SEVENTH FRAMEWORK PROGRAMME

FP7-KBBE-2009-3-1-02

Jatropha curcas – breeding strategy – towards a sustainable crop for biomaterials and bio-fuels – SICA (India and/or African ACP and/or Latin America)

Final Report

JATROPT

Jatropha curcas:
Applied and Technological Research on Plant Traits

www.jatropt.eu

1 January 2010 – 31 December 2013

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1. Publishable Project Summary

JATROPT aimed to improve jatropha production systems through developing advanced genetics tools for breeding and for development of jatropha agro-systems. The major achievements are:

1. A worldwide germplasm collection of *Jatropha curcas* (from TNAU, Quinvita, Embrapa and DLO/Biocombustibles de Guatemala), showed a high degree of molecular biodiversity in Central America and early growth traits, seed oil content and fatty acid composition. The few non-phorbol ester accessions are genetically very close and genetically separated from the rest. Final results in the Genotype x Environment trials in India, Mali, Madagascar, Cape Verde, Guatemala and Brazil show large variation in early growth and in seed and oil yield. Non-phorbol ester accessions showed lower yield potential than the phorbol ester containing accessions, demonstrating the need for plant breeding. Non-phorbol ester varieties will be the basis for use of seed meal as feed.

2. Four mapping populations (variable for seed yield, oil traits, flowering, morphological traits, phorbol ester) have been created and evaluated. A single QTL was found for phorbol ester that will be very valuable in breeding programs and multiple QTLs for many yield related traits, oil content and fatty acid composition. The world's first public genetic map (over 500 SNPs and SSR) of *Jatropha curcas* has been published by JATROPT (King *et al.*, 2013)

3. Agronomy trials India, Madagascar, Mali, Cape Verde, Brazil and Guatemala (fertiliser, irrigation, plant density, mono/intercrop, hedges) show the possibilities and impossibilities in jatropha production. Nitrogen, irrigation and planting density all affect early growth and seed and oil yield. The data have been used to parameterise a mechanistic crop production model driven by soil and weather data that can be used to assess feasibility of jatropha production for specific sites.

4. Designing farming systems without the food versus fuel dilemma

A quantitative Sustainable Livelihoods Analysis framework for optimising farming and bio-energy production systems was developed to find the optimal allocation of land (of varying soil quality) and of fertiliser to crops (jatropha, maize, grain legume and forage grass) and to analyse the effect of several strategy on economic and environmental livelihood outcomes. This primary production model was combined with a downstream economic and environmental cost/benefit analysis. The model sows it is possible with 30 % of the land allocated to (non-toxic) jatropha to produce as much food on the remaining 70 % as the system without jatropha, at almost the same net farm income.

5. Demonstration of the potential of local/regional use of produced biofuels to increase agricultural and general economic productivity will be investigated.

The potential under practical conditions of jatropha cultivation was studied. In the traditional systems, yields are up to 2500 kg/ha of seed and 1000 kg/ha of oil and the oil extraction efficiencies were high (standard screw press > 90 % of extraction efficiency). Analysis of the cost price showed that labour costs may be a large barrier towards economic feasibility with the standard yields except in when using own labour (as we assumed in the SLA-analysis).

6. High density jatropha yields twice the standard production: a breakthrough

A new production system with an ultra-high planting density was established that is amenable to mechanical harvesting. A new genotype was found (dwarf type with higher harvest index) that is optimally suited to this high density system with oil yields over 1500 kg/ha in the first year, and 3000 kg oil/ha in the second year, in a growing season of over 300 days (with irrigation). This system will cause a breakthrough in jatropha production, as costs of harvesting are very low and efficiency of inputs (water, nutrients, labour) was increased.

2. Project context and main objectives of JATROPT

Jatropha curcas shows a big promise towards sustainable and affordable biofuels. Small successes but also big failures have been reported when trying to set up jatropha bio-energy production chains. Reasons for lack of large scale success were the use of varieties of jatropha found locally, while this material in Asia and Africa was optimised for use as a live fence and not for jatropha oil production, and implementation of large scale jatropha plantation without sufficient evidence and knowledge on the agronomic suitability at the chosen locations. Jatropha was introduced without a domestication and plant breeding phase towards locally adapted materials optimally suited for jatropha oil production.

This lack of breeding and agro-systems development was a reason for the European Commission (and also a motivation for the partnership of JATROPT) to develop a project aimed at the development of improved breeding strategies and agro-systems that enable the sustainable use of the crop for biomaterials and bio-fuels, especially involving the countries were jatropha production is expected to occur (as this was a SICA call aimed to involve India, African ACP and Latin America). A partnership was developed consisting of research organisations and companies from eleven countries. Seven partners were located in Internationals Cooperation Partner Countries (ICPC) and four in the EU itself:

1 Stichting Dienst Landbouwkundig Onderzoek - Plant Research International B.V. (co-ordinator), Wageningen UR, The Netherlands
2 Empresa Brazileira de Pesquisa Agropecuária, EMBRAPA , Brazil
3 Centre for Novel Agricultural Products, University of York, CNAP, United Kingdom
4 Biocombustibles de Guatemala, S.A, BCGSA, Guatemala
5 BIONOR Transformación, S.A., BIONOR, Spain
6 Centre de coopération internationale en recherche agronomique pour le développement, CIRAD, France
7 Quinvita Plant Science, Ltd., Quinvita, United Kingdom
8 Facultad de Agronomía, Universidad de San Carlos, FAUSAC, Guatemala
9 Centre National de la Recherche Appliquée au Développement Rural, FOFIFA, Madagascar
10 KeyGene N.V, Keygene, The Netherlands
11 Tamil Nadu Agricultural University, TNAU,India
12 Universidad Autónoma Chapingo, UACh Mexico

These groups were working independently towards development of both agro-systems and high quality germplasm of jatropha, and downstream processing for the biodiesel markets. The challenges were to bring the big promise of jatropha come true: high oil yield, low competition with food crops, use in various agro-systems from monoculture plantations to mixed cropping and use in hedges around agricultural fields.

JATROPT has linked high quality research groups and companies operating in different continents and has achieved a large synergy in research and development of jatropha as a biofuel crop.

In five Work Packages (Breeding, Genetic tools, Sustainable Agro-systems, Demonstrating and Dissemination), the following aims were pursued:

1. Achieve a world-wide germplasm collection of *Jatropha curcas*, molecularly characterised in order to classify the collection into groups with similar genetic backgrounds; evaluation of elite germplasm of this collection in Asia, Africa and Latin-America; linking segregating population based on parents from different parts of the world and creating a global jatropha linkage map (WP1).

2. Develop genetic information and marker tools (genetics of toxic/low toxic trait, branching patterns; disease resistance) to speed up the breeding process (WP2).

3. Develop agro-systems that yield sustainable and affordable biofuels - and interesting uses of the co-products (biomass/protein residues after oil extraction), with a focus on Pro Poor development and on designing systems in which competition for food and fuel can be minimised (WP4)

4. Demonstration of the potential of local/regional use of produced biofuels to increase agricultural and general economic productivity will be investigated (WP4)

5. Achieve dissemination of knowledge on quality of germplasm, genetics and sustainable agro-systems, towards distribution of combined packages of agronomic guidelines and germplasm (WP5).

3. Main results and achievements of JATROPT

JATROPT aimed to improve jatropha production systems through developing advanced genetics tools for breeding and tools and knowledge to improve jatropha agro-systems. Here we describe the main Science & Technology results per Work Package.

WP1 Breeding for sustainable production systems

Integrated molecular biodiversity study

Development of a SNP marker set for use in genetic diversity analyses

For the generation of the SNP markers the CRoPSTM 1,2 technology developed by Keygene N.V.was used. This technology combines the AFLP® 3 technology and the sequence technology of 454 Life Sciences and it has proven to be a successful technique for the generation of many SNP loci in a variety of crops.
Also for Jatropha curcas the CRoPSTM technology resulted in more than a thousand SNP loci and 384 SNPs were selected for the design of an Illumina BeadExpress (BEX) assay.

Generation of the SNP marker data for the germplasm sets

The Jatropha001 assay was used for the analysis of the germplasm sets of the participants. The SNP markers in the different sets were codominantly scored using the BeadExpress genotyping software. For each germplasm set a score file with the codominant marker information of the 384 SNP markers is presented (in Appendix to D1.01). From the number of scored SNP markers per germplasm set and the number of polymorphic markers per set, it could be concluded that the Jatropha001 assay is useful for the analysis of the different Jatropha curcas germplasm sets. Furthermore, it could be concluded that there is a large difference in the percentage polymorphism between the different sets of samples ranging from 57% (TNAU samples) to 97% (BCGSA samples). Both the actual variation in regions where the samples were collected as well as the selection of the specific lines in the germplasm sets are responsible for these differences.

The non-phorbol ester accessions from Mexico generally showed a low degree of polymorphism with only a few accessions responsible for the polymorphisms found. This is already an indication of the low genetic variation within the set of non-phorbol ester containing accessions of Mexico. This is evidence of a genetic bottleneck from the toxic (phorbol ester containing) germplasm (which have a much higher degree of genetic variation) to the non-phorbol ester accessions. This is probably due to the fact that a single mutation is responsible for the absence of phorbol ester as is also proven in WP2 on the basis of the segregation of the trait in mapping populations and the finding that only a single QTL is detected for phorbol ester content. The reason for this bottleneck is probably the long term use by people of the non-phorbol ester containing seeds as a food snack. This can be viewed as a specific domestication process, in which indigenous people from Mexico selected this mutant and multiplied it through cloning and self-fertilisation, leading to a very narrow set of genotypes homozygous for the non-phorbol ester trait. Broadening the genetic variation by crossing with toxic jatropha germplasm must have been a dangerous route (when hundreds of years ago the use of this non-toxic jatropha as food snack started) and therefore today the genetic variation in the non-phorbol ester varieties is very low.

Biodiversity analysis on the combined core sets of TNAU, Quinvita, Embrapa and BCGSA/PRI)

The DNA-markers developed in WT 2.1 (> 100 SSR and 384 SNPs)were used to characterise the total combined set of in total > 200 accessions from across the world. Dendrogram and the cluster analysis based on pair-wise similarities of all pairs of accessions (based on the DNA-marker profiles of the accessions) show that accessions coming from different partners are genetically very different. Almost all material obtained from Asia, Africa and South-America show a large similarity (in DNA-markers) with Cape Verde material. The TNAU material from Asia also contains a group of material that is different from Cape Verde material. This group contains J. curcas x interregima hybrids and backcrosses. The Mexican collection containing about 25 different accessions showed little variation. Apparently, the different communities in the region where the non-phorbol ester accessions grow and are used to provide a food snack for human consumption use a very limited set of genetically very similar accessions.

