SUMMARY
AND
CONCLUSIONS
Castor (*Ricinus communis L.*) is a non-edible oilseed, cultivated around the world because of the commercial importance of its oil. India is the world leader in castor production and dominates the international castor oil trade. Advent of high yielding hybrids in castor crop has brought improvement and has boosted the yields significantly. Hybrid production is quite an error prone process. The castor hybrids production involves male sterile lines, which have been found to bear viable pollens and allow lot of self-seed settings. The manual emasculation, although is done by skilled labourers but always leaves enough viable pollen to allow self-pollination.

Therefore, the hybrid lots often bear mixture of parental genotypes among themselves. To decrease this mixing and to make the process of differentiating hybrids and parents at early stage faster the present work was taken under. The aim of the present work was to differentiate the hybrids and their parents through physical, physiological, biochemical and molecular parameters. With this in view the detailed study of hybrids and parents of castor was undertaken.

I: MORPHOLOGICAL STUDIES UNDER LABORATORY AND FIELD CONDITIONS

Physiological parameters like anthocyanin pigmentation and root volume at the 14-d stage in laboratory provided a little bit of help in differentiating hybrids and genotypes. Physiological studies at 14 d stage showed superiority of two hybrids GCH-4 and GCH-2, in terms of longer root length, shoot length and corresponding heavy plant parts. Hybrid GCH-5 showed poorest performance in terms of physiological parameters studied.

Physiological studies at 21-d stage in field condition showed over all superiority of hybrid GCH-4 considering all parameters studied. The 21-d old seedling grown in field exhibited prominent differences in hybrids and their parents of castor using characters like seedling pigmentation, bloom, leaf shape, internode and petiole length. On the basis of the morphological findings observed under grow out test to differentiate commercial hybrid lots of castor, similar results were obtained. The similarity of observations between authentic hybrids and commercial ones show that the Grow Out Test which is capable of identifying plants at 21d stage against 50-day, is highly reliable as the characters chosen are entirely genetically influenced and have
got enough potential to separate the desired genotype even among the commercial hybrid lots whose constituents generally are not known.

II: VIGOUR STUDIES
In an attempt to identify hybrids and their parents, seeds were subjected to various vigour tests like standard germination tests, electroconductivity and leachate analysis. All the hybrids proved the superiority of hybrids over their parents. Though dehydrogenase activity, electro conductivity along with leachate analysis could discriminate responses of hybrids and parents in decreasing order, but the differences were not found to be large enough to identify hybrids and parents distinctly. However it can be taken as a rough guide to differentiate hybrids and parents of castor. Thus vigour provided a crude physiological method for discrimination of hybrids and parents as it depends on various environmental factors and maturity of seeds besides genetic factors.

III: BIOCHEMICAL AND METABOLIC CHANGES
The hybrids and parents were tried to be differentiate using various biochemical changes. Biochemical parameters are useful parameters to differentiated parents and hybrids in different cultivars. But since the magnitude of the differences in the changes in metabolic and enzyme activities sometimes are very less and hard to be measured, then the discrimination becomes difficult. The biochemical changes in metabolites and enzymes did not give a perfect trend for checking viability and characterization of any genotypes. Sugars gave a mixed performance in all genotypes and thus could not be used a single parameter to differentiate hybrid and its parents. Moreover total protein and fraction protein also did not give proper results that can be used to differentiate hybrids and parents at an early stage.

Enzyme activities were analyzed at 48, 72 and 96h stages as cytoplasmic and wall bound fractions but they did not give good results. Castor hybrids showed different levels of enzymatic activities in comparison to their parents. All hybrids showed better metabolic machinery in terms of enzymes playing a major role such as peroxidase, IAA- oxidase and invertase. The various enzyme activities studied suggest that these activities can be used for varietal identification as one of the parameters but cannot be used single handedly. From the results obtained it can be
suggested that peroxidase and IAA oxidase enzymes can be used in differentiating hybrids and parents to some extent. The other enzymes gave very unstable results.

Thus the biochemical estimations provided simple and quick method for identification of hybrids and parents, which is a crude identifying index. The method cannot be taken as reliable one for varietal identification as it depends on seed vigour, seed maturity and post harvest history of the seed.

IV: ELECTROPHORESIS STUDIES OF PROTEIN AND ISOZYMES

Gel electrophoresis of seed proteins can be done in laboratory in a relatively short time and hence has been used a marker for varietal differentiation. Castor hybrids and parents presented with quite differential patterns for soluble seed proteins. The biochemical fingerprinting of proteins and fraction proteins provides a method as well as clue for identification of hybrids and parents of castor.

The isozymes of esterase, peroxidase and Glutamate Oxaloacetate Transaminase were examined in 7 days old seedlings in 10% polyacrilamide gels. In the present context any of the isozyme, showing variations in band spectrum, can be used, but combination of various variants always improves the rate of accuracy. Therefore, a single banding pattern of esterases or peroxidases cannot be used to differentiate hybrid and parents. But it certainly provided highly useful and accurate clues for identification of hybrids and parents of castor. Thus overall electrophoretic methods provided quick, simple and working clues for identification purposes.

