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IDENTIFICATION OF HYBRIDS AND PARENTS OF PEARL MILLET, COTTON AND CASTOR- A JOURNEY FROM SEED MORPHOLOGY TO MOLECULAR BIOLOGY THROUGH PHYSIOLOGY

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It is customary to deliver a talk based on one's researches on this occasion. I have pleasant memories of my brief encounters with late Prof. P. Maheshwari in 1963 when he came to Meerut College to conduct our M.Sc. Final Botany Practical as well as to examine my M.Sc. Thesis on Physiological studies on Cotton. I can never forget his dynamic personality, penetrative eyes and photogenic memory and I had brief encounters in Botany Department of Delhi University where I joined as a CSIR Research Fellow with Late Prof. B. M. Johri to learn techniques for controlled growth of ovaries and ovules of parasitic angiosperms. I would like to thank my parents, teachers, mentors, colleagues and dear students, for encouragement, cooperation, help, criticism and appreciation of our humble researches in this area of Seed Biology and Plant Physiology.

We have been working for many years on many aspects of Seed Pre-treatment/ presowing / hardening / soaking and drying / priming (Saxena 1974, 1979, 1985; Saxena and Pakeeriah 1986; Saxena *et al*. 2001, 2002 and 2002a); Seed size studies in Wheat, Mung, Maize, Soyabean, Groundnut, Cowpea and Gram (Garg 1981, 1984; Narula 1981; Kulkarni 1987; Bose 1998); Seed identification studies of Castor, Bajra, Cotton, Groundnut (John 1986, Nair 1990, Murali-Krishana 1993; Bose 1998, Bhatt 2001,2007; Shukla, 2004).

Finally, I have selected the topic today on seed identification and variety approaches due to genuineness of genotype is premier and perennial concern of seed quality control programmes as it is a yield determining feature of a particular genotype. In order to combat the odds of environment a number of efficient parental lines, hybrids and variety of other cultivars have been developed and released. India already has the distinction of being the first in developing and exploiting hybrids in crop like Pearl millet, Cotton, Castor and Pigeon Pea. Therefore it is necessary that the genotypes (hybrids and parents) once released must be identified by its distinct features. Also apart from Grow-out test, which is the standard method, quick and alternative method must be developed so the cultivars can be identified whenever it is required. Hence, it is essential to develop alternative methods that are rapid, reliable and less influenced by environment with the following objectives:

- To modify the Grow-out test and/or develop short term routine, reliable, reproducible and economical methods for the identification of Pearl millet, Cotton and Castor.
- To undertake biochemical studies for metabolites and enzymes in the seedlings.
- To identify hybrids and parental lines through electrophoresis for protein and isozymes patterns.
- To differentiate between hybrids and parents using PCR based RAPD (Cotton and Castor).

Prof P. Maheshwari award lecture delivered in November, 2007.

Studies conducted on hybrids and parents of Pearl Millet [*Pennisetum glaucum* (L.)R. Br.]

Five Pearl millet hybrids and their parents used for the study were:

Male	Hybrid	Female
K-560	BK-560	MS-5141A
D-23	P-23	MS-841A
ICMP-451	MH-179	MS-81A
H-90/4-5	HHB-50	MS-81A
H-77/833-2	HHB-67	MS-843A

Morphological Studies in Pearl millet were carried out for Seed Morphological studies (i.e. seed size, shape, colour, germ shape, seed volume, etc); Plant/Seedling morphology; Analysis of hybrid-parent mixture under field conditions; Evaluation of commercial hybrid seed lots under field condition; Seedling vigour and growth studies.

Based on the results it was concluded that

- Pearl millet hybrids and parents could be identified through modified 21-days long Grow-out test against the 60 days traditional Grow-out test.
- Known mixture analysis (of hybrids and parents) and commercial seed lot identification provided direct evidence of the reliability of modified Grow-out tests. The studies suggested that the markers selected at 21-days stage were specific and precise.

  Seed morphological features can be used as primary (seed size and shape) or secondary diagnostic features (colour, germ shape, seed volume).