Remarkably, the Brazilian material is very similar to standard Cape Verde materials. 'Cape Verde' is the denomination of most materials used in Africa and Asia. Several distinct types of 'Cape Verde' lines exist, coming from different islands. They are all highly homozygous due to a process of inbreeding. Apparently, only a limited amount of genetic variation was present in the initial jatropha materials brought to Cape Verde. This has presented a genetic bottleneck. The seed reproduction has led to further inbreeding apparently before materials were distributed to different Cape Verde islands. Continued inbreeding through seed production must have occurred, resulting in the current highly homozygous Cape Verde lines available worldwide It must be concluded that the collected Brazilian material consists mainly of imported Cape Verde materials and that not much original Brazilian material has been collected (yet?). Until now none of the Brazilian accessions of J. curcas show a distinct Brazilian origin that is different from Cape Verde materials. Scientists in Brazil now argue that J. curcas should no longer be considered a native species, although still some genetic variation may be present in Brazil that is unique to Brazil. Currently, an appeal is prepared to the Brazilian government to change the status of J. curcas again to non-native. This would create much easier opportunities to import and export jatropha seeds. The current status as an native species even blocks easy exploitation of jatropha for export purposes, and certainly delays all scientific exchange of germplasm of jatropha.

The DOPS/Quinvita germplasm consists of a large part that is Cape Verde like, which is logical as Cape Verde is the base of the plant breeding by DOPS/Quinvita. Another part consists of a wide set of Central American jatropha accessions (many from Nicaragua, but also some from Guatemala and Mexico). Also some non-phorbol ester accessions are present in this material.

The BCGSA/PRI germplasm collection consists of a large group from India. Most of that material is genetically a single, homozygous genotype and genetically hardly distinguishable from Cape Verde materials. Another part consists of Asian accessions from other countries than India. Also that material is genetically very close to 'Cape Verde'. From the almost 200 accessions, 70 accessions could not be distinghuished on the basis of prelimary AFLP, SSR (of PRI) and TRAP marker analysis. These 70 accessions were only represented by a few accessions in the further SSR analysis (by CNAP) and SNP analysis (by Keygene). Also the group of African accessions show little genetic variation and are genetically either identical to 'Cape Verde' material or very close to it. As in the DOPS/Quinvita germplasm, only the Central American accessions show a large molecular genetic variation.
A special position in the total germplasm set is taken by the non-phorbol ester accessions from Mexico and Guatemala. They all fall into a small cluster that is very distinct from all the phorbol ester containing accessions. However, within the non-phorbol ester group the amount of genetic variation (analysed with the current set of SSRs and SNPs) seems to be very small compared to the variation in the rest of the Central American materials. The non-phorbol ester accessions of both DOPS/Quinvita and BCGSA fall in the same cluster as the Mexican non-phorbol ester accessions of UACh, so apparently all partners have collected accessions coming from genetically very similar sources.

Publication on molecular and phenotypical variation in the JEP-collection

The JATROPT core collection is in part based on the JEP-collection of BCGSA and DLO. A paper on the molecular biodiversity and genetic variation in this JEP-collection for early growth traits, seed hull content, oil content and oil composition showed that most of the variation can be found in Central America while the majority of the accession from Asia and Africa are genetically fully uniform and should be considered to be a single fully inbred line. This line seems to originate from Cape Verde as the molecular marker pattern of almost all Asian and African (and even the South-American accessions) show a large similarity with Cape Verde material. It seems that the domestication process by the Portuguese has created a genetic bottleneck that annihilated all genetic variation. This stresses the importance of enriching the world's jatropha germplasm with the genetic variation from Central America (Montes et al., 2014).

Design of a coreset of jatropha curcas lines

The dataset generated on the germplasm lines was also used to advice on the construction of a core set of Jatropha curcas lines, which is a reduced set of lines capturing most of the genetic diversity.

The development of the core set of accessions was based on the genetic marker map generated for the Quinvita mapping population in which most SNP markers were mapped (see WP2 for the genetic marker map development). A statistical method (SSBL, sumsq bin length) was used to optimise the number of unique recombinations in the core set, with a bin being defined as a genome segment bordered by two recombinations (Vision et al. 2000). Different subsets were generated with increasing numbers of individuals. Using this method, which optimizes for the number of unique recombinations, and therefore for the distribution of markers over the genome, a subset of 70 to 90 individuals proved sufficient to cover all genetic variation; a list with this core set is provided in the Appendix to D1.01 Keygene - Molecular Biodiversity Analysis of JATROPT collection (uploaded as other document).

Maintaining the global core set after the JATROPT project

Maintaining the global core set after JATROPT is not a deliverable of JATROPT. The accessions in the core set are currently being maintained by the partners owning these accessions. A large part of the core set (> 50 accessions) is maintained in one location at BCGSA in Guatemala.

G x E-analysis of selected germplasm on different continents

Nine advanced or often used accession of jatropha were evaluated in India, Madagascar, Mali, Cape Verde, Brazil and Guatemala. Three accesssions were 'non-toxic' accessions (not containing phorbol ester in the seeds) and the other six accessions did contain phorbol ester in the seeds. Two non-phorbol accession originated from Mexico and one from Guatemala. Two accessions originated from Cape Verde and two from India (one a typical Cape Verde type and the other the result of interspecific hybridisation of *J. curcas* x *interregima* (backcrossed to *J. curcas*). In a separate test, three Brazilian accessions were compared to Cape Verde type accessions on Cape Verde an in Guatemala. These three Brazilian accessions proved to be genetically almost identical (on basis of the DNA-marker analysis in the biodiversity analysis) and also showed the same performance (in terms of morphological development and yield) as standard Cape Verde types (as available from Quinvita and BCSGA).

WP1 has demonstrated that GxE interaction in a set of nine advance accessions is not large for environment in which the productivity of jatropha is high. It proved that the locations in Mali and Madagascar had a slow development towards total crop coverage and total light interception, and thus the nine accesssion all showed depressed yields in these two countries. In these two locations, the order of performance deviated considerably from the order in the more productive locations. Apparently, both environment need locally adapted material and perhaps improved, locally adapted agronomy practices. Still, a local accession from Mali (included as a reference accession in the Mali GxE trial) , did not perform any better than the nine accessions tested there.

The performance of these nine accessions in Guatemala and Cape Verde showed a large positive correlation for seed and oil yield (with R2 = 85 %), showing an absence of GxE interaction for seed and oil yield (the order of performance of accessions is the same in Guatemala and Cape Verde). The order of seed yield performance of these nine accessions in Guatemala and Cape Verde correlated also highly with the performance in India (except for two accessions that underperformed in India). This last observation shows that GxE- for seed yield can occur. Although, generally, for the areas with higher productivity jatropha the performance order does not change, this is not true for all accessions. This means that prior to introduction of high yielding varieties developed in one region in another region the suitability for that new region should always be tested to avoid that farmers will run the risk of using an inferior genotype for their situation.

A yield component analysis revealed that genetic differences in seed and oil yield is almost only determined by the number of seeds per tree (and the plant density) and the weight per seed; the seed yield is the the product of number of branches, number of fruits per branch and number of seeds per fruit and the weight per seed. In all locations, but Mali, the number of seeds per fruit was three, but in Mali in the first year it was only about 2, but reached a value of 2.7 seeds per fruit in the third year. Oil content, estimated seed hull content and estimated seed meal content did not significantly differ between accessions. Apparently, the first goal in plant breeding should be to increased the number of fruits and seed per tree and to select accessions with the highest weight per seed.

Field trials for evaluations of 4 mapping populations

Field trials for the evaluation of the four mapping populations have been set up and evaluations results were obtained that were used in WP2 for the QTL-analysis on phorbol ester and yield related traits. A set of in total about 1000 F2-plants has been evaluated. The mapping populations of Quinvita can probably not be maintained as Quinvita Group had to stop its activities. The two mapping populations of BCGSA in Guatemala are maintained by BCGSA in collaboration with DLO.

Diallel crosses

Offspring of 14 F1-families of crosses (1 cross did not give seed) of the half diallel and six S1-offspring from selfings of the six acessions were obtained (about 100 seeds each). The six accessions used were: BCGSA101, BCGSA102, BRA101, BRA102, DOPS101, UACH101. UACH101 does not contain phorbol esters in the seed, while all other accessions do contain phorbol esters in the seed. A set of crosses between the six most important accessions of the GxE trial were made, including the selfings of these lines. This set of crosses constitutes a set of diallel crosses that will form a starting point for further breeding.

Conclusion on WP1

WP1 has analysed the genetic variation in world-wide collection of accesssion and has defined a core set of accession that when maintained can cover all genetic variation present in the total JATROPT collection.

WP1 has demonstrated that GxE interaction (even when testing accessions selected in one continent and evaluating them on other continents) in a set of nine advance accessions is not large for environment in which the productivity of jatropha is high. It proved that the locations in Mali and Madagascar had a slow development towards total crop coverage and total light interception, and thus the nine accesssion all showed depressed yields in these two countries. The performance of these nine accessions in Guatemala and Cape Verde showed a large positive correlation for seed and oil yield (with R2 = 85 %), showing an absence of GxE interaction for seed and oil yield (the order of performance of accessions is the same in Guatemala and Cape Verde). The order of seed yield performance of these nine accessions in Guatemala and Cape Verde correlated also highly with the performance in India (except for two accessions that underperformed in India). This last observation shows that GxE- for seed yield can occur. Although, generally, for the areas with higher productivity of jatropha the performance order of genotypes/accessions does not change, this is not true for all accessions. This means that, prior to introduction of high yielding varieties developed in one region into another region, the suitability for that new region should always be tested to avoid that farmers will run the risk of using an inferior genotype for their situation.

WP2 Genomics and genetic tools

Economic cultivation of *Jatropha curcas* is currently hampered by both lack of knowledge of suitable agronomic practices and the availability of purpose bred varieties or cultivars. Compared with most crop species, the cultivation of *J. curcas* has been rather sporadic, and it has not therefore benefitted from a sustained programme of plant breeding aimed at increasing traits such a yield. Furthermore, current cultivation of *J. curcas* has relied on very limited germplasm. In Asia, Africa and South America, the *J. curcas* plants currently grown appear almost isogenic (Basha *et al.*, 2009; He *et al.*, 2011; Sun *et al.*, 2008). This genetic bottleneck is likely to be due to the export of only limited quantities of germplasm during the “domestication” of *J. curcas* by the Portuguese (Heller, 1996). Much higher levels of genetic diversity have been observed in Mesoamerica, which is thought to be the origin of *J. curcas* (Basha *et al.*, 2009; He *et al.*, 2011; Ovando-Medina *et al.*, 2011).

The economics of *J. curcas* cultivation are also hampered by the presence of toxic diterpenoids (phorbol esters) within seeds. The seed meal from most oilseeds, which is rich in protein, can be converted into animal feed. The phorbol esters however, are not sufficient degraded by conventional seed processing methods, although they can be removed by performing an additional solvent extraction step (Brooker, 2009). "Non-toxic" types of *J. curcas* are known to occur, particularly within some regions of Mexico. These have been shown to lack phorbol esters (He *et al.*, 2011). These "non-toxic" types of *J. curcas* also show limited genetic diversity, and may not be suitable for commercial production of *J. curcas*. However, introgression of this trait into other genetic backgrounds could be a beneficial approach to creating new non-toxic cultivars.

