

**Final work done Report**

**UGC SPONSORED MAJOR RESEARCH PROJECT**

**F No. 40-40 / 2011 (SR) Dt 29-6-2011**

From

01-07-2011 to 31-12-2014

On

**Physiological and molecular characterization of root traits,  
Wilt and Charcoal rot resistance in Castor (*Ricinus communis* L)**

**Submitted by**

**Principal Investigator: Dr Ramesh Thatikunta**

**Professor**

**Department of Crop Physiology, College of Agriculture**

**Professor Jayashankar Telangana State Agricultural University (PJ TSAU)**

**Rajendranagar, Hyderabad, Telangana 500030**

Annexure -III  
 UNIVERSITY GRANTS COMMISSION  
 BAHADUR SHAH ZAFAR MARG  
 NEW DELHI – 110 002.

**FINAL REPORT OF THE WORK DONE ON THE MAJOR RESEARCH PROJECT**

1. Project report No. 1st/2nd/3rd/Final : Final
2. UGC Reference No. F 40-40 / 2011(SR) Dated 29-6-2011 (MRP)
3. Period of report: From 01-07-2011 to 31-12-2014
4. Title of research project : Physiological and molecular characterization of root traits, wilt and charcoal rot resistance in castor (*Ricinus communis* L.)
5. (a) Name of the Principal Investigator : Ramesh Thatikunta  
 (b) Dept. and University / College where work has progressed  
 Department of Crop Physiology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad 500030
6. Effective date of starting of the project 04-11-2011
7. Grant approved and expenditure incurred during the period of the report: Rs. 10,51,800/-
  - a. Total amount approved Rs. 5,70,300/-
  - b. Total expenditure Rs. 5,48,517/-
  - c. Report of the work done
    - i. Brief objective of the project : four objectives
      1. To identify germplasm lines for deep root mining abilities
      2. To validate Carbon (<sup>13</sup>C) and Oxygen isotope (<sup>18</sup>O) values with identified tolerant lines
      3. To study molecular diversity with SSR markers of selected germplasm lines
      4. To evaluate identified germplasm for yield
    - ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication) Separate sheet enclosed

iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons

Yes. The progress has been according to the original plan of work

iv. Please indicate the difficulties, if any, experienced in implementing the project

No difficulties have been encountered during the implementation of the project

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet

No, the project has been completed.

vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission.

Yes, final report of the work done is attached and being sent

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

a. Manpower trained

1. Project Associate, One M.Sc. and One Ph.D student has been trained to take data on crop plants, carry out data analysis, interpret the data, work with the root study structure. Details are given below

S No.	Name of the student and ID No.	Title of thesis	Year of submission
1	L Sagarika RAM/2010-61	Physiological and molecular characterization of root traits related to water use efficiency in genotypes of castor	2014

b. Ph. D. awarded: One. Work involved crossing of two parents with contrasting characters to generate mapping population, their molecular characterization along with phenotyping for root traits. Details are given below

S No.	ID No.	Name of the student	Title of thesis	Year of submission
1	RAD/ 2012-23	P Gouthami	Phenotyping and genotyping studies for physiological traits in castor ( <i>Ricinus communis</i> L.)	2019

c. Publication of results: Six papers have been published. Details are given below

1. Ramesh Thatikunta, A Siva Sankar, J Sree Lakshmi, Ch V Durga Rani, V Gouri Shankar, Gouthami Palle, C Leela and P Narayan Reddy. 2014. Genetic Divergence Studies for Yield components and Root Traits in Castor (*Ricinus communis* L.). Indian Journal of Plant Genetic Resources, 27(3): 271-276. DOI 10.5958/0976-1926.2014.00025.4. NAAS RATING: 5.12
2. Ramesh Thatikunta, A Siva Sankar, Gouthami Palle, J Sreelakshmi, C Leela, Ch V Durga Rani, V Gouri Shankar, P Narayan Reddy and MHV Bhawe. 2014. Research Article Genetic diversity analysis using shoot and root morphological markers in castor (*Ricinus communis* L.). Electronic Journal of Plant Breeding, 5(4): 695-701 ISSN 0975-928X. NAAS RATING:4.19
3. Ramesh Thatikunta, A. Siva Sankar, J. Sreelakshmi, Gouthami Palle, C. Leela, Ch. V. Durga Rani, V. Gouri Shankar, B. Lavanya, P. Narayana Reddy and M. Y. Dudhe. 2016. Utilization of in silico EST–SSR markers for diversity studies in castor (*Ricinus communis* L.). Physiology and Molecular Biology of Plants. 22(4):535–545. DOI 10.1007/s12298-016-0367-x. NAAS RATING: 7.35
4. Ramesh Thatikunta, A Siva Sankar, L Sagarika, Gouthami Palle, J Sreelakshmi, V Gouri Shankar, Ch V Durga Rani, and B Lavanya. 2016. Identification of genotypes for high water use efficiency and root traits in castor (*Ricinus communis* L.). Journal of Oilseeds Research. 33 (1): 87-90. NAAS RATING: 3.97
5. Gouthami Palle, Ramesh Thatikunta, S Narender Reddy, CH Durga Rani and V Gouri Shankar. 2018. Genetic Variability Studies for WUE Related Traits in F2 Mapping Population of Castor (*Ricinus communis* L.). 2018. International Journal of Current Microbiology and Applied Sciences. 7 (09):1809-1816 NAAS RATING: 5.38
6. Gouthami Palle, Ramesh Thatikunta, Narender Reddy S, Durga Rani Ch. V and Gouri Shankar V. 2018. Utilization of est-ssr marker for the generation of additional descriptor for the identification of elite material in castor (*Ricinus communis* L.). The Journal of Research PJTSAU. 46 (2&3) 89-94. NAAS RATING: 3.35