Chemical Identification methods used for Pearl millet hybrids and parents were:

Phenol Reaction; Alkali hydroxide Reaction; Ferrous Sulphate test; Peroxidase Activity

Based on the results of chemical identification it can be concluded that

Chemical reactions provided simple, quick, inexpensive, routine and reliable on-the-spot methods for identifying pearl millet hybrids and parents. Potassium hydroxide reaction though alone can differentiate the hybrids and parents but a combination of phenol test or ferrous sulphate test could be more meaningful.

Physiological Studies in Pearl millet were undertaken for

Storability studies; Germination and seedling growth performance; Leachate analysis; Electro conductance and Water soluble sugars; Hormonal treatment with gibberellic acid, kinetin and ethylene (10^{-4} to 10^{-8}M conc.) through direct germination, seed presoaking and foliar spray methods

Based on the Physiological Studies it can be concluded that

- Physiological studies provided insight into physiological nature (storability) of pearl millet hybrids and parents.
- Although, the physiological character is not much useful in varietal identification it showed that storability of hybrids and its parental lines were distinct.
- Exogenous application of plant growth regulators has significant and different effect on hybrids and parents. Thus it could be used as an ideal supporting characters only.

Biochemical Studies on Pearl Millet

- The direct genome products i.e. seed proteins and isozymes were studied through electrophoresis.
- Seed protein studied through vertical gel

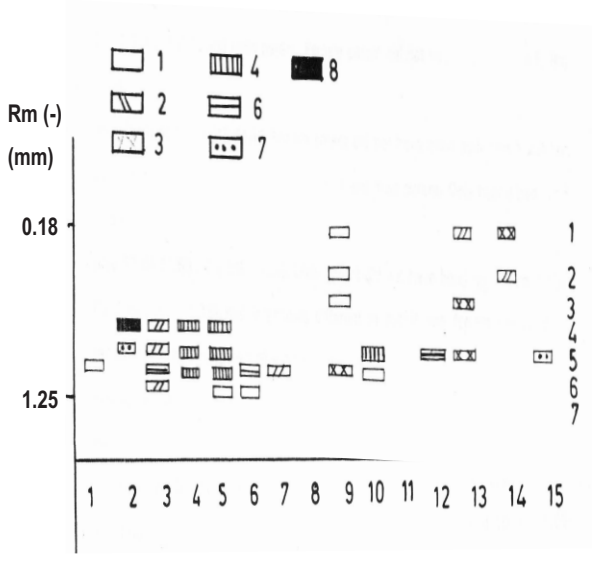
electrophoresis were: salt soluble, prolamin, cross-linked prolamin, glutelin-like and glutelins.

- 12 isozymes studied through horizontal gel electrophoresis were: Acid Phosphotase, Amylase, Catalase, Esterase, Glutamate dehydrogenase, Glutamate oxaloacetate transaminase, Malice enzyme, Malic dehyrogenase, peroxidase, Ribulose bis-phosphate, Shikimate dehydrogenase, Superoxidase dismutase.

Based on the Biochemical Studies it can be concluded that

- Electrophoretic study of seed proteins profile showed that the five protein fractions lacked adequate polymorphism for distinct identification of pearl millet hybrids and parents.
- Seedling isozymes: esterase,glutamate oxaloacetate transaminase and malic

FIG. 1 ESTERASE
BANK COLOUR INTENSITY



- enzyme provided highly polymorphicband profiles leading to complete identification

