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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.

s.no	Objective of Pure Seed Testing
1.	To identify the seed quality, problem and their probable cause
2.	To determine their seed quality, that is, their suitability for planting
3.	To determine the need for drying and processing and specific procedures that should be used
4.	To determine if seed meets established seed quality standards or labelling specifications.
5.	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.

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.

S. No	Importance Pure Seed Testing
1.	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.
2.	These quality attributes are seed moisture content, germination and vigour, physical and genetic purity, freedom from seed borne diseases and insect infestation. In India, seed testing is done mainly for moisture, germination and physical purity of seeds.
3.	Standard seed testing procedures for the evaluation of the seeds were developed by ISTA. It is obligatory on the part of the seed analyst to follow rules prescribed by 1STA (1STA, 1985) if the seed is moving to the International trade.
4.	Testing of seed to evaluate the planting value and the authenticity of the certified lot.
5.	Quality control of seed depends on the different seed testing protocols which determine the genuineness of the cultivar.
6.	Seed testing is required to assess the seed quality attributes of the seed lots which have to be offered for sale.
7.	The testing of seed quality is carried out on seed samples drawn from seed lot to be used for cultivation. The quantity of seed sample taken for testing in laboratory is minute compared to that of seed lot it represents.
8.	The seed testing procedures which are described below are based mostly on the international rules because most of our rules (Chalam et al.. 1967) are based on, 1STA, 1996. Economic yield of a crop depends on the quality of seeds which can be evaluated by seed testing (1STA, 1996).
9.	The testing of seed quality is carried out on seed samples drawn from seed lot to be used for cultivation. The quantity of seed sample taken for testing in laboratory is minute compared to that of seed lot it represents.

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

a) Testing the cleanliness of seed lot and

b) Testing the geneuiness of the cultivar

- Section for moisture testing
- Section for viability, germination and section for vigour testing.

Purity test of Soybean

s.no	Name of the test	Method of Purity test
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 group 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	Electorphoretic 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 Laemeli (l970) 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** Single seed of each genotype was crushed by pestle and mortar. The ground seed sample was put into eppendorf tubes for defatting. The seed powder was vigorously mixed with CMA (Chloroform, Methanol and Acetone) solution (5.0 ml) for 20 min at room temperature with simultaneous addition of sodium sulphite (1.5 mg) and sodium meta bisulphate (1.5 mg). The CMA solution was decanted off and the residue was again treated two more times with CMA solution (5.0 ml each) and centrifuged the contents at 10,000 rpm under refrigeration. Then the suspension was used for protein extraction. To the defatted seed material, 1.5 ml of extraction solution, 0.2 ml of tries glycine extraction buffer (25 Mm, pH 8.5) was added and agitated thoroughly and kept at 80 C overnight for protein extraction. Then the suspension was centrifuged under refrigeration at 10,000 rpm for 10 min and the clear supernatant was collected. This protein extract was dissolved in an equal amount of working buffer and kept in boiling water at 900 C for 10 minutes, cooled and again centrifuged at 10,000 rpm for 5 minutes and the supernatant was collected and used for loading on to the gel. **(b) Preparation of gel for electrophoresis** **i)** Resolving gel (12 %) was prepared by mixing 12.4 ml of 30 per cent acrylmide solution, 23.8 ml of resolving gel buffer, 13.8ml of water, 0.4 ml of 10 per cent SDS, 0.4 ml of 10 per cent 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. **ii)** Stacking gel (4%) was prepared by mixing 3.8 ml of 30 per cent acrylmide, 3.0 ml of stacking gel buffer, 8.0 ml distilled water, 0.2ml of SDS (10%), 0.4 ml 10 % APS and 0.04 ml TEMED. Top of the resolving gel was thoroughly cleaned before pouring the stacking gel, then poured the stacking gel solution on to the top of the resolving gel solution 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 for 30 minutes, then the comb was removed carefully and the wells were rinsed with distilled water. **(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 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 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) wasused. 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. Then 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 de-stained gel was preserved in 7 per cent Acetic acid and used for interpretation of protein bands.
5.	Electrophoretic analysis of isozymes	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 mortor with 50µl 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:**

		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. **Solution III:** 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. a) 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. 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. **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. **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. **a)** Staining solution for esterase (EST): 50 mg fast blue RR salt in 2 ml of 50 mg napthyl acetate in 50 per cent acetone added to 100 ml of 0.5 M sodium phosphate buffer (pH 6.2) for one hour. **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. **c)** Preparation of staining solution for peroxidase (POX): 50 mg 3-9 AEC (3-Amino-9-Ethyl-Carbozole) in 3ml of DMF (Dimethyl Formamide) was added to 100 ml of 50Mm Sodium acetate buffer (pH 4.5) and 1 ml of 30% H_2O_2 for 5 minutes. The solution was prepared in the dark just few minutes before staining.

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