Data presented in the national conference & two regional workshops. Details are given below

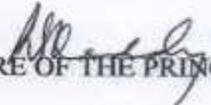
Sl no	Title of the paper	Presented in workshop / conference	Date and place
1	Identification of genotypes for high WUE and desirable root traits through genetic diversity analysis in castor ( <i>Ricinus communis</i> L.)	Frontiers of Plant Physiology Research : Food Security and Environmental Challenges	23-11-2014 to 25-11-2014, OUAT, Bhubaneswar
2	Genetic divergence studies with accessions of castor ( <i>Ricinus communis</i> L.)	Physiological and Molecular Interventions for Improving Crop Productivity	23-1-2014, Bapatla ISPP South Zonal Seminar
3	Identification of genotypes for high water use efficiency and root traits in castor ( <i>Ricinus communis</i> L.)	Crop Physiology – Emerging Challenges and Opportunities for Sustainable Agriculture	03-03-2015, Tirupati ISPP South Zonal Seminar

(d) other impact (Achievements from the project)

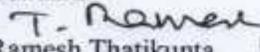
- 1. Identified the germplasm lines suitable for dry land situations
- 2. Identified contrasting parents viz., PCS 106 and PCS 245. These were crossed to develop mapping populations
- 3. Constructed a temporary root study structure which could be used for phenotyping of Root System Architectural (RSA) Traits

CONTRIBUTION TO THE SOCIETY (GIVE DETAILS)

A temporary root study structure constructed is under utilization with two other Departments of the College viz., Soil Science and Agricultural Chemistry and Agronomy

  
SIGNATURE OF THE PRINCIPAL

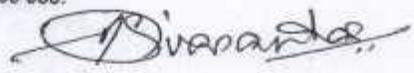
INVESTIGATORS

  
Dr Ramesh Thatikunta 13/11/14

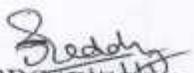
Dr. T. RAMESH  
PROFESSOR  
DEPARTMENT OF CROP PHYSIOLOGY

SIGNATURE OF THE COINVESTIGATOR

Dr A Siva sankar

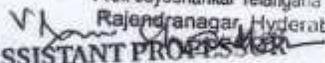


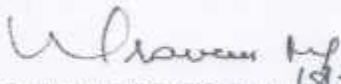
Dr P Narayan Reddy

  
PROFESSOR

Dept. of Plant Pathology  
College of Agriculture  
Prof. Jayashankar Telangana State Agril. University  
Rajendranagar, Hyderabad-500 030. T.S.

Dr V Gouri Shankar

  
ASSISTANT PROFESSOR  
Dept. of Genetics & Plant Breeding  
College of Agriculture, Prof. Jayashankar  
Telangana State Agril. University  
Rajendranagar, Hyderabad-500 030. T.S.

  
REGISTRAR/PRINCIPAL  
P.J.T.S. Agriculture Jn  
Rajendranagar 500 030

Annexure - IV  
UNIVERSITY GRANTS COMMISSION  
BAHADUR SHAH ZAFAR MARG  
NEW DELHI - 110 002

UTILIZATION CERTIFICATE

Certified that the grant of Rs 5,48,517 (Rupees five lakhs forty eight thousand five hundred and seventeen only) out of Rs 5,70,300 (Rupees five lakhs seventy thousand and three hundred only) sanctioned to Dr Ramesh Thatikunta, Professor Department of Crop Physiology, ANGRAU, Rajendranagar campus by the University Grants Commission vide their letter number UGC Ir no F 40-40 / 2011 (SR) and dated 29-6-2011 has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the Commission.

The university has so far not utilized the left over UGC grant amount of Rs 21, 783/- and interest obtained on UGC grant of Rs 14,811/- totaling to Rs 36,594/- (Rupees thirty six thousand five hundred and ninety four only). If as a result of check or audit objection, some irregularity is noticed at a later stage, action will be taken to refund, adjust or regularize the objected amount.

*[Signature]*  
SIGNATURE OF THE PRINCIPAL

INVESTIGATORS

*T. Ramesh*  
Dr Ramesh Thatikunta

*[Signature]*  
Dr. T. RAMESH  
PROFESSOR  
DEPARTMENT OF CROP PHYSIOLOGY  
COLLEGE OF AGRICULTURE  
RAJENDRANAGAR, HYDERABAD - 500 030.



SIGNATURE OF THE COINVESTIGATOR

Dr A Siva sankar

*[Signature]*

Dr P Narayan Reddy

*[Signature]*  
PROFESSOR  
Dept. of Plant Pathology  
College of Agriculture  
Prof. Jayashankar Telangana State Agril. University  
Rajendranagar, Hyderabad-500 030. T.S.

Dr V Gouri Shankar

*[Signature]*  
ASSISTANT PROFESSOR  
Dept. of Genetics & Plant Breeding

REGISTRAR / PRINCIPAL STAUTORY AUDITOR

*[Signature]*  
COMPTROLLER  
Prof. Jayashankar

Telangana State Agricultural University  
Rajendranagar, Hyderabad-30

*[Handwritten initials]*  
11/5/2015  
*[Handwritten initials]*  
11/5/15

*[Signature]*  
REGISTRAR  
P.J.T.S. Agricultural University  
Rajendranagar, Hyderabad-30 T.S.