FIG. 2 GLUTAMATE OXALO ACETATE TRANSAMINASE
BAND COLOUR INTENSITY

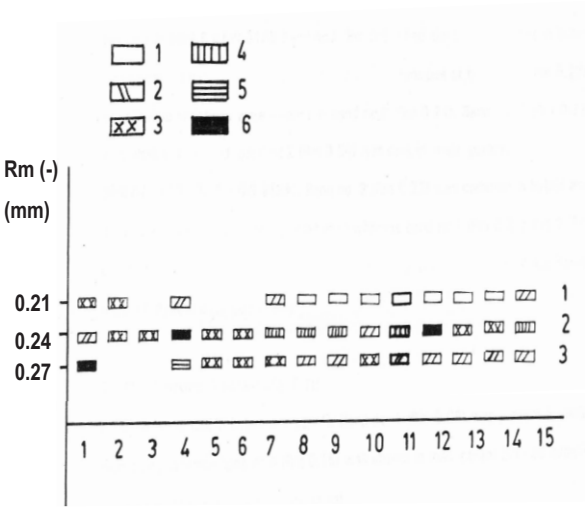
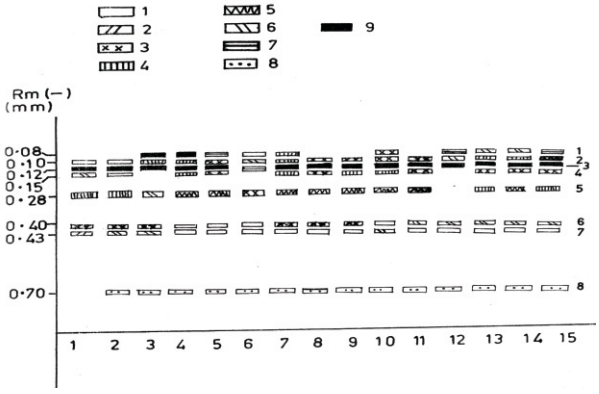


FIG. 3 MALIC ENZYME
BAND COLOUR INTENSITY



- of pearl millet hybrids and parents.

Studies Conducted on hybrid and parents of Cotton (*Gossypium hirsutum* L.)

Three Cotton hybrids and their parents used for

Male	Hybrid	Female
G.Cot-10	H-6	G.Cot-100
Surat dwarf	H-8	G.Cot-10
LRA-5166	H-10	BC-68-2

the study were:

Morpho-Physiological marker studies in Cotton were conducted for:

Morphology of seed and seed-coat; Morpho-

logical and physiological studies at 7-days stage; Morphological and physiological studies at 21-days stage; Morphological and physiological studies at 80-days stage; Known mixture analysis.

Based on the Morpho-physiological markers it was concluded that

- Cultivars of cotton (parents and hybrid) can be distinguished on the basis of seed shape, size, luster and seed coat studies successfully, as the seed coat pattern were genetically controlled and were constant for genotype.
- Parents and hybrids could be well discriminated at 7-days stage using morphological as well as physiological characters.
- More accurate cultivar identification was possible at 21-days stage using morpho-physiological markers.
- 80-days study provided vegetative as well as reproductive characters for quick morphological identification along with physiological markers.
- Known mixture analysis was good marker to check the reliability of the morphological studies.

Vigour Studies in Cotton showed that:-

Various germination tests like brick gravel test, paper piercing test, accelerated ageing test, etc.; Leachate analysis through electro conductance, soluble sugar, soluble protein, inorganic phosphates, phenols, etc.; Tetrazolium test and dehydrogenase activity and Seed moisture content, seed weight, seed volume, etc.

Based on the Vigour studies it can be concluded that

Various vigour tests conducted were good indicators of the quality and vigour of the seed. The values of the various parameters studied were comparable to its parents in the controlled tests. The percent germination of the hybrid seeds was higher in various vigour tests, like standard germination, brick gravel and paper piercing test. Thus overall it can be concluded that various vigour tests together can be used to predict quality and vigour of the varieties of the seed lots and can be used to distinguish parents and hybrid of cotton.

Hormonal and biochemical Studies in Cotton

- Hormonal treatment with gibberellic acid, kinetin and ethylene (10^{-4} to 10^{-6} M.) through direct germination, seed presoaking and foliar spray methods
- Various metabolic and enzymatic studies were carried on to differentiate hybrids and parents of cotton.

Based on Hormonal and biochemical studies it was concluded that

- Differences were observed in different cultivars,in terms of growth parameters, but superiority of any one of the variety among parents and hybrids was not marked in any particular treatment in field, however laboratory studies along with other markers can be helpful in distinguishing parents and hybrids.
- Biochemical analysis of protein and metabolites alone cannot be considered as an important tool for discriminating parents and hybrids, but if standardized can be used as an important supplement to other market tests.

Based on TLC and Tissue Culture studies it was concluded that

TLC run showed some spots on plates.