The application of plant breeding has improved the yields obtained from the major commodity oilseeds (Vollmann and Rajcan, 2010), and a similar approach in *J. curcas* should also be adopted in order to create agronomically improved varieties of plant. Modern plant breeding is underpinned by molecular biology, and in particular, the use of molecular markers which identify genetic variation within the genome of a plant. These molecular markers can be used to assess genetic variation within germplasm collections, and idenfity genetically distinct materials. The identification of this genetic variation is important both for the selection of materials to be conserved within germplasm banks, and the identification of materials to be used in plant breeding programs.
The relative location and order of these markers can be determined and used to create a genetic map, and statistical analysis can be performed on the association between a particular trait and markers on the genetic map to identify quantitative trait loci (QTL). New varieties of plant can be bred containing multiple QTL, resulting in the pyramiding of beneficial traits which may contribute toward yield, disease resistance, or other qualities of the harvested grain (seed) which may improve its value.

The aims of second JATROPT work package are to develop the tools required to perform marker-assistant breeding of *J. curcas*. These included

(a) A set of over 500 co-dominant molecular markers which can be used for genetic diversity analyses and plant breeding (*Deliverable D2.01*)
(b) The creation of a medium density genetic map for *J. curcas* using multiple mapping populations segregating for a number of key traits. (*Deliverable D2.02*)
(c) The identification of loci affecting a number of traits related to yield and seed toxicity (*Deliverables 2.03 and 2.04*)

Work Package 2 will therefore permit the modern plant breeding techniques that are currently used for other oilseed crops to be applied to *J. curcas*, facilitating the more rapid development of improved varieties.

Creation of a set of molecular markers for genetic diversity and linkage analysis

A number of different types of molecular marker are available, and excellent summaries of their attributes are available in the published literature (Nyugen and Wu, 2005). The JATROPT consortium focused on the development of Single Nucleotide Polymorphism (SNPs) and Simple Sequence Repeats (SSRs). These markers are superior to other forms of molecular marker, as they co-dominant (i.e., provide information on both loci in a diploid), highly reproducible between laboratories, and can readily be converted into high-throughput assays.

The project aim was to create over 500 markers (for the combined or integrated map), as this should provide a sufficient density of linkage map for QTL analysis, and permit robust error checking of marker data to allow correction or elimination of genotyping errors.

Using the CRoPS™ technique (van Orsouw *et al.*, 2007), KeyGene identified over 1000 SNPs from a reduced representation libraries obtained from two genotypes used in the mapping populations provided by Quivita. Of these SNPs, 384 were selected 384 SNPs were selected for the design of an Illumina BeadExpress (BEX) assay (Assay Jatropha001).

CNAP identified over 100 SNPs from seed transcriptome data obtained from both toxic (King *et al.*, 2011) and non-toxic (unpublished) types of *J. curcas*. Over 70 of these were converted into SNP assays based on allele discriminating PCR.

CNAP also developed a number of SSR markers. Early in the project, SSR markers were identified by mining transcriptome data (King *et al.*, 2011) or by sequencing an SSR-enriched library created using the FIASCO protocol (Zane *et al.*, 2002). During the JATROPT project, a draft genome sequence for *J. curcas* was released (Sato *et al.*, 2011). SSR markers were also mined from this data. A number of SSR markers developed by other research groups were also used, particularly for the purpose of maintaining consistent numbering and orientations of linkage groups between the output of the JATROPT consortium with an interspecific map produced by another research group (Wang *et al.*, 2011). In total, over 300 SSR markers were tested. Not all were found to be polymorphic, but 137 markers were used in the construction of linkage maps for Deliverable 2.02.

AFLP markers were also used locally by some of the JATROPT partners for the purpose of preliminary analysis of materials for inclusion within the global germplasm analysis.

The sequences of the SSR and SNP markers have been made publically available as part of an open-access peer-reviewed publication by JATROPT consortium members (King *et al.*, 2013).

Construction of four F_2 linkage maps for *J. curcas*

Both Quinvita and BCGSA provided two F_2 mapping populations containing at least 200 individuals per population. Each of these mapping populations were segregating for a number of traits, some of which are described in the consortium's 2013 publication (King *et al.*, 2013). DNA extraction and genotyping were performed by both KeyGene and CNAP. Individual F_2 linkage maps were then constructed using CRI-MAP software. After performing a number of error-checking steps using an iterative approach, a single consensus genetic map was then constructed using the data from all four mapping populations. The individual linkage maps of each of the mapping populations are available as part of the consortium's 2013 publication (King *et al.*, 2013). The total genetic distance of this integrated map was 717.0 cM, with an average marker density of 1.5 cM and 1.8 cM for all and unique loci respectively. There are relatively few gaps within the integrated map, with only two pairs of loci separated by more than 15 cM, 7 pairs of loci by more than 10 cM, and 30 pairs of loci by more than 5 cM.

Although marker densities and numbers were lower for the individual maps, the average density and length was in each case still high compared to first generation maps for most species. The least populated map (G51 x CV) still contained 253 markers and had a mean density of 3.3 cM based on unique loci. The marker coverage on all four maps is sufficient for interval mapping, where an interval of 10 cM is regarded as adequate (Mayer, 2005).

In conclusion, the linkage maps produced by the JATROPT consortium provide a valuable resource for the QTL mapping and subsequent marker assisted breeding. These are the first published maps created for populations obtained from crossing only within the *J. curcas* species, and these results will be valuable to those creating mapping populations using other *J. curcas* germplasm.

Identification of loci for quantitative traits

The four mapping populations described used in this study were segregating for a number of traits. QTL were identified for the following traits:

Plant height
Number of seeds per plant
Stem diameter
Fatty acid composition
Canopy diameter
Seed weight

Number of branches
Oil content of seeds

Significant correlations were observed between the vegetative traits and the overall seed yield per plant.
The consortium have identified a number of QTL for theses traits which can be introgressed into other genetic backgrounds to create improved varieties of *J. curcas* by using the flanking DNA-markers of these QTL in marker assisted selection and plant breeding.

Identification of a locus controlling seed toxicity in *J. curcas*.

As a prerequisite to mapping the locus (or loci) responsible for seed toxicity, the inheritance of the phorbol ester (PE) biosynthesis trait was studied. Our previous investigation into the distribution of PE within the seeds of *J. curcas* revealed that although most of the PE was present within the endosperm of mature seeds, the highest concentration of these diterpenoids was present within the maternally derived inner layer of the seed coat referred to as the tegmen. The distribution of phorbol esters within the endosperm is also not uniform; much higher concentrations were observed in the outer (seed coat facing) endosperm layers than the inner (embryo facing) layers (He *et al.*, 2011). Sujatha *et al.*, have demonstrated previously that non-toxic plants pollinated by toxic plants bear non-toxic seed, and vice versa (Sujatha *et al.*, 2005). Together, these observations suggest that the PE biosynthesis may only occur within the maternal tissues.

The phorbol ester content of seed produced from reciprocal crosses between toxic and non-toxic varieties of *J. curcas* was analysed). Genotyping of the endosperm from F_1 and F_2 seeds was also conducted using a subset of SSR markers to confirm that crosses were genuine (data not shown). PE-ve maternal plants cross-pollinated with pollen from PE+ve plants produced only seeds lacking PE, whereas PE+ve maternal plants cross-pollinated with the pollen from PE-ve produced seeds contain PE. Self-pollination of F1 plants derived from both PE-ve (female) x PE+ve (male) and PE+ve(female) x PE-ve (male) crosses resulted in F2 seed all of which contained PE, because the resulting F1-plants from these crosses all are heterozygous (PE+/PE-) and when these F1-plants are used as female (with selfing these plants operate as male and female) all selfed F2 seed would be containing PE under a maternal control model. Open pollinated seeds were collected from F2 plants. 31 out of 120 plants analysed produced seeds lacking phorbol esters. The trait did not segregate within seeds collected from an individual plant. These observations are consistent with a genetic locus causing seeds to lack phorbol esters being encoded on the nuclear genome, and the phorbol esters being synthesised only within maternal tissues. The 3:1 segregation also confirms that PE biosynthesis is a dominant monogenic trait.

The maternal control of phorbol ester biosynthesis has some important implications for cultivation of this crop. These data on the inheritance of phorbol ester biosynthesis indicate that as the embryo/endosperm genome does not control PE biosynthesis, there is no risk of maternal non-toxic plants bearing seed containing phorbol esters due to cross-pollination by a toxic plant. This observation means that guaranteeing production of non-toxic seeds should be feasible even in locations where genotypes producing toxic seed are growing.

To conduct linkage analysis for loci controlling phorbol ester biosynthesis, the data from the 120 F_2 plants in mapping population G33 x G43 was analysed. Although the presence of phorbol esters is a qualitative trait, the data was scored quantitatively as the actual concentration of PE present may be influenced by whether the plant is heterozygous at the PE locus. The QTL analysis was performed using GridQTL, and resulted in the identification of a single locus at 41 cM on linkage group 8 of the G33 x G43 linkage map. The 95 % confidence intervals for the QTL position were 31 to 49 cM (18 cM length). On the map for this population, the two flanking markers were positioned at 34.8 cM (JCT3) and 47 cM (1401433|12335072) respectively, which is a gap of 12.2 cM. Further analysis of the data confirmed that all F_2 individuals which were homozygous for the G43 (non-toxic) alleles at both these positions yielded seeds which did not contain PE. This confirms that the mutation responsible for the lack of PE within non-toxic seeds must reside between these two markers. The mean values for the PE content of the F2 plants which were heterozygous at these two alleles was also significantly less than the plants which were homozygous for the G33 (toxic) alleles ($p<0.01$).

The QTL analysis of phorbol ester content based on seeds collected from 120 F2 plants in mapping population G33 x G43 for linkage group 8 showed DNA-markers very closely linked to the monogenic phorbol ester traits. Further, genotypes were found from the cross that had zero kernel phorbol ester in the seeds collected from F2 plants which were homozygous at the two markers

To conduct finer mapping of the locus controlling phorbol ester biosynthesis, two strategies were used. First, blastn searches were performed against the J. curcas genome sequence (Hirakawa et al., 2012; Sato et al., 2011) using the markers which mapped in at least one of the other populations in this study, or had mapped in the interspecific cross produced previously (Wang et al., 2011). The scaffolds identified from these blastn searches were then mined in-silico for SSR sequences. Those sequences found to be polymorphic were genotyped in mapping population G33 x G43 then added to the linkage map. As a second approach to increasing the map density in the region containing the locus responsible for PE biosynthesis, a comparative mapping approach was used which exploited the microsynteny (co-linearity) between the J. curcas and Ricinus communis genomes. Where synteny exists between chromosomal regions of these two species, it is possible to infer the relative positions of J. curcas genes based on the larger R. communis assemblies. The synteny analysis revealed a number of syntenic blocks on linkage group 8 of J. curcas. In particular, the R. communis scaffold 30174 mapped to two regions of J. curcas linkage group 8, one of which was located near the locus for PE biosynthesis. A number of additional SSR markers were created from J. curcas scaffolds which were syntenic with this R. communis scaffold. Analysis of the seed PE content data with the genotyping data indicated that genetic locus conferring absence of PE biosynthesis resides between makers NG285A and G273A. The genetic distance of this region is 2.3 cM. Our ability to map this trait to less than this distance is limited by a breakdown in synteny to the castor genome in this region (and a lack of further progeny with informative recombination events in this region.