Annexure - V  
 UNIVERSITY GRANTS COMMISSION  
 BAHADUR SHAH ZAFAR MARG, NEW DELHI – 110 002  
 STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR RESEARCH PROJECT

1. Name of Principal Investigator Ramesh Thatikunta  
 2. Deptt. of University/College : College of Agriculture, PJTSAU, Rajendranagar  
 3. UGC approval No. and Date F 40-40 / 2011(SR) Dated 29-6-2011(MRP)  
 4. Title of the Research Project Physiological and molecular characterization of root traits, wilt and charcoal rot resistance in castor (*Ricinus communis* L.)  
 5. Effective date of starting the project 04-11-2011  
 6. a. Period of Expenditure: From 1-7-2011 to 31-12-2014

b. Details of Expenditure \_\_\_\_\_

S No.	Items	Amount approved Rs	Expenditure incurred so far
1	Books & Journals	--	--
2	Equipment	--	--
3	Contingency	276000	259151
4	Field Work/Travel	15270	13434
5	Chemicals & Glassware	130000	129454
6	Hiring Services	28000	28000
7	Overhead	88000	57030
8	Any other items (Please specify)	--	--
9	Honorarium to Principal Investigator (from – to )	--	--
10	Staff date of appointment (from 1-12-2011 to 30-9-2012) annexure IX as per XI plan guidelines of Major Research Project)	80000	80000
	Total	570300	567069
	Unspent amount received from executive engineer, ANGRAU		18552
	Total expenditure (567069 – 18552)		548517
	Amount remaining (570300 - 548517)		21783
	Bank Interest		14811
	Total returned as DD to UGC Rs 21783 + 14811		36594

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.
2. It as a result of check or audit objective, some irregularly is noticed, later date, action will be taken to refund, adjust or regularize the objected amounts.
3. Payment @ revised rates shall be made with arrears on the availability of additional funds.

4 It is certified that the grant of Rs 5,48,517 (Rupees five lakhs forty eight thousand five hundred and seventeen only) received from the University Grants Commission under the scheme of support for major research project entitled on "Physiological and molecular characterization of root traits, wilt and char coal rot resistance in castor (*Ricinus communis* L)" vide UGC Ir no F 40-40 / 2011 (SR) Dated 29-6-2011 out of an sanctioned amount of Rs 5,70,300 (Rupees five lakhs seventy thousand and three hundred only) has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

The university has so far not utilized the left over UGC grant amount of Rs 21, 783/- and interest obtained on UGC grant of Rs 14,811/- totaling to Rs 36,594/- (Rupees thirty six thousand five hundred and ninety four only). If as a result of check or audit objection, some irregularity is noticed at a later stage, action will be taken to refund, adjust or regularize the objected amount.

*T. Ramesh Babu*  
 Principal Investigator  
 (Signature with seal)  
 Dr. T. RAMESH  
 PROFESSOR  
 DEPARTMENT OF DROOP PHYSIOLOGY  
 COLLEGE OF AGRICULTURE  
 RAJENDRANAGAR, HYDERABAD - 500 030.  
 SIGNATURE OF PRINCIPAL INVESTIGATOR

Registrar  
 (Signature with seal)  
 P.J.T.S. Agricultural University  
 Rajendranagar, Hyderabad-30 T.S.

*Reddy*  
 PROFESSOR  
 Dept. of Plant Pathology  
 College of Agriculture  
 Prof. Jayashanker Telangana State Agri. University  
 Rajendranagar, Hyderabad-500 030. T.S.

*V. Sankar*  
 ASSISTANT PROFESSOR  
 Dept. of Genetics & Plant E  
 College of Agriculture, Rajen  
 Rajendranagar, Telangana State Ag  
 Hyderabad-500 030. Telang

SIGNATURE OF THE COINVESTIGATOR

*A. Sankar*  
 Dr. A. SIVA SANKAR  
 Associate Professor  
 Department Of Crop Physiology  
 Acharya N G Ranga Agricultural University  
 College Of Agriculture  
 Rajendranagar, Hyderabad - 500 030.)

REGISTRAR/PRINCIPAL

*W. Sankar Babu*  
 REGISTRAR 18/5/14  
 P.J.T.S. Agricultural University  
 Rajendranagar, Hyderabad-30 T.S.

## Annexure-IX

PROFORMA FOR SUPPLYING THE INFORMATION IN RESPECT OF THE STAFF  
APPOINTED UNDER THE SCHEME OF MAJOR RESEARCH PROJECT

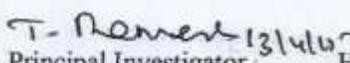
UGC FILE NO F 40-40 / 2011(SR) Dated 29-6-2011 (MRP)  
YEAR OF COMMENCEMENT : 2011

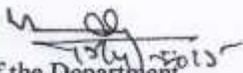
TITLE OF THE PROJECT: Physiological and molecular characterization of root triats, wilt and charcoal rot resistance in castor (*Ricinus communis* L.)

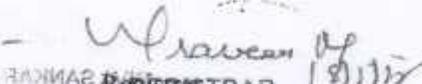
1	Name of the Principal Investigator	Ramesh Thatikunta, Professor				
2	Name of the university / college	Professor Jayashankar Telangana State Agricultural University (PJTSAU), College of Agriculture, Rajendranagar				
3	Name of the research personnel appointed	Miss J Srilakshmi				
4	Academic qualifications	S No	Qualifications	Year	Marks	% age
		1	M Sc (Biotechnology)	2009	8.9 CGPA	89
		2	M Phil	--	--	--
		3	Ph D	--	--	--
5	Date of joining	1-12-2011				
6	Date of birth of research personnel	15-6-1987				
7	Amount of HRA drawn	Not drawn				
8	Number of candidates applied for the post	Three				

CERTIFICATE

This is to certify that all the rules and regulations of UGC Major Research Project outlined in the guidelines have been followed. Any lapse on the part of the university will be liable to terminate the said project.

  
 Principal Investigator  
 Dr. T. RAMESH  
 PROFESSOR  
 DEPARTMENT OF CROP PHYSIOLOGY  
 COLLEGE OF AGRICULTURE  
 RAJENDRANAGAR, HYDERABAD - 500 030.

