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**Jatropha curcas L. accessions in central-Java, Indonesia;  
A selection for future breeding programs.**



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Cover: Picture of the team and myself during  
fieldwork in the breeding trial. (27-12-2013)

**Jatropha curcas L. accessions in central-Java, Indonesia; A selection for future breeding programs.**

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## Disclaimer

The original subject for this thesis was on the comparison of differing *Jatropha*-inclusive farming systems found in Gunungkidul province using a biophysical and economical assessment aiming to comment and compare the productivity and profitability of each system. After a month in the field it proved to be practically impossible to assess and the research proposal was altered. Before the used research proposal and methodology were developed and field work could begin, around 9 weeks of time were “lost” and below a description is given as to how and why.

As a starting point a list of thirty farmers located throughout the Gunungkidul province, gotten from a 2011 Msc thesis from students of the Universitas Gadjah Madah Jogjakarta (UGM), was provided by supervisor Juliana Tjieuw. From this list 21 out of the 30 farmers were visited, each with a similar story and situation. The *Jatropha* grown in their fields was dead, uprooted, diseased or overgrown by jungle making analysis of the crop impossible.

Once it became clear that the allocated fields were unusable, new research areas and research questions were needed. By this time the raining season and florescence had started and great haste needed to be made to label/maintain the plots and collect the harvest. This was a stressful period which I spent driving around at random with scooter and GPS looking for suitable areas. Thanks to Juliana, Maja, Ibu Rully and the Gunungkidul forestry department, two new research areas (The breeding trial which originally contained 12 accessions all 3 years old and the seed garden containing 2 accessions aged 5 to 7 years) were found and the focus of the study quickly changed from farming systems to accession breeding. Unfortunately both sites were in poor condition (7 out of the 12 accessions in the breeding trial were dead/unusable and in the seed garden >90% of the trees had died). Due to the timeframe, workers were needed to help with measuring which required the selection of suitable candidates as well as giving a 3 day training to explain and check their work.

By this time it was the end of December and the research could be started. Below a list of moments/events that were cause for additional delay:

- Beginning of study. I had some trouble with obtaining my research visa and needed to fly back to Jakarta costing about a week.
- Whole period. Trees started shedding leaves upon florescence due to nutrient shortage and at the end of the fieldwork all LAI measurements required a do over.
- Whole period. The seed garden was a piece of government owned land containing 13ha of *Jatropha* trees and was located next to a small village. Despite of governmental warnings, farmers reclaimed pieces of land piece by piece. Random patches of land became fertilized throughout the experiment and the trees were either uprooted or became intercropped. This caused that a new selection of trees needed to be made and that all measurements needed to be done again.
- January 13<sup>th</sup>. A tropical storm had knocked off most of the harvest making it hard to properly determine harvest per tree as well as accounting for the difference in seed weight (since some capsules were soaked and covered in dirt).
- January 28<sup>th</sup>. A rainstorm caused the whole breeding trial to become waterlogged causing trees to die and shed what little leaves they had.
- February 13<sup>th</sup>. Volcano on Mt. Kebul erupts covering everything in a thick layer of ash as well as spewing ash for 5 days. Everything had come to a halt for a full week.
- March/April. All gathered data was written down into logs and was manually entered into excel. Processing the data and creating the needed excel-template took up a lot of time. This because it was sometimes hard to follow the logs and the amount of information per tree was huge.

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## Abstract

This study, conducted in Gunungkidul Indonesia, focused on six *Jatropha Curcas* L. accessions and analysed performance in accordance with the central-Javanese climate and the aim to create a selection for future breeding programs. Through non-destructive aboveground measurements and observations nine parameters were determined and used to compare accessions, labelled A through F, on productivity and vegetative traits. Accessions A to E were organized in 4x4 plots with 2 repetitions each and located in a breeding trial, F was located in a seed garden where 45 trees were hand-picked.

For B, D and E recorded harvest was low in comparison to planting density. In contrast to B and D, E scored poorly on vegetative traits as well having the lowest average accumulated biomass tree<sup>-1</sup>. Accessions A and C showed a contrasting pattern with lower biomass, surface area per tree and higher yields implying a (genetic) prioritisation for the allocation of resources to the generative traits making them suitable candidates for cultivation. A itself has a deviating tree shape with relatively low biomass and surface area per tree but a large tree height. No significant differences were found between A and C and data on F proved to be inconclusive due to a large variability amongst trees.

Further findings include the confirmation that the “quick and dirty” canopy volume estimation is an accurate method for predicting tree biomass, a relation exists linking the fraction of total accumulated biomass volume (fr. BO) to each branching order group where  $\text{fr. BO}_n \approx \frac{1}{2} \text{ fr. BO}_{n+1}$  and that this relation is influenced by tree age. Final analysis comparing vegetative traits with allometric equations obtained from literature showed that (Ghezehei et al. 2009) is more in line with data than (W.M.J. Achten, Maes, Reubens, et al. 2010) These findings can be used as a stepping stone for harvest prediction as well as increasing the level of detail used in formulating allometric equations.