The identification of a locus controlling phorbol ester biosynthesis also permits the “non-toxic” seed trait, which occurs naturally within only a limited set of germplasm, to be introgressed into other genetic backgrounds. The ability to improve the economics of J. curcas cultivation by allowing the meal to be converted into animal feed is also a significant advance in J. curcas breeding. The mapping of the mutation responsible for the lack of PE biosynthesis to within a relatively small region of the genome means that it will now be possible to breed new high oil yielding varieties of J. curcas which lack phorbol esters. This 2.3 cM region is likely to represent less than 1 % of the entire 717.0 cM genome, which means it will be possible to remove large portions of the remaining genome of the non-toxic variety, which may contain many undesirable alleles, by backcrossing.

Conclusion on WP2

WP2 has achieved the development of a publicly described set of DNA markers (> 100 SSR and 384 SNP markers). These markers have been applied to a global biodiversity analysis (see WP1) and the analysis of segregation of agronomically important traits in four mapping population. The world's first public J. curcas genetic marker map was published by JATROPT (previous marker maps were generated using a J. curcas x interregima cross, and could not show segregation of genetic traits variable in J. curcas as no such large genetic variation within J. curcas was ever shown. A single QTL (with very closely linked DNA-markers) was found for the non-phorbol ester trait of the Guatemalan non-phorbol ester accessions and multiple QTLs were found for seed and oil yield and yield related traits.

WP3 Sustainable *Jatropha curcas* production systems

WP3 has set out to collect experimental data on agronomy and jatropha production system (including data on crop production of other crops used in the areas where jatropha production is investigated). Large scale agronomy trials have been carried out in India, Madagascar, Mali, Brazil, Mexico and Guatemala. A standard experimental design was developed to enable the estimation of production functions of jatropha in various environments. Data were collected on the initial growth curves of jatropha using a combination of destructive and non-destructive harvests to determine the development of the leaf area index of jatropha. Light interception by leaves of a crop are the main driving force of plant production.

The ultimate aim of WP3 was to develop experimental dataset that will help to assess the feasibility of jatropha production. To this purpose, it is insufficient only to use experimental production curves, as experimental production functions are only valid for the specific location, with the specific management and weather conditions of the season in which such experimental production functions are measured. For a general assessment of feasibility of jatropha production, functions predicting the production of jatropha biomass and valuable products need to be developed that can also predict this production for a new location on the basis of climate data or weather data, and the specific soil qualities and management applied. Therefore, WP3 aimed at abstracting from the individual experimental trials to a formulation of crop physiological, mechanistic model to estimate jatropha production, also for situations were no experimental data are yet available. The use of such a simulation model can help in deciding whether specific locations are suitable for jatropha production and in assessing the level of jatropha production that can be obtained. For that purpose, three levels of production need to be determined: 1. the potential production level only limited by the amount of light interception, 2. the water-limited production that depends on the availability of water for crop evapotranspiration and the water use efficiency of jatropha, 3. the plant nutrient limited production (for which nitrogen proves to be the most limiting factor, but only when an adequate amount of potassium and phosphorus has been applied). As nitrogen inputs and nitrogen cycling are by far the most energy intensive inputs and cause the largest CO2-equivalent emission, through the energy use in production of nitrogen fertiliser and the N2O-emission from N-cycling, we have given priority to investigating the impact of nitrogen nutrition.

The impact of potassium and phosphorus application can, however, be very large and the costs of potassium fertiliser can have a very high impact on the feasibility of jatropha. For the moment, these effects have not been taken into account as a limiting factor, but in the later economic analysis, cost of potassium (K) and phosphorus (P) fertiliser have been taken into account. The modelling approach can already be helpful not to estimate a limiting effect of K and P, but to estimate the necessary amount of K and P that should be supplied to be able to reach the nitrogen or water limited production levels.

The agronomic data contain a wealth of information that is not even fully explored yet. All data collected from 2010 until the end of 2013 are centrally stored and available standardised Excel formats.

Agronomy trials in India, Madagascar, Mali, Brazil, Mexico and Guatemala

First, a set of agronomy trials was set up. A variety of factors determining the growth of jatropha were investigated: 1. growing jatropha as mono-culture, with an intercrop or in hedges, 2. the effect of planting density (1,250 trees per hectare of 2,500 trees per hectare) and the effect of interplant distance in the hedges, 3. the effect of nitrogen fertiliser (0, 90 or 180 kg N per ha in the later years of production and half these rates in the first or first two years), 5. growing jatropha under rain-fed conditions or with irrigation.

Crop physiological crop model development

Second, the trial data were analysed to parameterise a new mechanistic crop model for jatropha, and third, the crop model was used to make a world map indicating for 50 x 50 km blocks the suitability for jatropha production. Special attention was given to the restart of production of leaf area after the dry period as leaf production is not driven then by light interception, but by remobilisation of reserves from the stem and branches. This last process, typical of perennial crops, is especially important for a correct estimation of leaf area index development (and therefore of light interception that drives the production in a new season). It proved necessary to use experimental data on this regrowth after a rest period to initialise this regrowth pattern in the model.

A validation of this new crop model was carried out by using the outcomes for the specific soil and weather conditions in the agronomy trial areas and to compare the outcomes with the real experimental data, for the data from the India trial which was the most advanced trial (planted already early in 2010). It proved that the using the measured leaf area index development and the radiation and rainfall data, that the new model based on an adaption for jatropha of the LINPAC simulation model (Jing et al., 2012) could predict the water-limited production of the India trial satisfactory. The model predicted rain-fed production levels of about 3,500 kg of seed per ha, which was also the experimental level of yield obtained.

Jing Q., Conijn J.G., Jongschaap R.E.E., Bindraban P.S. (2012) Modeling the productivity of energy crops in different agro-ecological environments. Biomass & Bioenergy 46:611-633.

A global map indicating suitability for jatropha with a 50 x 50 km resolution

Third, using this validated, mechanistic model, for all 50 x 50 km blocks in the world, the production potential of jatropha was calculated. Besides using the productivity level that would be possible, other criteria were used that should first be fulfilled, before it may be assumed that any jatropha production would be successful (e.g. temperature below zero, at least one month without rainfall). The outcome of this research is a global map indicating the suitability for jatropha production on a scale of 50 x 50 km. Jatropha is assumed to be feasible between the tropics of Cancer and Capricorn and generally no areas were found were jatropha would be feasible outside these tropics. However, within the tropics, the suitability for jatropha varies enormously. This global map is presented in the Deliverables of the project and will be published together with a publication on this new crop physiological, mechanist crop production model for jatropha.

The results confirm the large potential area for jatropha cultivation. However, when taking into account some jatropha specific criteria concerning absence of frost, the availability of at least one month without (excessive) rainfall the really suitability area becomes smaller. No cross-checking of the suitable areas found has been done yet with the existing land use (e.g. natural, protected areas, other crops). Such cross-checking will further limit the potential area, until a sustainable and economically feasible potential growing area will remain.

Link jatropha accessions (G) with information on growth performance and development in different soil and climate conditions (E) and management (M)

For each of the trial locations of the GxE and agronomy trials, the estimates on LAI development were determined on the basis of newly developed allometric relationships between the number of branches, the number of leaves per branch and the average size of leaves.

Using the weather data for these locations, the new crop model predicted the course of LAI in time, and the biomass and seed yield development. In this way, a reference yield level was determined with which to compare the actual yield levels obtained as an indication of whether either light was limiting, water was limiting or nitrogen was limiting. If yields would in practice be even lower than the predicted nitrogen-limited yield, this would be an indication that the specific soils, climate/weather induce the wrong type of development or that biotic factors (pests and diseases) play a role.

For the India trial, a good correspondence between the outcome of the model and the experimentally determined yields was found. In some other places, the model predicted that moderate yields would be possible, but in practice hardly any yield was obtained. For example, in Guatemala, at the location of FAUSAC, specific conditions prevailed that caused leaf loss during the season and abortion of flowers (for example very strong mountain winds, very high humidity in some parts of the season, and (too) low temperatures in other parts of the season). In Brazil, a biotic factor was responsible for problems in yield formation (a new disease in jatropha Phakopsera arthuriana was the cause of problems with black spots and no seed yield was obtained).

In the absence of such factors, the jatropha LINPAC model adequately predicts LAI, biomass and seed yield formation.

Life Cycle Analysis, including energy and GHG balances of jatropha production systems

As a basis for Life Cycle Analysis (LCA) of jatropha products (and bio-energy products derived from jatropha products data were collected for the situations in India, Mali and Brazil on environmental effects of jatropha production systems (energy costs, CO2-emission from nitrogen cycling and nitrogen fertiliser use and other factors) and converted to EXCEL sheets with to evaluate LCA for different jatropha cultivation systems and towards different end-products (e.g. bio-electricity, bio-diesel, bio-gas from lignocellulose). Already two LCA analysis publications have appeared, a first publication on an LCA for the Mali case (which was already prepared before JATROPT) and one paper directly resulting from the work in JATROPT (from Embrapa, Brazil). Details on all LCA-analysis performed (India, Brazil) are in individual partner reports and draft publications uploaded as 'other documents' in the ECAS reporting system.

The results of the analyses on Life Cycle Analysis and the energy and Greenhouse Gas (GHG) balances were input for the final integration of all data in a quantitative optimisation analyses framework (JATROPTIMAL) to be discussed later.

Evaluate socio-economic impacts of jatropha production systems

For all countries were agronomy trials were carried out, data have been collected on the local farming systems. The net returns on competing crops have been determined and compared with the cost/benefit analysis of jatropha production. Especially, the data from India provide a rich database of the cost of production and benefits from which clear conclusions have been drawn on the profitability of jatropha (relative to other crop choice for the same land). It proves that the regional opportunities with other crops play a decisive role in whether or not jatropha will be feasible. In regions with lower productivity of the other crops, jatropha will sooner be profitable for a farmer than in areas with production opportunities for higher value crops.

In Mali, an analysis was made of the labour productivity in different crops for different areas. Also, in Mali it proved that in some cases jatropha can be more profitable than the current worst option to a farmer.

Next to determination of the cost of production and the benefits of primary production of jatropha, economic data on world market prices for plant oils for bio-diesel, on fertiliser costs, labour costs and processing costs of the downstream processing (which together with the LCA data give the opportunity to carry out an integrated economic and environmental cost/benefit analysis. Also, food prices play a role in answering the question on whether jatropha will be an improvement to the livelihood of farmers and local consumption patterns. Farm gate prices and consumer prices for the main foods (cereal grains like maize, legume grains such as soybean or other beans and animal products like milk and meat) have been assembled to incorporate in the optimisation model that integrates the whole farming system (including food and feed crop production and animal production) and downstream processing until the final bio-energy products from jatropha.

JATROPTIMAL: a simulation tool to assess economic and environmental sustainability of jatropha farming systems and downstream processes toward bio-energy products.

In order to achieve goal 3.2 Simulation tool to assess viability of different jatropha production and supply chains, quantitative optimisation model was developed for the Sustainable Livelihood Analysis of different designs of jatropha production system across a range of annual rainfall and with soils from poor to very good. This model (called JATROPTIMAL) integrates the knowledge gained from the experimental agronomy trials, the information on Life Cycle Analysis and the socio-economic information collected.

The production function used in JATROPTIMAL are derived from the production simulated with the mechanistic LINPAC based Simulation module for jatropha biomass production and Simulation module for valuation of jatropha feedstock diversification (from Deliverables D3.08 and D3.09).