  
 Head of the Department

  
 REGISTRAR  
 P.J.T.S. Agricultural Uni  
 Rajendranagar, Hyderabad

PROFESSOR & HEAD  
 DEPARTMENT OF CROP PHYSIOLOGY  
 COLLEGE OF AGRICULTURE  
 RAJENDRANAGAR, HYDERABAD - 500 030.

## **Work done so far and results achieved in UGC Major Research Project**

The work done in the project titled “Physiological and molecular characterization of root traits, wilt and charcoal rot resistance in castor (*Ricinus communis* L.)” has been carried out as an interdisciplinary approach. The details of carried out has been given separately for each of the four objectives listed below.

1. To identify germplasm lines for deep root mining abilities, wilt and charcoal rot resistance
2. To validate Carbon Isotope Discrimination (CID) values ( $^{13}\text{C}$ ) values with identified tolerant lines
3. To study molecular diversity with SSR markers of selected germplasm lines
4. To evaluate identified germplasm for yield.

**1<sup>st</sup> objective: To identify germplasm lines for deep root mining abilities, wilt and charcoal rot resistance**

### **Introduction**

Castor a non edible oilseed crop is cultivated in tropical and sub tropical regions of the world. Under rainfed situations the crop yields are low. Genetic enhancement programmes aim at improvement in seed yield, oil content and tolerance to biotic and abiotic stresses. For crop improvement, identification of variability among the genotypes and those possessing superior traits is a research priority. Measurement of relevant crop physiological responses can lead to further insights into various aspects of crop growth and development. Therefore, the present study was taken up to identify genotypes with superior shoot and root system architectural (RSA) traits that contribute to high water use efficiency and seed yield coupled with wilt and charcoal rot resistance.

### **Materials and methods**

Thirty five accessions were planted in Randomized block design in a specially designed temporary root study structure 25 m length, 4m width and 1.5 m height with a permanent wall separating the replications to enable root studies (Fig.). Crop was raised with two replications

with spacing of 90 x 45 cm during late *Rabi* 2011 and 2012. Observations were recorded on four plants in each replication for 15 quantitative traits. Data were recorded on standing crop with respect to SPAD chlorophyll meter readings (SCMR) in the morning (8.00 to 9.30 hours) with the help of hand held Minolta SPAD chlorophyll meter (Minolta Corp., Ramsey, New Jersey, USA), relative water content (RWC) using the formula (fresh weight – dry weight / turgid weight – dry weight) x 100, PS II efficiency (Fv/Fm), plant height up to primary raceme, number of nodes up to primary raceme, effective length of main spike, 100-seed weight and seed yield. Structure was dismantled 110 DAS when root growth was maximum and data recorded on RSA traits viz., root diameter at crown region, root length, number of laterals, total root length, root dry weight and total dry weight.



Fig: Inauguration of the temporary root structure and the completed structure

### Results obtained

The data obtained on fifteen quantitative traits with respect to the variation in morphological (Table 1) and root traits (Table 2) which correlate with Water Use Efficiency and their contribution to seed yield in castor genotypes is presented. In the present study eight characters viz., greening index ( $>49$ ), SLA ( $<2.64 \text{ dm}^{-2} \text{ g}^{-1}$ ), TDM ( $>314.5 \text{ g}$ ), root length ( $>192.6 \text{ cm}$ ), root volume ( $>249 \text{ cm}^3$ ), extrinsic WUE ( $>8 \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ ) and intrinsic WUE ( $>45 \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ ) and CID ( $<18.01 \text{ \%o}$ ) have been considered for selection of genotypes with superior WUE traits combined with seed yield have been outlined in the present study. Six genotypes showed at least two superior characters. Three advanced breeding lines with superior characters included PCS 171 with high tap root length, root volume, seed yield; RG 48 with high tap root, root volume, greening index, low SLA, total dry matter, seed yield and SKI 215 with high tap root, root volume, greening index, total dry matter and seed yield (Table 1).

**Table1. Castor genotypes with better root and shoot characters - summary table**

Sl no	Character	Value	Superior genotype
1	Extrinsic WUE (A E <sup>-1</sup> )	>8	PCS 320, RG 67, JP 65, M 574 and Kiran
2	Intrinsic WUE (A g <sub>s</sub> <sup>-1</sup> )	>45	PCS 320, PPL 109, M 574, PCS 265 and PCS 106
3	Greening index	>49	RG-48, Kranthi, PCS-230, SKI-215 and Haritha
4	Low specific leaf area (dm <sup>-2</sup> g)	< 2.64	RG-48, Kranthi, PCS-230, SKI-215 and Haritha
5	13C (‰)	<18.01	PCS 330, Kranthi, PCS-230 and PCS-265
6	High tap root length (cm)	>192.6	PCS 324, Haritha , Kranthi, PCS-171, PCS 328, PPL 109, RG-47, RG-48 and SKI-215
7	High root volume (cm <sup>3</sup> )	>249	RG-48, Kranthi, SKI-215, Haritha and PCS-171
8	High total dry matter (g)	>314.5	Kranthi, RG-48, Haritha, SKI-215, PCS-328, RG-47and PCS-324
9	Seed yield (g plant <sup>-1</sup> )	>102	RG-48, Kranthi, SKI-215, Haritha, Kiran and PCS-171

**Table 2. Castor accessions with better shoot and root characters**

High shoot dry weight	> 80g	Haritha, RG 48, RG 47, RG 67, PCS 252
High root dry weight	30g	Kranthi, Haritha, RG 48, PCS 252
High RWC	> 80%	Kranthi, Haritha, RG 48, RG 43
High SCMR	>50	Kranthi, Haritha, RG 48, RG 47, RG 67, PCS 230, SKI 215, PCS 171, , PCS 265, DCS 78, PCS 302
High root diameter	> 8cm	Kranthi, Haritha, PCS 236, PCS 252, SKI 215, PCS 171
More root laterals	> 40	PCS 293, RG 1354, PCS 312, RG 1, PCS 236, PCS 252, SKI 215
High root length	> 180cm	Kranthi, PCS 106, SKI 215
High total root length	> 1000cm	Kranthi, Haritha, RG 48, RG 43, PCS 312, PCS 252, RG 1686 SKI 215, PCS 171

### Materials and methods for disease reaction

The genotypes under study are being characterized for tolerance / resistance to diseases in specially developed plots that are sick for wilt and charcoal rot diseases in RARS, Palem centre, ANGRAU.

