eight hours.

(d) Staining and destaining For staining the gel, Coomassie Brilliant Blue R-250 (CBB) was used. The gel was removed after the run and kept in Coomassie Blue solution prepared by dissolving one gram of CBB in 100 ml methanol, 20 ml Acetic acid and 80 ml of distilled water. After dissolving CBB, it was filtered through Whatman No.1 filter paper and used for staining the gel. The gel was immersed in CBB solution overnight at room temperature and then washed with distilled water two to three times and incubated in destaining solution prepared by mixing 227 ml of distilled water until the bands were clearly visible. The destained gel was preserved in 7 per cent Acetic acid and used for interpretation of protein bands.

5. | Electrophoretic analysis of enzymes | Three isozyme viz., Esterase (EST), Malate Dehydrogenase (MDH) and Peroxidase (POX), were analyzed for isozymic pattern as described by Glaszman et al. (1988) with slight modification Seedlings were raised at 250C and eight to ten days old seedlings were used for this purpose. Single seedling was used for extraction of these isozymes.

(i) Sample preparation
Eight days old seedling was ground thoroughly in a pestle and mortar with 50 µl of extraction buffer under ice condition. The extract was taken in 2 ml eppendorf tubes and centrifuged at 10,000 rpm for 15 minutes. The supernatant was collected and 10 µl of tracking dye (1% bromophenol blue) was added into each tube. 50 µl of sample extract was used for loading.

(ii) Preparation of gel (8%)
Running and spacer gel solutions were prepared by using the solutions I, II and III as given below:

**Solution I:**
Acrylamide 30.08 g Bis-acrylamide 0.8 g Distilled water to make 100 ml

**Solution II:**
### References


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**Preparation of gels and staining solutions:***

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<tr>
<th>Step</th>
<th>Description</th>
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<td>i</td>
<td>Prepare 1.5 M Tris by dissolving 18 g in 80 ml distilled water and adjust the pH to 8.8 with 1 N HCl, make up the volume to 100 ml.</td>
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<td>ii</td>
<td>Prepare 0.5M Tris by dissolving 6 g in 80 ml distilled water and adjust the pH to 6.8 with 1 N HCl, make up the volume to 100 ml.</td>
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<td>iii</td>
<td>i) Resolving gel (8%): It was prepared by mixing 10.4 ml of solution I, 10.4 ml of solution II, 18.4 ml of distilled water, 1.2 ml of Ammonium Per Sulphate and 30 µl TEMED. The solution was shaken and poured quickly into the gel plates leaving a margin of 2.0 to 3.0 cm on upper side. The gel was allowed to polymerize for 15 minutes.</td>
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<td>iv</td>
<td>b) Stacking gel (5%): It was prepared by mixing 2 ml of solution I, 3 ml of solution III, 6.8 ml of distilled water, 140 µl Ammonium Per Sulphate (APS) and 30 µl TEMED. Pour the solution on to the top of the resolving gel and immediately a comb was inserted to form the wells of 1.5 cm depth taking care not to trap the air bubbles underneath the comb. The gel was allowed to polymerize, then the comb was removed carefully and the wells were rinsed with distilled water.</td>
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<td>v</td>
<td>iii) Electrophoresis: The upper and lower reservoirs of electrophoretic apparatus were filled with electrode buffer. Then 50 µl of isozyme extract was loaded into the wells of stacking gel using micropipette. A current of 1.5 mA per well with a voltage of 80 was applied until the tracking dye crossed the stacking gel. Later the current was increased to 2 mA per well and voltage up to 100. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel, which took five to six hours.</td>
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<td>vi</td>
<td>iv) Staining of gel: After the gels were removed, staining solution of respective enzyme were added (as given below) and kept in dark and periodically shaken. Bands appeared within 25-30 minutes and the gels were photographed immediately.</td>
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<td>vii</td>
<td>a) Staining solution for esterase (EST): 50 mg fast blue RR salt in 2 ml of 50 mg naphthyl acetate in 50 per cent acetone added to 100 ml of 0.5 M sodium phosphate buffer (pH 6.2) for one hour.</td>
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<td>viii</td>
<td>b) Staining solution for Malate dehydrogenase (MDH): Nitro Blue Tetrazolium 20mg (MTT) + 4 mg PMS (Phenyl Methyl Sulphate) +3 ml of 1M Malate (pH 7.5) added to 0.1M Tris buffer for 2 hours. The solution was prepared in the dark just few minutes before staining.</td>
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<td>ix</td>
<td>c) Preparation of staining solution for peroxidase (POX): 50 mg 3-9 AEC (3-Amino-9-Ethyl-Carbazole) in 3ml of DMF (Dimethyl Formamide) was added to 100 ml of 50Mm Sodium acetate buffer (pH 4.5) and 1 ml of 30% H₂O₂ for 5 minutes. The solution was prepared in the dark just few minutes before staining.</td>
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