The primary production system in JATROPTIMAL, consisting of three soil types from poor to good (but can be specified by the user). The farmer has a choice from four crop categories: cereal (maize), legume grain (soybean), forage grass and jatropha. Forage grass is used for dairy production.

The model determines on an annual basis how much water will become available for crop evaporation given the water holding capacity of the soil, the rootable depth of the soil and the rooting depth of the specific crop and information on annual rainfall and the distribution of rainfall, and the length of the rainy season. For each crop, specific parameters for water use efficiency, nitrogen response curve and dry matter and nitrogen distribution to different plant parts are specified. The model calculates the yields of food, feed and animal product (milk, or if desired by the user, meat), and the yield of jatropha, if chosen.

JATROPTIMAL analyses the result for a set of livelihood outcomes for different strategies (e.g. with and without jatropha, use of seed meal as feed or fertiliser, local bio-energy use or export of oil to Europe for bio-diesel), not only on farm income, but also on the farmers' food production, food supply for the region, the long term organic matter and nitrogen status of the soil, N-cycling in the system and the CO_2-emission reduction and bio-energy production. The model, implemented in EXCEL, optimises net farm income, but with a range of conditions to ensure that production is not only sustainable economically. Any of the livelihood outcomes can

be selected as the goal for optimisation, but we think that if a system is not better for a farmer, the farmer will not chose that strategy.
The model consists of several modules on the water balance, the nitrogen cycling, plant production, animal production, local processing (oil extraction and production of bio-electricity), international bio-diesel production, trade of food and jatropha products and a module that calculates the costs and benefits of all processes in financial terms, but also in terms of net CO2-emission reduction and net bio-energy production of the whole system. Environmental costs are allocated to food or bio-energy production on the basis of the farm gate value of the products for the farm production system and all costs of jatropha processing are allocated to jatropha bio-energy. In order to analyse the trade-off between bio-energy and food production, a module was added to determine for how many people the farm system can supply food on the basis of Daily Reference Intake values. The food demand of the farm is determined (on the basis of family size and farm size) and any surplus is sold.
The plant production module uses a summary model (based on a weekly water balance model) to relate annual rainfall to the amount of water available for crop evapotranspiration and converts this using the water use efficiency of the different crops to biomass and products. Soils and crop combination differ considerably in the efficiency with which they can ' catch' rainfall for crop evapotranspiration. The effect of nitrogen supply is calculated using saturation type functions relating crop total N-uptake and total biomass production and a fixed distribution of biomass and nitrogen to different plant parts.
The final – optimisation part – of the model consists of defining a goal function (in our case we have used net farm income, or farm value, which is net farm income plus the value of the production of food consumed on the farm) and a set of conditions, and a set of choice variables. Most conditions are concerned with the minimum and maximum values (variable should be higher than zero and no more land and nitrogen can be allocated than is available). One condition is special (and may be relaxed if desired). As our farming system is supposed to currently supply the region with food in proportion determined by the average diet, we have required the farming system to produce food in proportion to this average diet (determined by the Daily Reference Intake). Without this condition, the product mix of the farming system could become very unbalanced, when farm income is optimised.
Using the production functions, the value of the goal variable is calculated for a set of trial solutions f to find the set of farmer's choices (allocation of land and nitrogen to crops and soils) that maximises the goal function (here, net farm income, but other goals can be used easily); the model is implemented in EXCEL and the SOLVER addin is used for the optimisation. Calculations have been performed for a range of annual rainfall from 200 to 1800 mm per year (with a highly erratic rainfall distribution, that shows how important a good water holding capacity is for plant production).
In this simulation/optimisation, all four crops were available to choose from. The optimal allocation of different crops to land on the different soils depends highly on annual rainfall, because of differences in the use of water and nitrogen by the crops on the different soils. The proportion of jatropha (that maximises farm income) in the optimal solutions (with maximised net –farm income) is about 30 % (not all land is used for jatropha, as jatropha was most profitably grown – in the model – on the poorest soil).
This optimisation framework has been used to answer questions on whether it is economically or environmentally better to use jatropha for local or international energy supply, or to what extent the farmers' net income would be effected by the possibility of using seed meal as fertiliser only or also (first) as feed for animals and how this would affect the net to gross ratios of CO2-emission reduction and bio-energy production. It proves that in most scenarios analyses a level of net bio-energy and net CO2-emission reduction is reached that is comparable to the levels of rapeseed (at least when seed meal can be used as feed) under conditions of generally low productivity and low input levels. The efficiency of bio-energy production is about 88 % (net output/gross output), when also lignocellulose is used for bio-energy. The net/gross CO2-emission reduction is between 65 and 70 %. These efficiencies are high enough to conform to the RED-criteria of the EU (for the period after 2017).

As an example (full report in Deliverable Report D3.10 and uploaded separately as other document in the Project reporting portal), we analysed the difference in outcomes of only using jatropha seed meal as fertiliser versus using it as feed (with non-toxic jatropha) and then as fertiliser through manure. The net CO2-emission reduction and bio-energy production are hardly affected, but net farm income and the efficiency of CO2-emission reduction and bio-energy are lower as the same amount of fertiliser N is needed, but less food is produced per kg of N input on the farm. N-losses are lower when using seed meal as feed, because with seed meal as feed part of the nitrogen ends up in animal product (milk). The number of capita/ha that can be fed is reduced without seed meal as feed from 3.3 to 3.1 capita/ha. All calculations were carried out assuming the input from own labour at the farm and a farm size at which the farm family is not fully employed on the basis of the farm activities. A farm cost of production level was used that is representative for Africa. A traditional jatropha production system was assumed with 1,250 trees/ha and the use of an intercrop during the first year of establishment. The situation would change enormously for large plantation with hired labour, especially when labour costs per hour are larger than in Africa. With wages and cost of land such as occur in Guatemala, the net-farm income would be negative in our model. This is also the actual situation in Guatemala. There a totally new production system is needed to obtain economic feasibility. Such a new, high density system with improved yields (with the use of a novel dwarf genotype) has been developed and the cost/benefit analysis shows that the traditional system is indeed not feasible (leads to a farm loss) and the new high density system is very profitable. This situation can be modelled in our optimisation model by changing the biomass production function and by using the experimentally determined higher fraction of seed production in total biomass production.

This optimisation model will be very helpful in designing jatropha production system in concrete situations as it is able to show potential outcomes of different strategies.

Conclusion on WP3

WP3 collected a wealth of agronomic data on jatropha production. These data were used to parameterise a new crop physiological, mechanistic model that calculates potential production and water-limited production and can estimate the necessary amounts of fertiliser (NPK) needed to achieve these production levels. This model was used to create a world map indicating the suitability of jatropha with a resolution of 50 x 50 km. Economic data were collected to be able to determine the competitiveness of jatropha in various countries (and regions within countries). Data were collected on the environmental impact through LCA. All data were integrated into a quantitative optimisation model for a whole farming system (in which also food and feed crop and animal production are included) and including the downstream logistics and processing until bio-energy products are obtained.

WP3 has shown that in low income countries and countries with soils that are fast draining with a low water holding capacity, jatropha can contribute to increased net farm income, deliver a high yield of bio-energy per hectare and contribute to an efficient CO2-emission reduction.

WP4 Demonstrator Jatropha curcas supply chain

Two production systems demonstrated at 1 ha level

Two production systems were demonstrated at a 1 ha level: the traditional system with a relatively low density in which manual harvesting and pruning is needed and a high density system with mechanical harvesting

Large uncertainty exists about the optimal density for jatropha cultivation. Until now, densities of about 1,250 and 2,500 trees per hectare have been promoted and used. With such densities, trees can reach large heights, making it very expensive to harvest the fruits by hand and making it necessary to prune the trees annually. Therefore, a novel production system was tested with much higher densities and shorter heights. With a density of ca. 100,000 trees per hectare, much smaller trees can be used and mechanically harvested. This system was compared to the traditional planting system of 1,250 trees per hectare. The traditional systems with densities of 4.0 m x 2.0 m meter can be combined with other crops (intercropping) or cattle. These practices may reduce agronomic costs for weed control and improve the profit per area.

A novel system was designed in which seeds are directly sown into the soil at high density, which can be mechanized by seed sowing machines. Harvest machines from sugar cane or sorghum were used simultaneous harvest of branches and the attached fruits. This reduces the costs of harvesting to a great extent.

Traditional genotypes (Cabo Verde type) were used in the traditional planting pattern (with 1,250 trees per ha) and in the new high density system. Seed yields in the traditional planting system were similar to what normally is obtained. In the harvest year in which these yields were obtained yields were 1500 kg of seed per hectare and 500 kg of oil per hectare. However, in the initial trials with the high density system with the traditional Cabo Verde type, hardly any yield was obtained. In this system, with planting at 10 x 10 cm or 30 x 30 cm it proved the plants do hardly initiate flowering and so hardly any yield was obtained (only 300 kg seed per ha and 100 kg oil per ha).

The trials, however, gave a good indication of the costs of the two systems. The total cost of the traditional system was 1,565 €/ha and in the high density system only 1,081 €/ha, so a cost reduction of about 33 %. Still, the operational cost price per kg oil was too high to be competitive against other plant oils used for bio-fuel at 3,130 € per ton oil for the traditional system and even 10,810 €/ha for the high density system. This does not even take into account that a return on investment is needed on the initial investment 4740 € per hectare.

A new genotype needed for a successful high density system

Evidently, a new type of jatropha genotype was needed that has enough flower induction in the high density system to bring any yield. Biocombustibles de Guatemala (BCGSA) started to search for smaller genotype with shorter branches that would also give less pruning wood and would have a higher harvest index (seed weight to total biomass production). Among the very large collection of plants (from the original practical plantation) some mutant plants were found that should dwarfed growth and extensive flowering. The next step was to multiply these genotypes by selfing to obtain sufficient sowing seed for testing these genotypes in the high density system.

Breakthrough for the high density system: new dwarfed genotype flowers and brings a very high yield

Enough offspring seed of five offspring families (F2-families derived by selfing from the S1 seed of the original dwarf genotype) was available for a large scale demonstration of the high density system with these new genotypes. A slightly lower density was used now with seeding in a pattern 55 x 55 cm, achieving a sowing density of 83,000 seeds per ha (planted in January 2013). Germination rate was 80 % so initially 66,000 plants per ha starting growing. During the end of the first year, some plants died, apparently as a result of self-thinning, so at the end of the first year, 33,000 plants survived.

This plant started flowering already a few months after planting. In total seed yields of over 800 plants were obtained, from border plants of the 5 x 40 m plots and from plants inside the plot. A clear edge effect was found as the border plants had about double the seed yield of the inside plants. For the analysis of the attainable yield with the dwarf genotypes, therefore, only the plants inside the plots were used for the yield analysis. The average seed and oil yield per hectare obtained with the five F2-families derived from this single mutant dwarf type was amazingly high at 3,714 kg seed per hectare and 1,300 kg oil per hectare. One F2-family even had a yield of 4,354 kg seed per hectare and an oil yield of 1,524 kg per hectare, in the first year after seeding. The mechanical harvesting was tested on part of this trial and proved to be very feasible. Separation of the branches and fruits mechanically was also a simple process.