**Results :** The summary of disease reaction is presented in the table given below

Sl no	Description	Wilt reaction	Charcoal root rot reaction
1	No of lines evaluated	35	35
2	Resistant lines	13	6
3	Susceptible lines	6	4
4	Medium resistant	4	9

**Diversity index and Principal component analysis:** Realizing the importance of shoot and root characters, ANOVA, Principal Component Analysis (PCA) was carried out to study the variation and extent of relationship between yield and the morphological or root characters.

Shannon and Weaver diversity index was calculated to compare the phenotypic diversity index ( $H'$ ) for morphological characters. The index is generally used in genetic studies to measure both allelic richness and evenness. However, because of log transformation it is not readily interpretable in genetic terms. A low  $H'$  indicates an extremely unbalanced frequency class for an individual trait and a lack of genetic diversity.  $H'$  estimates for fifteen traits showed significant variation (Table 2).  $H'$  ranged from 0.446 to 0.586. High values for  $H'$  were observed for traits like CID, root diameter at crown region, Fv/Fm ratio, SCMR, total root length, effective spike length, shoot dry weight and root dry weight.  $H'$  values were low for plant height, number of laterals and node number. Also very few divergence studies were carried out in castor based on root related characteristics which have direct relevance with the physiological studies for the identification of the water use efficient genotype. Hence, present studies gains importance in understanding the root related traits and their role in quantifying the genotypes in terms of divergence.

**Table 3. Shannon Weaver diversity index of crop characteristics**

Plant traits	$H'$ index
Shoot dry weight (g)	0.547
Root dry weight (g)	0.544
Relative water content (%)	0.524
SCMR	0.570
Fv/Fm ratio	0.580
Plant height (cm)	0.497
Node number	0.443
Effective spike length (cm)	0.556
100 seed weight (g)	0.539
CID (%o)	0.586
Root diameter (cm)	0.584
Number of laterals	0.494
Root length (cm)	0.525
Total root length (cm)	0.568
Seed yield (kg ha <sup>-1</sup> )	0.446
Mean	0.533
SE $\pm$	0.012

PC1 which is the first and most important component accounted for 44.52% variation followed by PC2 with 15.93% and PC3 with 10.54%. Accessions that show high values for various characters were shoot dry weight (> 80g), root dry weight (> 30g), RWC (> 80%), SCMR (> 50), root diameter (> 8cm), number of lateral roots (40), root length (> 180cm) and total root length (>1000cm). At least seven accessions have > 2 common superior traits. Five lines recorded both high shoot and root traits and included Kranthi, Haritha, RG 48, PCS 252 and SKI 215 (Table 3). Based on the analysis of the data involving the castor accessions it can be concluded that nine traits viz., root dry weight, shoot dry weight, root length, total root length, root diameter at crown region, SCMR, effective spike length, node number to primary spike had high weight in the first three PCs indicating their importance for selection in castor improvement.

## **2<sup>nd</sup> objective: To validate Carbon (<sup>13</sup>C) values with identified tolerant lines**

### **Materials and methods**

The actively photosynthesizing leaf of each genotype was selected for study. Leaf was dried and approximately 2 g of sample after removal of veins was sent to the National Facility for estimation of Carbon Isotope Discrimination (CID expressed in per mill (‰) with Isotope Ratio mass Spectrometry (IRMS) in GKVK campus, Bengaluru. Measurement of water used by the crop in field situations is not feasible in all the studies. Alternate approaches to quantify WUE also have gained importance as in this case CID, SLA and greening index (table-4).

### **Results**

PCS-330 showed significantly lower <sup>13</sup>C values (17.49‰) followed by Kranthi (17.88‰), PCS-230 (17.94‰) and PCS-265 (18.01‰). These four genotypes which showed less discrimination are considered to have high WUE trait. Among these genotypes two of them Kranthi and PCS 230 showed low SLA and high greening index (Table 1).

## **3<sup>rd</sup> Objective: To study molecular diversity with SSR markers of selected germplasm lines**

Crop presents a wide variation regarding vegetative traits such as leaf and stem colors, number and size of leaf lobes and presence of wax covering the stem. Most conspicuous

variability is related to reproductive characters, as color shape and size of seeds, number of flowers per raceme, peduncle length and fruit dehiscence. The remarkable phenotypic variation observed in castor bean does not seem to reflect a high genetic diversity. SSR markers available for castor are not sufficient to distinguish the variation when compared to the numbers available in other crops. Hence, EST-SSR markers were used in this study to evaluate the levels of genetic variation among different castor accessions.

### Materials and methods

Leaf material was collected at 30 DAS. DNA was extracted by CTAB method and utilized. A total of 63,852 ESTs available in National Center for Biotechnology Information (NCBI) were downloaded in FASTA format on 30<sup>th</sup> Dec.2012. Total length of EST sequence was 45046231bp with average of 706 space length base pair. All EST sequences were assembled by CAP3 software to seek unigene sequences. These unigene sequences were used to identify microsatellites utilizing SSR Locator. SSRs (motifs ranging from 1 to 10 nucleotides) were searched to evaluate the pattern of EST-SSR distribution. Since, longer SSRs had higher probability of being polymorphic, longest sequence used for SSR identification was 1,361bp. EST assembled and EST - SSR distribution characters is summarized and given in Table.