Similarly tissue culture showed development of callus, plant and multiple shoot but clearly did not prove to be good tool for distinguishing parents and hybrids of cotton.

Molecular Studies in Cotton on the direct genome products i.e. seed proteins and isozymes were studied through electrophoresis. Seed protein studied through vertical gel electrophoresis were: salt soluble, prolamin, cross-linked prolamin, glutelin-like and glutelins. PCR based RAPD studies were conducted from DNA extract of leaves of parents and hybrids and using 6 primers.

Based on Molecular Studies it was evident that Electrophoresis and RAPD-PCR, showed differential banding patterns in the intensities of bands and proved to be very important tool to discriminate cultivars.

Studies conducted on Hybrids and Parents of Castor (*Ricinus communis* L.)

Following hybrids and their parents were

Male	Hybrid	Female
VI-9	GAUCH-1	VP-1
JI-35	GCH-2	VP-1
48-1	GCH-4	VP-1
SH-72	GCH-5	Geeta G
JP-65	GCH-6	JI-96

selected for studies in case of Castor:

Morpho-physiological markers studied in Castor were:

Morphological and Physiological Studies viz. Anthocyanin pigmentation at 14 days stage in laboratory, bloom, leaf shape, pigmentation, etc. at 21 days stage in field. Grow out Test of commercial hybrid lots at 30 days and 80 days stage.

Based on Morpho-physiological markers it was concluded that

- Genetic purity can be reliably estimated using morpho-physiological markers.
- Remarkable anthocyanin pigmentation was observed in the 14-days stage, which proved to be a key of differentiating hybrids from parents.
- Differential pattern of bloom proved to be a significant character that could be used singly for differentiating castor hybrids and parents.
- Commercial hybrid seed lots analysis showed that modified Grow-out tests can be applied at commercial scale.
- The Grow out Test proved to be the most important parameter in differentiating hybrids and parents at 30-days stage. Results of female parent and hybrid in the field correlated with the laboratory germination data.

Biochemical Studies in Castor

- Various germination tests, Leachate analysis through electro conductance,soluble sugar, soluble protein, inorganic phosphates, phenols, etc.
- Tetrazolium test and dehydrogenase activity,seed moisture content, seed weight, seed volume, etc.
- Various metabolic and enzymatic studies were carried on to differentiate hybrids and parents of castor.
- Hormonal treatment with gibberellic acid, kinetin and ethylene (10^{-4} to 10^{-6}M conc.) through direct germination, seed presoaking and foliar spray methods.
- Seed protein studies through vertical gel electrophoresis were: salt soluble, prolamin, cross-linked prolamin, glutelin-like and glutelins.
- 3 isozymes studied through horizontal gel electrophoresis were: Esterase, Glutamate

oxaloacetate transaminase and Peroxidase.

Based on Biochemical Studies it was concluded that

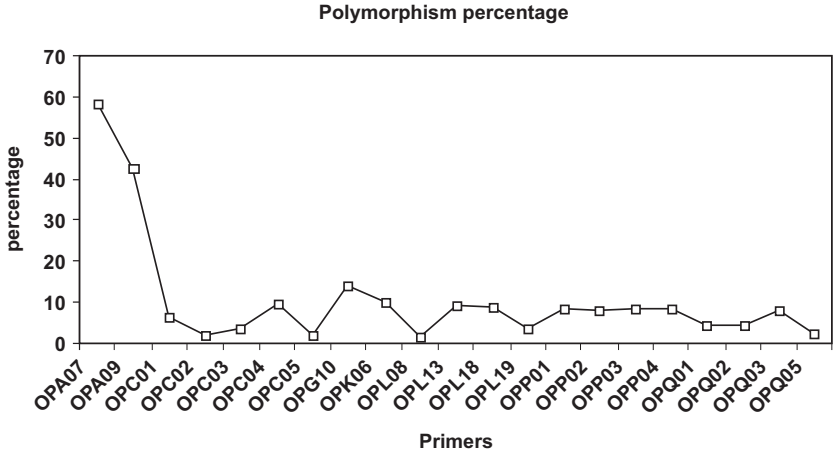
- Varietal differences were observed in respect of biochemical parameters such as Electro conductance and leachate study.
- Dehydrogenase activity helped in differentiating hybrids and parents in some cases.
- Biochemical analysis of metabolically active enzymes and electrophoresis of soluble seed proteins as well as isozymes provided quick clue for genotypic identification.
- Exogenous application of hormone do induce significant differences among hybrid and parents but seedlings cannot be identified confidently. The effects of PGRs depends on many factors extrinsic, intrinsic, plant age, etc. the requirement and response of plants also varies and therefore results of the applied PGRs also varied.
- Hence, hormones treatment alone cannot be used, but with other markers they can be used as important supplement to them to mark distinction between hybrids and parents.