The F2-family with the highest yield even showed segregating for seed yield in a 1:2:1 ratio, with about 25 % of the trees having a higher yield (of 3,000 kg oil per ha and 9,000 kg seed per ha) than the other 75 % indicating the segregation of a single, recessive gen. This means that in the breeding process towards a stable, uniform variety, it will be possible to select for an even higher yield in the first year than the already high average of about 1,500 kg oil per ha and 4,400 kg seed per ha. However, when during this breeding process the yield of a first year harvest would not increase, the yield levels are already very promising. The effects on the cost/benefit analysis when using this new genotype will be discussed later.

Oil pressing data and products from demonstrator production: oil and meal

The full post-harvest processing was demonstrated. The process consists of the following steps:
1. Fruit reception (in the high density system after separation of the branches and the fruits)
2. Separation of seed and fruit coats
3. Drying of seeds (in order to make the seed shell also called seed hull crispy)
4. Shelling the seeds
5. Separation of kernel and seed shell (to improve the efficiency oil pressing)
6. Oil extraction using a screw press expeller
7. Oil filtering

Seeds from the BCGSA plantation were subjected to the standard oil extraction process of BCGSA that consists of a screw press expeller. The costs of this process were estimated and were included in the initial cost price mentioned above. The oil efficiency extraction was determined on seeds from fruits with different degrees of maturity (green, yellow, brown and dark brown, in increasing order of maturity). It proved that the maximal oil extraction efficiency was found with seed from brown fruits. The amount of oil extracted as percentage of the dry weight of seed was maximal with seeds from brown fruit (33 % of the present 35 % of oil as extracted, which is a remarkably high 95 % efficiency). Still, at this high efficiency an amount of oil of 2 % of the seed weight ends up in the seed meal fraction which means the oil content of the seed meal will be 5.5 %.

Seed hull separation efficiency crucial to obtain high oil extraction efficiency

Oil extraction efficiency was also determined on the nine accessions tested in the GxE trials (see WP1) and it proved that oil extraction efficiency varies among these nine accessions, probably because of differences in the efficiency of seed shell separation. The non-phorbol varieties BCGSA103 and UACH102 had the lowest separation of seed hull from the seed and as it was lower than 100 % of the seed hull fraction in the seed, some seed hull remained in the kernel fraction prior to oil extraction. With most Cabo Verde like accession (BCGSA101, DOPS101, DOPS102 and TNAU101) more than 100 % of the seed shell fraction was removed, which means that also part of the kernel was lost in the separated seed shell/seed hull fraction. The highest efficiencies of oil extraction occurred when the fraction of seed hull separated was close to 100 % or lower.

BCGSA103 had by far the highest efficiency of oil extraction, but still the extraction efficiency was only 76 %. The operational efficiency of seed hull separation and of oil expelling needs to be improved. In breeding, a low seed hull fraction in the seed should be a target to both improve the oil content of the seed and reduce the problems with seed hull separation. An optimised screw press expeller of this scale has a reported operational extraction efficiency of 80 to 90 %.

The conclusion from this oil extraction efficiency experiment is that the process of separation of seed kernel and seed hull should be very carefully optimised: too strict separation leads to loss of kernel in the seed hull fraction and too lenient separation will lead to higher forces needed to press out the oil and also then the oil extraction efficiency in total may be lower. The results indicate that it is better to leave some seed shell in the kernel fraction than vice versa.

Seed meal of these oil extraction trials was kept for the evaluation of seed meal as fertiliser.

Use of seed meal from demonstrator

A field test was carried out on farm in an established jatropha plantation (4 years old and fully mature) to evaluate the fertiliser value of jatropha seed meal in comparison with an equivalent NPK-supply with artificial fertiliser and in comparison with no application of fertiliser. The three levels of nutrient applications were: 1. No fertiliser, 2. artificial fertiliser (150 kg N/ha, 75 kg K/ha, 30 kg P/ha, and 3. jatropha seed meal (152 kg N/ha, 28 kg K/ha and 12 kg P/ha, based on protein content in seed meal of 50 %), all per year. The field test was located at a farm of Biocombustibles de Guatemala ('Las Canoas'). The seed cake was produced from jatropha seeds from this farm and defatted on farm with a screw press.
The seed yield was determined of each of 16 trees per treatment (grown at a density of 1250 trees/ha). Nitrogen content and oil content of the seeds was rather constant (at 2.23 % N in seed with seed hull and 5.6% N in seed kernel). N-uptake in seed yield was estimated and marginal N-recovery calculated.
The total seed an oil yield and the estimated N-uptake in the seed were much lower without fertiliser than with seed meal or artificial mineral fertiliser on a per area basis. No significant difference between the yields with mineral fertiliser and jatropha seed meal as fertiliser was found (seed yield was about 1200 kg per ha and oil yield 420 kg/ha, while with zero fertiliser the seed yield was only 420 kg seed per ha and 147 kg oil per ha) . Also N-uptake in seeds was significantly higher with mineral fertiliser and seed meal than without fertiliser (38 kg N/ha with both fertiliser treatment and only 13 kg N/ha with the zero fertiliser treatment). The marginal N-recovery of mineral was on average 16.4 % (this means that 16.4 % of the applied nitrogen was taken up in seed) and with jatropha seed meal it was slightly lower, 13.0 %). The total N-application was the same for the mineral fertiliser and the seed meal. As the seed and oil yields were also the same, this implies that organic N in

seed meal, it can be concluded that the fertiliser value of jatropha seed cake is equivalent to the fertiliser value of mineral fertiliser (at the same rate of NPK). .

Economic analysis on basis of demonstrator results

A full cost/benefit analysis was carried out to determine the feasibility and profitability of the two systems, using the cost estimates from the demonstrator trials with the standard density system and the improved high density system (with the new dwarf genotype).

This analysis followed the price structure for products as specified in JATROPTIMAL. These prices are based on five year average world market price for vegetable oil (with palm oil as a substitute product), artificial fertiliser N price as substitute product for the fertiliser value of seed meal.
A prospective analysis was made on the further advantage of future use of a dwarf genotype crossed with the non-phorbol ester line to obtain a dwarf, non-toxic genotype. When such genotypes become available, a more valuable use of the seed meal from a high density system will become possible. We have calculated how large the extra value would be from using such a non-toxic, dwarf type genotype that is amenable to use in a high density system with mechanical harvesting.

So, three cost/benefit analyses were made: 1. for traditional planting systems, with standard yields of about 2,500 kg seeds per ha and 750 kg extractable oil per ha, 2. the currently available combination of the new high density system with use of the new, dwarf genotype and 3. a future system with a high density in which a non-phorbol ester, dwarf genotype is used.

The cost of production determined from the demonstrator trial was used in the calculations (see above). To determine the benefits, historical market prices were used of substitute products. Palm oil as the substitute product for jatropha oil (five year average price of 650 € per ton palm oil), fertiliser price (taken at 1.00 € per kg N) and soybean meal (at 312 € per ton) was the substitute product for a non-toxic jatropha seed meal. The value of jatropha seed meal was even taken at 62 € per ton less, to account for a potential disadvantage of a new feed product in the market. It has been assumed that valuable use can be made of the lignocellulose co-products (harvested branches, fruit coats and seed hull). The current price of lignocellulose is about 50 € per ton and this value has been added to the benefits. A life cycle duration of 10 years was taken in all three cases. All investment costs were taken at the start, and benefits were converted to net present value using a discount rate of 10 %.

It proved that with the cost structure of BCGSA in Guatemala, the traditional planting systems has to high variable costs and even those variable costs are not covered from the proceeds of the sale of products at current prices. So, the traditional planting system leads to a net loss and is therefore not feasible (unless the price of jatropha oil would be above 2,500 € per ton).

The current new high density system with the new dwarf type has a good profitability with a net present value above zero already within a period of a couple of years. When the cost of acquiring land would not be pressing only on the first year (e.g. when an existing farm converts to jatropha production), a farm can already make a profit in the first year after sowing. The internal rate of return of this existing new system for a 10 year period is 22 % at a jatropha oil price of 650 € per ha. The benefits of a future system of a high density system with a non-toxic, dwarf genotype are much higher with an internal rate of return of 41 % for a 10 year period (and when the full cost of land acquisition is taken in the first year).

Conclusion on WP4

The combination of new, dwarf genotypes of jatropha and new high density cultivation gives huge opportunities of establishing profitable jatropha plantations, even in regions with higher wages as the cost of production is much lower as mechanical harvesting is possible and no pruning is needed. This may cause a breakthrough in the advancement of jatropha as bio-energy and feed crop.

4. Potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and the exploitation of results

JATROPTIMAL: a quantitative framework for analysing the impact of innovations in jatropha

JATROPT aimed to bring about an improvement in the use of Jatropha as industrial feedstock through innovations in genetics and agrosystem development. Various new technologies are now available to improve the efficiency of jatropha breeding and reduce the time to market when developing new varieties. A wealth of data has been generated on agronomy of jatropha and on the agro-systems in which jatropha is considered as an option. Before we discuss the potential impact of the technical results of JATROPT individually, we first discuss how the wider impact can best be analysed.

The potential impact of introduction of jatropha in production systems and the potential impact of individual technical innovations coming from JATROPT can only be analysed properly when the whole production system in which jatropha is introduced is taken into account. Jatropha introduction will change the whole farming system as it requires land and inputs which competes with land use and inputs for food production. Also, benefits for a farming system can be brought about as it can be envisaged that a jatropha production chain will be able to provide the necessary inputs to optimise jatropha production, for example by making fertiliser available for jatropha production. Such fertiliser input on jatropha may well improve the total plant mineral nutrient supply to the whole farming system, and food and feed crops may benefit from this. Therefore, it is insufficient only to analyse the economic and environmental net benefits of jatropha itself, as would be done in a Life Cycle Cost Analysis, but an analysis of the whole production system is needed (including the economic and environmental cost/benefit analysis of all downstream processes until the final bio-energy product as would be done in a standard LCCA). For this impact analysis, we have developed a quantitative analytical framework. The conceptual framework of the Sustainable Livelihood Analysis (SLA) was used. The SLA framework distinguishes between several aspects that should be included in analyse and action program for introduction of change (originally aimed at poverty reduction in developing countries). SLA requires the specification of several types of Assets, of the social, economic and institutional Context in order to allow the analysis of impact of alternative strategies (called Livelihood Strategies) for improving a set of goals (called Livelihood Outcomes). The set of livelihood outcomes we analysed consists of:

1. Income, 2. Employment, 3. Food supply, 4. Conservation of the natural assets (nitrogen and carbon stocks in the soil), 5. Net bio-energy production and 6. Net CO_2eq-emission reduction (including the N_2O-emission effect from N-cycling and fertiliser).

For 5 and 6, also the ratios of net output/gross output are determined as these ratios are criteria for bio-energy products under the RED policy of the EU). This approach has been implemented in a quantitative optimisation model (JATROPTIMAL) for farming systems (with or without jatropha) and the full jatropha production chain to enable the analysis of the impact of jatropha and jatropha innovations on this set of targets.