Feature	Statistics
Total number of EST used	63,852
Average EST length	720 bp
Singleton identified	20,495
Total number of EST SSR identified	1672
Longest sequence used for SSR identification	1361
Total number of microsatellite or EST designed	1107
Primers synthesized	12
Polymorphism identified	2

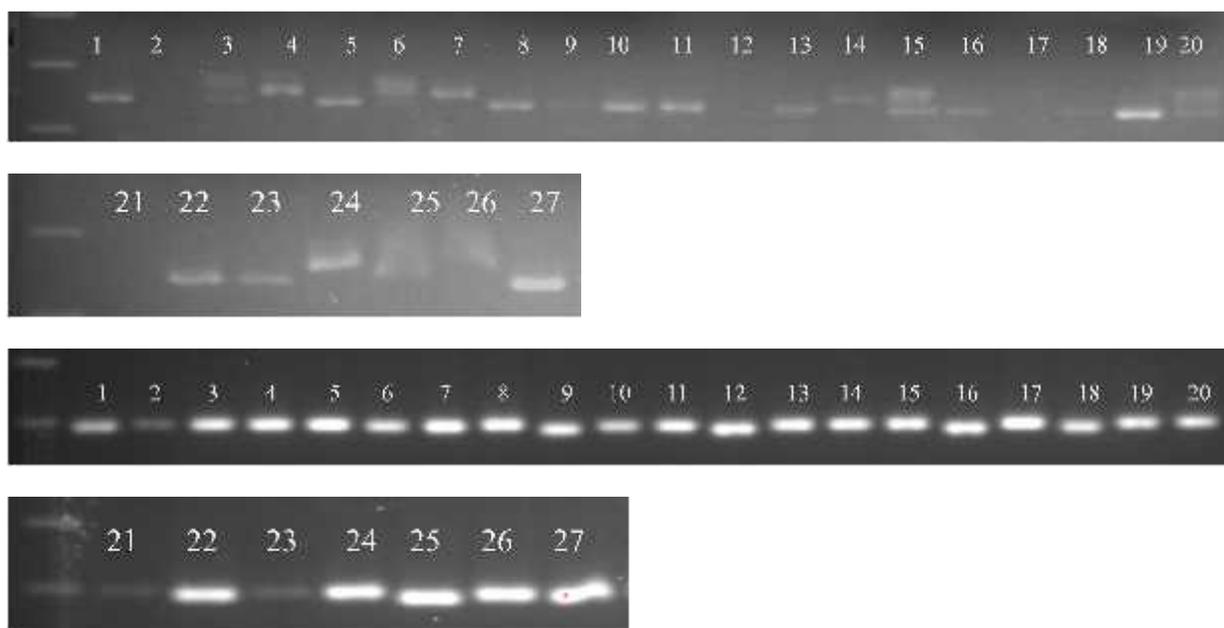
General classification of the 28 EST – SSRs is represented (Table 4). Accessions were genotyped for each EST SSR based on the presence (1) or absence (0) of peaks represented as bands. Faint or unclear bands were not considered for scoring to calculate the polymorphic

information content (PIC) value. The qualitative data generated was used to generate a matrix determining similarity among the samples. The similarity matrix was used to draw a dendrogram with UPGMA clustering method via the SHAN module of NTSYS-pc version 2.2j soft ware, to show a phonetic representation of genetic relationships as revealed by the similarity coefficient.

## Results

In present study, 12 polymorphic EST- SSRs, detected twelve alleles. A wide range of fragments were observed from 150 to 400bp. The gel picture of two primers GBRC 80 showing polymorphism is given (Fig).

Fig GBRC 80 primers exhibiting polymorphism (3% agarose gel) between 27 castor accessions (100 bp ladder)



1.Kranthi, 2. Haritha, 3. DPC-9, 4.RG-48, 5.RG-43, 6.PCS-293, 7.RG-1354, 8.RG-47, 9.PCS-312, 10.RG-1, 11.PCS-106, 12.RG-67, 13.Kiran, 14.PCS-236, 15.PCS-252, 16.RG-1686, 17.PCS-230, 18. SKI-215, 19.PCS-171, 20.PCS-265, 21.DC-578, 22.JP-65, 23.RG-20, 24.PCS-302, 25.M-574, 26.PCS-228, 27.PCS-224.

27 castor genotypes were tested with 28 primers sets. Among them 12 primers sets showed polymorphism with PIC values to range from 0.28 to 0.49 with an average of 0.339 and

remaining primers set doesn't show any polymorphism. The number of alleles detected by individual EST-SSRs averaged 2.3.

Cluster analysis was performed to generate a dendrogram by using 27 castor genotypes (Fig). Cluster I included 10 accessions (37.0%) while cluster II accommodated a maximum of 16 accessions (59.2%) followed by cluster III with single accession (3.70%). Cluster II can be further sub divided into IIa (Haritha, JP-65, RG-48, RG-1354, RG-47, PCS-236 and PCS-312) and cluster II b (RG-47 and PCS-265, RG-67 and DC-578) with 0.83% similarity coefficient. M-574 a single genotype formed separate OUT in cluster showed less similarity coefficient of 0.24%.

28 primer pairs were chosen from SSR-containing ESTs for characterization of 27 castor accessions. 12 SSRs showed polymorphism. Their Polymorphism information content (PIC) values ranged from 0.28 to 0.49. Number of alleles ranged from 2 to 3 with an average of 2.33 per locus. The allele size varied from 150 to 400bp. A dendrogram analysis grouped the 27 genotypes into three separate clusters. Dendrogram genetic similarity coefficient ranged from 0.24 to 0.83. PIC value of 0.28 to 0.49 revealed diversity to be medium in castor.