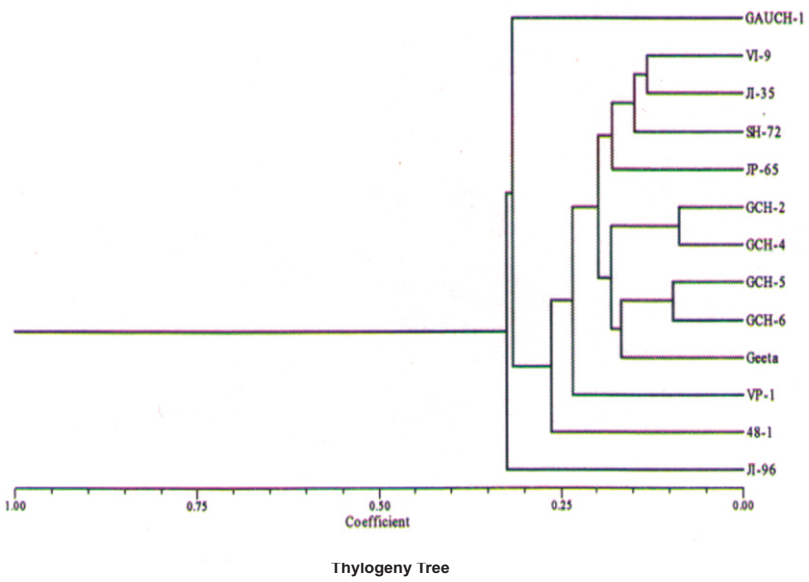
Molecular and Tissue Culture Studies in Castor

- PCR based RAPD studies were conducted from DNA extract of leaves of parents and hybrids and using 21 primers which yielded polymorphic products resolvable by gel electro-phoresis.
- Castor hybrids and parents seeds were used as explants for tissue culture studies.

Based on Molecular and Tissue Culture Studies it can be concluded that

- The RAPD analysis showed clear banding patterns but the ratio of polymorphic bands as compared to monomorphic bands was very less.
- The phylogeny tree was developed on the basis of presence and absence of amplified DNA bands in the gel, using Jaccard's similarity/dissimilarity matrix and UPGMA cluster analysis.
- The phylogenetic tree showed that all hybrids were in closer proximity to each other in comparison to their nearness of their parents.
- Though RAPDs are time saving and cost effective technique as compared to Grow out tests for genetic purity testing of seed lots which is not as reliable as it is based on a dominant marker and the results are not reproducible.
- Tissue culture studies are time consuming and comparatively tougher method for varietal identification as it takes more time for differentiating hybrids and parents as compared to the modified grow out tests.
- Thus Tissue culture techniques too can be





used for varietal differentiation but not single handedly.

Polymorphism Percentage in all primers

Finally it can be concluded based on above studies that:-

- No single parameter can be used independently for quicker identification of hybrids and parents and
- Morpho-physiological para-meters need more critical confirmation under varying environmental conditions; while molecular studies are useful but requires costly equipments and technical expertise.

I would like to thank all my former researchers in this area especially Dr. (Ms) Shaista Halim, Dr. (Ms) Chitra Shukla and Dr. (Ms) Avni Bhatt for their dedicated and sincere studies on pearl millet, cotton and castor respectively as well as other crops. I thank Dr. Santoshkumar Singh and Dr. (Ms) Zankhana Rathod for help in various ways in the preparation of this lecture.

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