Using JATROPTIMAL, we have shown that jatropha can increase farm income of small farmers when they introduce jatropha in their farming systems that now only produce plant based foods and animals products. This contributes to poverty reduction. It proves that the trade-off with food production is less severe than might be expected (given that in our optimisation about 30 % of the farming system could be used for jatropha, when farmers optimise their net farm income). Food production in a well-designed system with jatropha can still be at 90 % of the food production of the same system without jatropha.

This remarkable result can only be obtained when full use of all harvestable jatropha biomass is made. Only using jatropha oil (and perhaps jatropha seed meal as fertiliser) leads to infeasibility of jatropha in the system as the value of products are not high enough then to cover the cost of production, even the farming systems we describe that only uses labour input from the farm family. Use of seed meal as feed and harvestable lignocellulose for bio-energy production improves the feasibility of jatropha to a great extent (allowing also lower jatropha oil prices, if need to improve the competitiveness of jatropha oil versus other plant oils or fossil oil).

The extra income with full use of jatropha allows higher nitrogen inputs, thus increasing the productivity per hectare and hence the profitability of jatropha. The extra nitrogen input also increases the total nitrogen input

level of the whole farming system. Crop residues and manure from using seed meal as feed can be used not only to fertilise jatropha but also the food and feed crops. JATROPTIMAL calculates how a farmer should optimally allocate the available nitrogen inputs to jatropha and food and feed crops. This redistribution of N-inputs through N-cycling of crop residues and manure brings an advantage to food and feed production.
Local processing of jatropha can contribute to employment and income from the oil extraction from jatropha seed and from the generation of bio-electricity from either jatropha oil with electricity generators that can use pure plant oil (system demonstrated in the Mali site of JATROPT). The local bio-electricity also brings indirect advantages locally that were not taken into account. The lignocellulose residues of jatropha are a large part of the biomass produced (27 % of the annual biomass production) and consists of pruning wood, fruit coats and seed hull. In comparison, the oil production of jatropha of traditional varieties is only 11 % of the annual biomass production. The amount of energy produced in harvestable jatropha lignocellulose is therefore about the same as in jatropha oil. Making optimal use of this lignocellulose bio-energy source (available processes have been reviewed by Jingura et al., 2010) is essential for a good environmental performance of jatropha as we have shown with JATROPTIMAL.

Here, we discuss one example of how impact of innovations can be analysed using JATROPTIMAL: the comparison of a system with and without jatropha with three jatropha variants. The calculations were done for a system with three soils types, a good clay soil, a shallow soil with medium water holding capacity and a deep sandy soil with poor water holding capacities. This is a farming system that could easily be found in large parts of Africa and thus not yield very high yields at the chosen annual rainfall of 800 mm (with an erratic rainfall pattern)..

From this analysis, it is clear that
1) Jatropha can improve the livelihood of farmers in areas with low quality soils or low or erratic rainfall,
2) In the optimal solution, jatropha will be grown on the most marginal land,
3) Jatropha bio-energy has a high net to gross output ratio, 4) the net CO_2eq-emission reduction is about 66 %,
4) Use of lignocellulose and of seed meal as feed contribute strongly to the feasibility of jatropha,
5) Increasing seed yield per ha should have a high priority in breeding programs (for example by improving the harvest index of seed to biomass),
6) Tools like JATROPTIMAL that integrate all knowledge on primary production, environmental processes and economics are essential enabling technologies for designing optimal jatropha systems

Specific impacts from technical S&T results

Impact 1: Diversity studies and global evaluation of germplasm

The molecular genetic biodiversity analysis of world-wide germplasm of jatropha achieved in this project allows selection and development from a broader genetic variation and the sharing of knowledge allows more efficient breeding.
The Genotype x Environment analysis (continents, countries, different climates within countries) has shown that in high yielding environments most suitable to jatropha, GxE interaction is present, but does not have an over-riding effect on genotype performance order. Although locally adapted varieties will be needed to attain the final genetic potential, existing advance germplasm developed in different locations show the same ranking in general when tested at various locations.

Impact 2: Discovery of a new, dwarfed genotype, high yield in a new high density system

In Guatemala, the standard production system of jatropha (with 1,250 trees per ha) is too expensive as it requires too much hand labour and the wages in Guatemala are too high for such a system to be profitable. A new high density system has been developed, but it proved that with standard varieties no flowering occurred. A remarkable discovery was done by finding new, dwarfed genotypes that can be used in a high density system (33,000 plants per ha). The jatropha crop can be established using direct seeding. Fruits can be harvested by harvesting the new branches using a sugar cane cutter or maize cutter and the fruits can be mechanically separated from the branches. From that point, standard process for seed and fruit coat separation and oil extraction can be used. This new system has proven to give a yield of 1,500 kg oil per hectare in the first year after sowing (4,500 kg of seed per ha) and 3.000 kg of oil per hectare (9,000 kg of seed per ha) in the second year (and probably also in the years after). The cost of production per hectare with this system is 30 % lower than with the traditional system and because the production per hectare is so much higher, this system has a very high profitability as determined using a discounted cash flow analysis.

This innovation can cause a breakthrough in jatropha production. The dwarf varieties will be developed into registered varieties and Biocombustibles de Guatemala will start commercial exploitation after plant breeders' rights have been obtained.

Impact 3: A genetic map of J. curcas and QTL markers

JATROPT has produced the world's first public genetic map of *J. curcas* only (previously an interspecific cross with J. interregima had to be used due to lack of genetic variation in Asian jatropha materials). The DNA-markers for the non-phorbol ester trait and the quantitative yield related traits will allow plant breeders to start marker assisted breeding, which will allow rapid introgression of desirable traits into 'elite' lines and decrease the time-to-market of breeding efforts.

Impact 4: Sustainable agro-systems

The project has collected a wealth of agronomic data which will increase the adoption and sustainable use of Jatropha. The data have been used to develop a mechanistic (based on crop physiological process quantification) simulation tool with which a world map of suitability for jatropha has been created and which can serve people wanting to start up a jatropha production chain to estimate the production potential in their region.

Impact 5: Ability to use jatropha meal as animal feed after oil extraction

The project has identified non-toxic jatropha lines and evaluated their performance in various field conditions. The Guatemalan non-toxic line already has a medium to good productivity, while the Mexican non-toxic lines have a poor yield performance outside Mexico. DNA-markers have been identified that can be used for introgression of this trait into high yielding varieties. It is now technically possible to use jatropha meal as an animal feed after a toasting process similar to that needed with soybean meal (Mexican local communities have used the non-toxic jatropha seeds already as a food snack for a very long time) . However, in many countries evidence on the safety of using the seed meal as feed has to be formally provided before it will be allowed to feed animals with jatropha seed meal.
The analysis with JATROPTIMAL shows how big the advantage will be on farm income, food production (as seed meal now is converted to animal products) and bio-energy production.

Impact 6: Economic cost/benefit, Life cycle and Sustainable Livelihoods Analysis

The project has provided an economic cost/benefit analysis of various jatropha production systems in India, Mali and Madagascar. The data have been used to feed JATROPTIMAL which can now serve as a routine analysis tool to study economic and environmental costs and benefits for a variety of jatropha systems. This allows people to carry out economic

Special attention has been given to the food versus fuel debate as fears exist that introduction of large scale jatropha production will be detrimental to food supply.

We have shown using JATROPTIMAL that with full use of all valuable products of jatropha (lignocellulose co-products, seed meal as feed and of course jatropha oil for bio-fuel) a jatropha/food/feed farming system can be designed that whilst optimising net farm income (by using about 30 % of the poorest soil in the system for jatropha) the system can still supply 90 % of the food a system – also with an optimised net farm income - without jatropha can produce. However, when very highly productive jatropha varieties would be created, jatropha could become so competitive that regionally jatropha could drive out food production. Still, as food production is not always maximised per hectare of land, there is room for increased productivity of food production. Our analyses have shown that jatropha indeed can bring about an increase in the mineral nutrient supply to the farming system as jatropha introduction leads to higher nitrogen inputs (and other plant mineral nutrient as P and K) and improved productivity for example compared to extensively grasslands in the farming system.

Impact 7: Contributing to Millennium Development Goal 1 'Eradicate poverty and hunger'

Depending on the method and scale of bio-energy production in developing countries, bio-fuels may replace expensive fossil fuel imports, generate foreign currency, and / or may be used for different purposes in rural areas, such as the mechanisation of agriculture, transportation, irrigation, (food) processing, electrification (lighting) and small industrial activities. The production of bio-energy is an attractive option for developing countries to increase income of their poor rural population that currently depends on agriculture for their food and income (as shown in the Indian economic studies in JATROPT and shown with the more general analysis tool JATROPTIMAL).

Impact 8: Contributing to Millennium Development Goal 7 'Ensure environmental sustainability'

JATROPT has analysed various sustainability aspects of introducing jatropha. The analysis with the assessment tool JATROPTIMAL shows that jatropha can provide bio-energy and CO2-emission reduction at high efficiency (88 % net/gross outputs for bio-energy and 65-70 % net/gross CO2-emission reduction) above the levels required from 2017 under the RED policy of the EU.

Impact 9: Impact on EU bio-energy and climate change policy

The project has contributed to improvement of jatropha production system. This will help to increase the bio-fuel output, net energy production and greenhouse gas-emission reduction per hectare and the efficiencies of bio-energy production and CO2-emission reduction as required by the EU sustainability criteria for bio-energy and climate change.

Direct impact immediately after the project

The direct impact of the project will be:
1) Development and dissemination of improved genotypes,
2) Use of the of improved agrosystems and tools for designing locally adapted optimal agrosystems,
3) Use of the data and tools to determine economic cost/benefits and to evaluate sustainability criteria.

Special attention should be given to promote the first phase of the further development of jatropha. It will take about five years before new varieties can be fully commercialised. The development of new production chains with jatropha will require a high level of co-operation between end users and all stakeholders in jatropha production chains. Investments are needed in plant breeding and local processing facilities for oil extraction and facilities that make full use of all jatropha products, including seed meal as feed and lignocellulose co-products as source of bio-energy. The potential is present, but developing all knowledge and tools from JATROPT in innovations that are practically commercialised will not only require investments by private investors, but also support from public sources. Without a good support environment, the risk is high that the innovations prepared by the results of JATROPT will end up in the 'Valley of Death'. International policy support will be extremely helpful overcoming the 'Valley of Death' which often destroys the possibility of bringing the innovations to commercialisation and practical use.

Dissemination from JATROPT

Publications in peer reviewed journals

Andrew J. King, Luis R. Montes, Jasper G. Clarke, Julie Affleck, Yi Li, Hanneke Witsenboer, Edwin van der Vossen, Piet van der Linde, Yogendra Tripathi, Evanilda Tavares, Parul Shukla, Thirunavukkarasu Rajasekaran, Eibertus N. van Loo, Ian A. Graham. 2013. Linkage mapping in the oilseed crop *Jatropha curcas* L. reveals a locus controlling the biosynthesis of phorbol esters which cause seed toxicity. Plant Biotechnology Journal, 11: 986-996.

R. E. E. Jongschaap, R. A. R. Blesgraaf, T. A. Bogaard, E. N. van Loo, H. H. G. Savenij. 2009. The water footprint of bioenergy from Jatropha curcas L. Proceedings of the National Academy of Sciences of the United States, 106: E92-E92.