Fig. UPGMA dendrogram of genetic relationship among 27 castor accessions analysis using 28 EST SSR markers

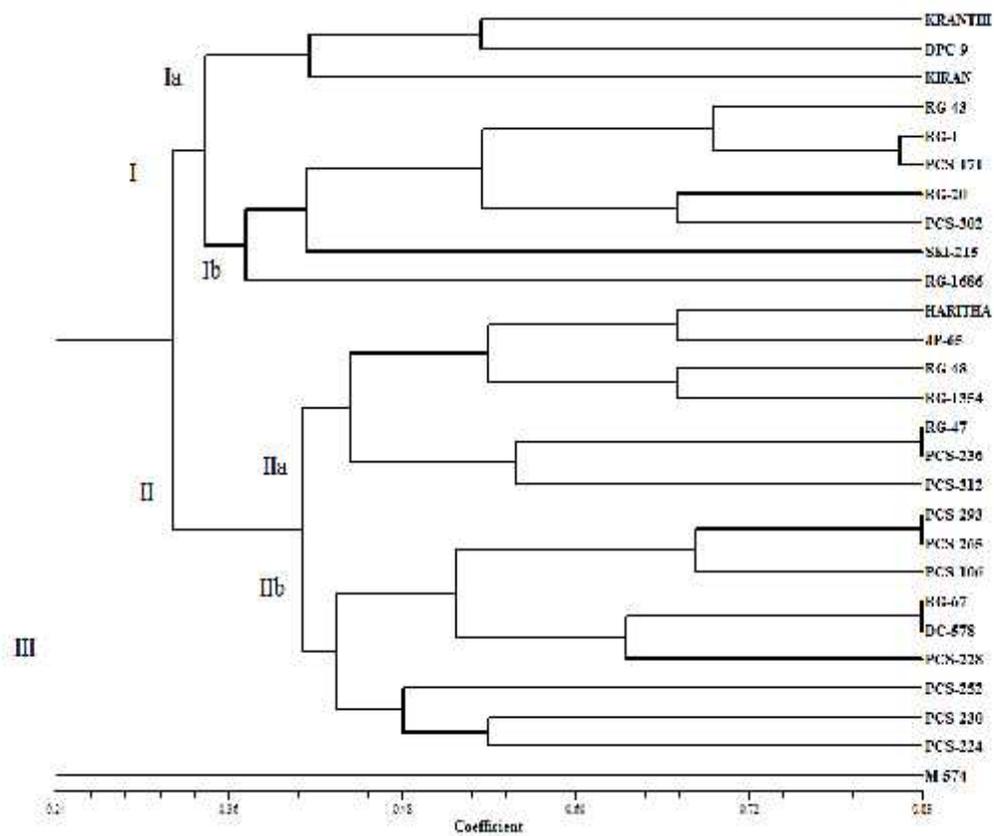


Table 4. Carbon Isotope Discrimination (CID) values of castor genotypes

Sample No.	Genotype	D13C
1	RG-178	18.10732616
2	PCS-293	18.51186382
3	RG-72	19.10409244
4	RG-1	19.21822264
5	PCS-236	18.93660817
6	PCS-171	18.99312796
7	RG-43	17.87541082
8	RG-1686	19.37008683
9	PCS-106	18.27976415
10	PCS-312	18.57879508
11	RG-1354	18.25049783
12	RG-1696	18.44912374
13	DPC-9	17.93494657
14	SKI-215	19.20565659
15	M-574	18.94393446
16	RG-47	17.9976233
17	RG-67	19.15399979
18	KRANTHI	19.47554595
19	RG-2810	19.51850396
20	RG-2758	19.65159198
21	RG-20	19.60757477
22	KIRAN	19.21263804
23	RG-30	19.25033762
24	D-578	18.31182002
25	PCS-252	17.48668515
26	PCS-265	18.60354828
27	HARITHA	18.3985891
28	JP-65	18.53243065
29	RG-2035	18.82323897
30	PCS-230	19.14771752
31	RG-48	18.19684763
32	PCS-224	18.73744371
33	PCS-278	18.72698186
34	PCS-228	19.22310986

Internal precision of the IRMS using CO<sub>2</sub> for 13C gas was better than 0.06 per mill. External precision for 13C using Starch standards: better than 0.15 per mill

## SUMMARY OF THE FINDINGS (IN 500 WORDS)

Castor yields are low under rainfed situations. To bring about improvement, it is necessary to quantify the available variability and make effective use in planning the crossing programme. To meet the objective, the magnitude of genetic divergence among twenty seven castor accessions was studied in a specially designed temporary root structure. Crop was raised with two replications with spacing of 90 x 45 cm during late *rabi* 2012 and 2013. Structure was dismantled at 110 DAS when root growth was maximum and data was recorded on root system architectural (RSA) traits. Group distance was measured on multiple characters. ANOVA, Principal Component Analysis (PCA) and diversity index ( $H'$ ) was used to measure of the phenotypic diversity of each trait. Leaf material was collected at 30 DAS and DNA was extracted. Expressed-sequence tags (ESTs) were generated and used for genetic analysis. 28 primer pairs were chosen for characterization of accessions. The qualitative data generated was used to generate a matrix to determine similarity among the samples and to draw dendrogram.

### Results

#### Diversity analysis

Significant difference among the accessions for the characters was studied. Based on the  $D^2$  values, genotypes were grouped into seven clusters. Cluster I included highly divergent genotypes vis a vis cluster VII indicating the scope of generating heterotic hybrids in a crossing programme. The contribution of individual character towards the divergence was maximum for seed yield (31.19%) followed by total dry matter (23.36 %). Root traits together contributed to 8.54 %. Nine genotypes possessed maximum root length of > 192.6 cm. Four lines recorded <18.01 for CID (%). Relative water content (%) ranged from 61.41 to 78.8. Disease screening revealed 13 and 6 lines to be resistant to wilt and charcoal rot.

