

## Laboratory Methods for Characterization of Cotton and Maize Hybrids

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The success of agriculture depends largely on the development of genetically improved varieties which can fulfill the specific requirements with respect to availability of the inputs, climate of the region, marketability of the produce and other related socio-economic parameters. Variety improvement programmes for cotton and maize have become highly competitive.

India became signatory member to the Trade Related Intellectual Property Rights Agreement (TRIPs) in 1994. According to the agreement the member countries require to provide protection of plant varieties either by a patent or by an effective sui generis system. The UPOV convention [1] (Article 7(1) of the 1996/1972 and 1978 Acts and article 12 of the 1991 Act) requires that a variety be examined for compliance with the distinctness (D) uniformity (U) and stability (S) criteria. Variability in the colour reaction of the seeds is a simple method to group large number of genotypes into distinct classes or to differentiate between genotypes. There are numerous ways to test seeds for cultivar purity e.g. morphological characters, laboratory tests, growth chamber tests and electrophoresis. Chemical tests or laboratory tests are tests which rely on the reaction of a specific chemical compound with some component of the seed. These tests depend on the reaction of a specific seed enzyme with its substrate like- hydrochloric

acid test for Oats, peroxidase test for Soybean [2], phenol test for Barley, Blue grass, Oats, Ryegrass, Wheat [3], potassium hydroxide test for rice.

Cotton and maize are two important cash crops playing a key role in the economic and social affairs of India. Cotton (*Gossypium hirsutum* L.) is the most important textile fiber crop and the world's second-most important oil seed crop. Maize (*Zea mays* L.) the third most important cereal crop plays a significant role in human and animal nutrition world wide. Maize is cultivated for food, fodder; feed, industrial use and more than 3500 value added products of daily application are derived from maize.

There is a huge demand for hybrid cotton and maize seeds world over. This has led to the release of a large number of hybrids both by the private and public sectors. Hence, reliable characterization of cotton and maize hybrids and their parental lines based on physiological and biochemical attributes would be useful in establishing their identity. Purity of an individual is maintained by growing individuals in isolation which is a labour intensive and time consuming. Rapid and reliable testing like intensity of anthocyanin pigments, peroxidase test, phenol test etc are available which are necessary for in house quality control.

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### Intensity of Anthocyanin pigments

A wide array of pigments and colouring compounds from different parts of plants like leaf, petals, berries, seeds etc. can be used for characterization of different plant species and varieties. Colour intensity of anthocyanin and phenolic compounds in berries were utilized for varietal characterization of grape vines [4]. Gerbera cultivars were differentiated on the basis of pigmented compounds found in the ray florets [5]. Here we grouped cotton hybrids and their parental lines based on intensity of anthocyanin pigments.

### Procedure

200 mg of the seed material cotton hybrids and parental lines were hydrated for 72h and decoated seeds were crushed in a pestal and mortar and transferred to 10 ml test tubes. To this 5ml of acidified methanol was added. Test tubes wrapped in black paper and kept at 4°C overnight. Supernatant was decanted and sediments if any were separated by centrifugation. The colour variation of methanolic extract ranging from yellow to green [6] was recorded by visual comparison (Table 1).

**Table 1. Grouping of cotton genotypes by anthocyanin intensity**

S.No.	Hybrids	Male parents	Female parents
1	Anjali (++)	LRA 5166 (+)	Surabhi (++)
2	GCH-10 (+)	LRA 5166 (++++)	BC-68-2 (+)
3	PKVHY-4 (++++)	AKH07R (++)	CAK-23A (++)
4	HHH 81 (++++)	GC-182 (+++)	H-777 (+++)
5	PHH-316 (++)	PH-325 (++)	PH-93 (+)

(Yellow = +, Olive = ++, Lemon yellow = +++, Green = +++)

### Peroxidase test

Peroxidase test is one of the standard test, which was first used to identify soybean cultivars.

Soybean cultivars were distinguished into two groups based on the presence of either high or low seed coat peroxidase reaction with oxidized guaiacol [2, 7]. A genetic analysis revealed that seed coat peroxidase activity is controlled by a major gene. Outcome of the seed coat peroxidase test is unaffected by seed quality or by seed storage condition [8]. We examined cotton hybrids and their parental lines using peroxidase tests.

### Procedure

Seedcoat removed from the cotton seeds placed into a test tube, 10 drops of 0.5 per cent guaiacol added to the test tube. After 10 min 0.1 per cent hydrogen peroxide was added. Data recorded on the basis of peroxidase positive (high peroxidase activity) indicated by a reddish-brown solution or peroxidase negative (low peroxidase activity) indicated by a colourless solution in the test tube (Table 2).

**Table 2. Response of cotton genotypes to peroxidase test**

S.No.	Hybrids	Male parents	Female parents
1	Anjali (+)	LRA5166 (+)	Surabhi (+)
2	GCH-10 (+)	LRA5166 (-)	BC-68-2 (-)
3	PKVHY-4 (-)	AKH07R (+)	CAK-23A (-)
4	HHH81 (-)	GC-182 (+)	H-777 (-)
5	PHH-316 (-)	PH-325 (+)	PH-93 (-)

(High peroxidase activity = +, Low peroxidase activity = -)

### Phenol test among maize genotypes

Phenol test is a quick and easy method for determining cultivar purity. Phenol reacts with enzymes in the pericarp and other seed coat structures. It has been reported that phenol reaction is a chemical reaction rather than an enzymatic reaction [2]. Phenol does not react in the seed as a solution. It is the vapor form of the solution that produces the colour reaction.

The phenol colour reaction depends on the quantity and quality of oxidative enzymes present in the seed coat, which oxidize phenol producing melanin pigment as the final end product. Phenol test was used to classify wheat cultivars into different groups [9, 10]. UPOV [1] considered phenol colour reaction as one of the descriptors for DUS testing of wheat varieties. The genetics of phenol colour reaction [11] concludes that it is controlled by a single gene. Phenol reaction can be useful for DUS testing among maize genotypes. We used this test for differentiating different hybrids and their parental lines.

#### Procedure

Ten seeds per replication (two replications each) were hydrated on moist blotters for 24h at 25°C, followed by incubating over 1 per cent phenol solution in tight lid Petri plates for 24h at 35°C. Based on the change of seed colour, five hybrids along with their parental lines were classified into four colour groups i.e. no colour change, light brown, brown and dark brown (Table 3).

Table 3. Response of maize genotypes in phenol test

Hybrid	P1	P2	P3	P4
VL-Makka-42 (+)	CM126 (+++)	CM127 (+++)	CM128 (-)	CM129 (+)
Vivek Hybrid9 (+)	CM212 (+)	CM145 (-)		
Pusa early hybrid Makka-2(+)	CM137 (+)	CM138 (+)		
Shaktiman 2 (+)	CM176 (++)	CM 186 (+)		
Ganga 11 (+)	CM202 (++)	CM111 (+)	CM501 (-)	

(No colour change = -, Light brown = +, Brown = ++, Dark brown = +++)

#### CONCLUSION

Chemical tests are rapid and easy for testing individual seeds. These tests are simple to conduct and are not expensive. Chemical tests can be employed with other identification tests for cultivar identification.

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