Luis Montes Osorio, Andres Torres Salvador, Raymond Elmar Jongschaap, Cesar Azurdia Perez, Julio Berduo Sandoval, Luisa Trindade, Richard Gerardus Visser, Eibertus van Loo. 2014. High level of molecular and phenotypic biodiversity in Jatropha curcas from Central America compared to Africa, Asia and South America. BMC Plant Biology, 14: 77

Y.B. Vera Castillo, H.W. Pritchard, A. Frija, P. Chellattan Veettil, J.A. Cuevas Sanchez, P. Van Damme, G. Van Huylenbroeck. 2014. Production viability and farmers' willingness to adopt Jatropha curcas L. as a biofuel source in traditional agroecosystems in Totonacapan, Mexico. Agricultural Systems, 125: 42-49

Conference papers

E.N. van Loo (Robert), A. Kobayashi, A. King, R. Pirot, P.van der Linde, H. Witzenboer, L. Montes, J. Berduo, D. Ramanana, J. Axayacatl Cuevas, M. Paramathma, R.E.E.Jongschaap. 2011. Jatropha curcas: Applied and Technological Research on Plant Traits. II Brazilian Congress on Jatropha Curcas Research.

Van Loo, E.N. Salentijn, E.J.M., Krens, F.A., Jongschaap, R.E.E. 2011. Novel Oilseed Crops Breeding for Industrial Use in Calendula, Crambe and Jatropha. 9th Euro Fed Lipids Congress: Oils, Fats and Lipids for a healthy and sustainable world. Rotterdam.

Other publications

Marília Folegatti Matsuura, Gil Anderi Silva, Luiz Alexandre Kulay, Bruno Galvêas Laviola. 2011. Life Cycle Inventory of Physic Nut Biodiesel: Comparison Between the Manual and Mechanised Agricultural Production Systems Practiced in Brazil. In: Towards Life Cycle Sustainability Management. Springer Netherlands, Dordrecht, p. 425.

J. Axayacatl Cuevas. 2013. Jatropha curcas Handbook in the Totonacapan. I Introduction; II Chut´a (Jatropha curcas) and its functions in the sustainability of traditional agroecosystems; III. The protection of Jatropha curcas non-toxic genotypes. Universidad Autónoma de Chapingo, Chapingo, Mexico.

MSc-theses

Ten MSc-theses have been written on results analysed of the trials in Mali of CIRAD.

Phd theses

Two Phd theses are currently being prepared, one by Luis Montes (director of Biocombustible de Guatemala) on biodiversity and QTL-analysis in jatropha and one by Jaspeer Clark at the University of York on QTL-mapping in jatropha.

Disseminatinon activities

Further, about 21 other dissemination activities have been organised or contributed to from invited speaker presentations at congresses, organizing workshops, publications in national newspaper and in the popular scientific press, TV-broadcasts, DVDs and movies.

Exploitable results

The following exploitable foregrounds have been recognized and exploitation plans towards their valulable use have been made:

1. New dwarf genotype that allows mechanical harvesting, has seed yields of 4.5 ton/ha and oil yield of 1.5 ton/ha in the first and 3-4 ton of oil per ha in second and later years.
2. Integrated molecular biodiversity study a world-wiede collection of jatropha genotypes.
3. Genotypes and knowledge on feasibility of their cultivations across a wide variety of climates and soils from the GxE trials performed.
4. DNA-markers linked to the non-phorbol ester trait.
5. A DNA-marker map with QTL for important agronomic traits of jatropha
6. New crosses in a diallel scheme (e.g. non-phorbol ester x advanced toxic jatropha)
7. Agronomy data in a wide variety of environments.
8. Outcome of Life Cycle Analysis for specific regions and routes to bio-energy products.

9. A crop physiological simulation model calculating the potential (light limited) biomass and seed yield of jatropha and the water-limited yield levels.
10. A quantitative optimisation analysis framework for developing optimal designs of jatropha production systems. The model integrates crop and animal production, the full downstream processes towards bio-energy products and a full economic and environmental cost/benefit analysis.
11. A descriptor list to facilitate morphological assessment of distinction, uniformity and stability of jatropha accessions and varieties (useful for plant breeders and variety registration offices).

5. Acknowledgements

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Various partners have receive funding to contribute to the own contribution needed next to the EC grant and the partnership acknowledges the support from national governments in India, Madagascar, Brazil, Mexico, Guatemala, the United Kingdom, Belgium (supporting the Quinvita Group), France and the Netherlands. Also the companies that were partner in JATROPT - Biocombustibles de Guatemala, Bionor Transformacíon, Quinvita Plant Sciences Ltd and Keygene - have contributed with own funding and this support to JATROPT has been of tremendous importance.

ANNEX 1. Compliance with the Ethical Review Committee

In the reporting, it was indicated that it should be made clear how the project has complied to the recommendations of the Ethical Review Committe. The questions of the Ethical Review Committe were already answered prior to the final preparation of the Grant Agreement and the assiociated Description of Work. Here we cite the discussion and resolution of the issues raised by the Ethical Review Committee from the Description of Work.

During the preparation of the Grant Agreement, the Ethical Review Committee (ERC) of the European Commission has reviewed ethical issues of JATROPT. JATROPT uses a world-wide collection of accessions supplied by the Beneficiaries. One beneficiary is involved in collecting new accessions in local communities in Mexico (dr. J. Axayacatl Cuevas of the University of Chapingo and head of the jatropha germplasm collection of the University of Chapingo). The germplasm bank has full authority and permission from the local communities to carry out this germplasm collection in the local communities. He is an ethnobotanist, specialised in analysing biodiversity of local communities and in his projects with local communities he is supporting the local use of jatropha and helps the communities to optimise their production system. Because such collection would take place in JATROPT, the ERC wanted to be convinced that we have the proper authorisation to use the full collection of accessions and that we would make sure the results would also benefit local communities in which these accessions (sometimes long ago) were collected.
Below you will find our response to questions from the ERC on this (excerpt from the Description of Work of JATROPT) with some additions on how JATROPT has operated.

Use of local resources

For the biodiversity and breeding of jatropha, use will be made in part of local resources. All partners with germplasm collection are owners of the plant materials or have explicit written permission of the donors to the collection for using their materials in research. The consortium follows all relevant national laws on biodiversity and respects the Convention on Biological Diversity ideas.

Benefit to local communities

The project will lead to capacity building local research organisations (partners in this project), access of local research to international scientific community in jatropha and access to high tech equipment and capacity building in use of genomics and genetic research technologies.
After questions raised in the ESR, the Ethics Review Committee has reviewed our ethics principles. Here we summarise the basic conclusions of that discussion:

1. Ethically justified conduct within JATROPT requires various levels of permissions from authorities to obtain approval for carrying out the research:

Level 1 Permission: ('license to produce')

Partners carrying out field trials should make sure that cultivation of jatropha is allowed by law or by explicit approval by the relevant authorities. The co-ordinator has obtained documents from all partners involved as to the form of approval all partners have. All partners have obtained such approval.
Level 2 Permission (Access Rights to local materials for research)
For new materials brought in by partners, partners will seek to obtain Access Rights in accordance with the Bonn guidelines derived from the Convention on Biological Diversity. This means that for all materials to be used in research collected after 1993, permission for collection and use in research should be obtained from a Competent National Authority or government body with similar authority. Notification of this CNA (or similar body) should preferably be done prior to collection or use (Prior Informed Consent should be obtained). Partners have indicated that they have obtained such permission. The way such permission can be obtained is very different in different countries. The co-ordinator is informed about all permissions by partners. For Jatropha materials brought in at the start of the project, partners have indicated to have obtained permission for use in research of have indicated that by law there is no need for an explicit permission as the research is anyhow permitted.
The Bonn guidelines suggest including a Benefit Sharing Scheme in the process to obtain Access Rights. The responsibility for the procedure to obtain Access Rights lie with the governments and these may deviate from the Bonn guidelines.

JATROPT intends to make public all information obtained in the project. New material developed from crosses will be owned by JATROPT partners and they may seek to obtain Plant Variety Protection. This is laid down in the Consortium Agreement. Genetic maps and first QTLs found have been published in Free Access journals and also other results have been made public through websites, articles in the popular press, videos and TV-broadcasts, presentations on congresses and scientific publications. Also after the end of JATROPT new publications on the results of JATROPT will be published with free access.

Level 3 Permission (Right to commercially exploit locally collected materials)

In case - to be expected only after the project - commercially viable materials are identified or developed, and only Access Rights for research are obtained for such materials, partners will seek to obtain permission to commercialise from the relevant CNA, using the Bonn guidelines as a guideline to develop Access Rights for commercialisation with a proper Benefit Sharing Scheme. It remains the responsibility of each partner to make sure the relevant regulations and laws are obeyed.

4.1 Ethics Review Report (ERR)

The Ethics Review Report indicated the following requirements:

1. Clarify whether personal data in the farmer survey will be collected and obtain appropriate authorizations copies of which should be forwarded to the European Commission.
2. Forward to the European Commission copies of all relevant Material Transfer Agreements obtained as part of the research.
3. Provide the Commission copies of all IPR exploitation agreements (including Benefit Sharing provisions) that are being made between partners in this project.

4.2 JATROPT reply to the Ethics Review Report

1. It is not planned that personal data in the farmer survey will be collected, and in case it would be required to collect these data, proper authorization will be sought.
2. Relevant Material Transfer Agreements will be copied to the European Commission; rules for Material Transfer for this project will be arranged in the Consortium Agreement, which will be sent to the European Commission after signing by all partners.
3. Agreements on existing IPR exploitation agreements between partners that were in place before the start of this project are considered confidential and cannot be disclosed. In case Background falling under such agreements is brought, this Background is explicitly described in the Consortium Agreement, which will be sent to the European Commission. The Access and Benefit Sharing agreements are probably most relevant between a partner or partners on the one hand and Competent National Authorities on the other hand. Relevant documentation will be provided on material used in JATROPT by partners bringing in such jatropha materials. As material can continuously be brought into the project, such documentation will be provided in the Annual Reports of the year in which such material was brought in. Considering IPR exploitation agreements between partners for Foreground of this project, the Consortium Agreement describes the Access Rights and remuneration to owners of Foreground, according to principles set out in the IPR management part of this document.

4.3 ERR remarks to the JATROPT reply on the Ethics Review Report

The Ethics Review Report states that 2 points are still not clear:

1. The suggested germplasm bank to be located in Mexico would presumably need appropriate national authorization. There is no mentioning of this issue.
2. It is not clear whether personal data in the farmer survey will be collected, in case of which appropriate authorization should be obtained."

4.4 Reply to ERR remarks on comments ERR report

1. Mexico already has an existing germplasm bank for national collections of Jatropha with a mandate to collect and maintain. This national germplasm bank has strict rules on how to give access rights to the Mexican material. In case, partners of JATROPT want to contribute materials to this existing gene bank, it should be made clear by the contributing partner they have a right to submit the materials and the appropriate export/import regulations should be followed; no further national authorization for the establishment of that germplasm bank is needed. This would be different if the Mexican germplasm bank would want to adopt a strategy of free access to all materials in the germplasm bank, this would require proper national authorization.
2. It is not planned that personal data in the farmer survey will be collected, and in case this would become the case, proper authorization will be sought.