#### Variation in characters

Variation in characters accounted to range from 3.62 to 50.29%. High variance was recorded for shoot dry weight (45.12%), root dry weight (50.29%), number of lateral roots (44.25%) and seed yield (38.57%).  $H'$  ranged from 0.446 to 0.586. High values for  $H'$  were observed for ten traits. Three PCs explained 70.99% of variation. Nine traits had high weight in the first three PCs indicating their importance for selection in castor improvement.

## Molecular variation

The remarkable phenotypic variation observed in castor bean does not seem to reflect a high genetic diversity. 12 SSRs showed polymorphism. Their PIC values ranged from 0.28 to 0.49. Despite the use of a number of DNA markers the variability could not be observed at molecular level as the genotypes showed less PIC (gene diversity) and clustered into few groups. A dendrogram analysis grouped the 27 genotypes into three separate clusters. PIC value of 0.28 to 0.49 revealed diversity to be medium in castor.

## Genetic Variability Studies for WUE Related Traits in F2 Mapping Population

400 castor F2 mapping population generated from a cross between PCS 106 and PCS 345 was studied for the variability in ten WUE related physiological traits and six yield contributing traits. The estimates of genotypic and phenotypic variances were worked out for all the characters. While considering the WUE traits high GCV estimates were observed for five characters. WUE related and yield characters showed high heritability (>60%) indicating low environmental effect and high capacity for the transmission to subsequent generation.

## Conclusion

Castor genotypes could be characterized for diversity. Haritha, RG 48, Kranthi and SKI 215 showed superior root and shoot characters. PCS 106 and PCS 345 identified with at least five contrasting characters were crossed. F2 mapping population was generated and characterized.

**Statement of expenditure and balance for the period 4-11-2011 to 31-3-2012 and  
31-3-2012 to 31-12-2012 in the University Grants Commission - Major Research Project**

*Enclosure-5*

**Statement of Expenditure and Balance for the period 4-11-2011 to 31-3-2012 and  
31-3-2012 to 31-12-2012 in the University Grants Commission Major Research Project**

Head of account  
270000 Department sponsored scheme  
270454 Project on "Physiological and molecular characterization of root traits, wilt and char coal  
rot resistance in castor (*Ricinus communis* L)"

Principal Investigator : Ramesh Thatikunta  
Professor  
Department of Crop Physiology  
College of Agriculture, ANGRAU  
Rajendranagar, Hyderabad 500030

S No.	Item	Amount approved Rs	Previous expenditure incurred in Rs (from 4-11-2011 to 31-3-2012)	Present expenditure incurred in Rs (from 31-3-2012 to 31-12-2012)	Progressive expenditure incurred in Rs from 4-11-2011 to 31-12-2012	Balance in Rs
i	Books & Journals					0
ii	Equipment					0
iii	Contingency	260000	240599		240599	19401
iv	Field Work/Travel	15270	13434		13434	1836
v	Hiring Services	28000	16980	11020	28000	0
vi	Chemicals & Glassware	130000	129454		129454	546
vii	Overhead	57030	57030		57030	0
viii	Any other items					0
ix	Project associate fellowship @ 8000/- pm	80000	32000	48000	80000	0
	<b>Total</b>	<b>570300</b>	<b>489497</b>	<b>59020</b>	<b>548517</b>	<b>21783</b>
	Interest upto 31-1-2012	5106	--	--	--	5106
	Interest upto 31-7-2012	6187	--	--	--	6187
	<b>Total Interest</b>	<b>11,293</b>	<b>--</b>	<b>--</b>	<b>--</b>	<b>--</b>
	<b>Balance</b>	<b>5,81,593</b>	<b>489497</b>	<b>59020</b>	<b>548517</b>	<b>33076</b>

UAIN: 21230800AAB  
AEAS845

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**Month wise and year wise detailed salary statement of Project associate fellowship  
@ Rs 8000/- per month**

*Enclosure 51*

**Month wise and year wise detailed salary statement of Project associate fellowship  
@ Rs. 8,000-00 per month**

**Head of account**

270000 Department sponsored scheme

270454 Project on "Physiological and Molecular characterization of root traits, wilt and char coal rot resistance in castor (*Ricinus communis* L)"

Principal Investigator: Ramesh Thatikunta

Professor, Head and PI

Department of Crop Physiology

College of Agriculture, PJTSAU

Rajendranagar, Hyderabad 500 030

Month and Year	Salary Drawn Particulars	
	4-11-2011 to 31-3-2012	31-3-2012 to 31-12-2012
December(2011)	Rs. 8,000-00	
January (2012)	Rs. 8,000-00	
February (2012)	Rs. 8,000-00	
March (2012)	Rs. 8,000-00	
April (2012)		Rs. 8,000-00
May (2012)		Rs. 8,000-00
June (2012)		Rs. 8,000-00
July (2012)		Rs. 8,000-00
August (2012)		Rs. 8,000-00
September (2012)		Rs. 8,000-00
October (2012)		
November (2012)		
December (2012)		
<b>Total</b>	<b>Rs. 32,000-00</b>	<b>Rs. 48,000-00</b>
<b>Grand Total</b>		<b>Rs. 80,000-00</b>

*Verified and found correct*

*Jesha*  
**ASST. COMPTROLLER (PENSION)**  
P.J.T.S. Agricultural University,  
Rajendranagar, Hyderabad-30.

*10*  
*21/1/2012*

Principal Investigator

*T. Ramesh*

(Ramesh Thatikunta)

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RAJENDRANAGAR, HYDERABAD - 50

*Nikunj*  
**NIKUNJ NEWATIA**  
Chartered Accountant  
M.No:230800



**UDIN: 21230800AAAA023790**