V: PLANT GROWTH REGULATORS STUDIES

Hybrids were found to be more sensitive to exogenously applied PGRs in comparison to their parents. The differential responses of hybrids and parents in terms of root-shoot lengths, their weights including leaf were found to be highly useful in differentiating hybrids and their parents at a particular stage. Hybrids and parents of castor had specific requirements for their physiological performances; however, hormones did not alter any morphological character such as bloom, pigmentation, leaf size and shape.
Hormonal screening thus provides a comparatively less reliable method for identification of hybrids and parents as their responses depend on several physiological features as well as history of seed. Therefore individual seedling cannot be identified by their hormonal responses. The hormonal screening can be a possible technique for identification of hybrids and parents mainly due to their mutual comparability, but to achieve this, physical factors such as light and temperature must be controlled strictly. The dependence of varietal screening on many factors make this technique of limited importance in varietal discrimination.

VI: RAPD STUDIES

RAPD studies was carried out to discriminate the hybrids and parents of castor using more recent and very advanced molecular technique, which included study of polymorphism at nucleotide level. Seed genomic DNA for developing RAPD fingerprints based on arbitrarily primed PCR reaction was used to test genetic purity testing of seed lots for seed certification. In this study we employed 21 specific or non specific primers of known length of oligonucleotide.

The reliability of these molecular techniques is marked in the field of genetics. Thus, RAPD-PCR, molecular study techniques are well-documented techniques in cultivar identification and phylogenetic studies. Moreover, it can be concluded that sharp bands are naturally different from faint bands and results show that using total bands for cluster analysis is better than using just sharp bands. The results suggest that RAPD analysis can be effectively used for the characterization and discrimination of parents and hybrid of castor or any closely related or distant species/varieties of same species. They are simple and quick markers but cannot be applied to large samples due to its tendency of non reproducing the same bands again. Moreover it requires trained technicians and a well-equipped laboratory also.

VII: TISSUE CULTURE STUDIES

Hybrids and parents of castor showed considerable variation in their culturability, morphology and biochemical analysis of calli, which was found to be highly useful for their identification. Thus the differential culturability and regenerating capacity of hybrids and parents provided a remarkable identifying index for their discrimination.
It is an expensive, 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.

The projection of present work could also be useful to seed certification agencies in identification of hybrids and parents using combination of various parameters for certification purposes. The certification agencies conduct grow out tests for checking genetic purity of hybrids, and the seed lots falling below the standards are rejected. The Grow Out Tests, especially in castor are long, tedious and expensive procedures and it identifies the genotypes on the basis of their floral traits which apparently take up long durations, crop maintenance and labour charges. Thus the morphological, biochemical, molecular and the related findings of the study can be directly used by certification agencies and commercial hybrid growers as the studies done herewith do not involve heavy expenditure and long duration which is after all highly important for a technique to be qualified as standard genetic purity test.

Based on the above findings following prominent features of the total work done can be drawn:

1. Genetic purity can be reliably estimated using physio-morphological characters. Remarkable anthocyanin pigmentation was also observed in the 14-day stage, which proved to be a key of differentiating hybrids from parents. As a general observation anthocyanin pigmentation was found more in female parent followed by hybrid and male parent in major cases. Castor seedlings can be identified at 21-day stage in field conditions.

2. Differential pattern of the bloom proved to be a significant character that could be used singly for discrimination of castor hybrids and parents.

3. The wide morphological screening of hybrids and parents of castor provided a set of morphological parameters for differentiating hybrids from parents although some of the parameters alone can be used, but combining of all the significant features appeared to be important for attaining maximum.
4. Commercial hybrid seed lots analysis shows that modified grow out tests can be applied at commercial scale. The Grow Out Test provided as the most important parameter in differentiating hybrids and parents at 30-days stage. Results of female and hybrids in the field correlated with the laboratory germination tests.

5. Varietal differences were also observed in respect of electro conductance (EC) and leachate study. E.C. provided direct information about the seed quality. Dehydrogenase activity in some cases helped in differentiating between hybrids and parents.

6. Biochemical analysis of metabolically active enzymes and electrophoresis of soluble seed proteins as well as isozymes provide quick clue for genotypic identification.

7. Electrophoresis of seed proteins gave differential band patterns with respect to hybrids and parents. The number of bands as well as band intensities differs with genotype. Differential banding patterns of isozymes provide helpful indices for cultivar differentiation.

8. Exogenous application of hormones 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.

9. 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.

10. 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.

11. Though RAPDs are time saving and cost effective technique as compared to Grow Out tests for genetic purity testing of seed lots it is not a reliable as it a dominant marker and the results are not reproducible.

12. 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.

Thus from the above studies conducted, it can be concluded that no single parameter can be used independently for quicker identification of hybrids and parents of castor. Moreover, it can be concluded that for testing of genetic purity at an early stage, biochemical markers are more reliable than molecular markers.