**Keywords:** *Jatropha Curcas* L., accessions, productivity, allometric equations, Indonesia

## List of abbreviations

BT	= Breeding Trial	(-)
SG	= Seed Garden	(-)
SD	= Stem Diameter	(mm)
TH	= Tree Height	(mm)
PD <sup>-1</sup>	= Surface area per tree	(m <sup>2</sup> tree <sup>-1</sup> )
PD <sup>-1</sup> -T	= Surface area per tree with Jatropha trees only	(m <sup>2</sup> tree <sup>-1</sup> )
PD <sup>-1</sup> -OC	= Surface area per tree including other crops	(m <sup>2</sup> tree <sup>-1</sup> )
D	= Disease expressed as percentage of total tree	(%)
TCV	= Total Canopy volume	(m <sup>3</sup> )
LAI	= Leaf Area Index	(m <sup>2</sup> leaf m <sup>-2</sup> )
TAB	= Total Accumulated Biomass Volume	(cm <sup>3</sup> )
Model total	= Total modelled harvest per plot	(g)
Actual total	= Total measured harvest per plot	(g)
SDW	= Seed Dry Weight	(g)
RB	= Representative Branch	(-)
x.x.x	= Branch with nr of x's stating order	(-)
L	= Branch length	(mm)
b <sub>1,2</sub>	= Branch bottom diameter	(mm)
t <sub>1,2</sub>	= Branch top diameter	(mm)
CB (#)	= Capsule bundle	(# capsules)
tBL	= Total Branch length	(mm)
IBL	= Leafy Branch length	(mm)
BOx	= Branching Order group x	(-)
#L	= number of leaves	(-)
L <sub>x</sub>	= Leaf number x	(-)



## Introduction

*Jatropha curcas* L., also known as physic nut, is classified as a tropical bio fuel crop originating from Mexico and Central-America. It belongs to the Euphorbiaceae family (Table 1) and was first discovered by the Portuguese in the 16<sup>th</sup> century for its medicinal properties. Later on it was cultivated to produce soap and lamp oil. *Jatropha*'s germ plasm was spread across the globe via Portuguese colonies such as Mozambique, Angola and later also via trade to India and Indonesia (W.M.J. Achten *et al.*, 2010; PROTA Foundation, 2008). Throughout the years it has served many functions including erosion control, living fence, green manure and combustibles, but the focus of cultivating *Jatropha* purely for bio fuel is something from the last two decades (Jongschaap *et al.* 2007). The biofuel is made by pressing the harvested seeds to extract an oil which can be processed into a liquid bio-fuel or biodiesel meeting European standards with reduced greenhouse gas emissions. The side products consist of a press cake that can be used as fertilizer with the possibility to digest any remaining organic waste to produce biogas (Achten *et al.* 2008). Besides the energy products *Jatropha* possesses water conserving strategies which, in combination with its tapped rooting system, make it an effective measure in preventing soil erosion and maintaining soil quality (Díaz-lópez *et al.* 2012). These benefits showed promising traits for *Jatropha* as a biofuel-crop with relatively low agro-economical demands and explains why the small tree or large shrub had become the focus of large planting programmes in several tropical countries (PROTA Foundation 2008). In 2006 *Jatropha* was identified as the undiscovered star in the alternative-fuel market (Barta 2007) and a total of 103 large scale cultivation sites were established in Africa, Asia and Latin America between 2006 and 2011 (Wahl *et al.* 2012). Once it became apparent that expected production levels were not being reached, *Jatropha* lost its title due to insufficient economic viability resulting in discontinued projects and the absence of new investments (FAO 2013; Slingerland & Schut 2014; Vel *et al.* 2013).

Table 1 Overview of *Jatropha* nomenclature. (TropCrop2.3.5 2014)

Species	<i>Jatropha curcas</i> L.
Subfamily	Crotonoideae
Family	Euphorbiaceae
Class	Dicotyledonae
Plant type	Shrub to small tree
Common name	Physic nut

The “current” plantations show large fluctuations in both crop characteristics and yields and in spite of several breeding programs, no high oil producing stable strain has been developed so far. Although climatic conditions are well documented, the response of *Jatropha* remains unknown thereby inhibiting yield predicting methods from future plantations (Jongschaap *et al.* 2007). The lack of knowledge on crop management and cultivar breeding can be seen as a knowledge gap remaining the largest issue in the establishment of long-term viable *Jatropha* production systems. The selection of certain accessions fitting a multitude of climates and agro-ecological zones is a topic which will need more study before any success can be achieved (W.M.J. Achten, Maes, Aerts, *et al.* 2010). In order to produce *Jatropha* in commercial quantities, marginal soils with low input will not suffice and knowhow is needed to determine best management practices and key factors impacting yield (Iiyama *et al.* 2012). Examples here are the effects of irrigation frequency, nutrient application, pruning intervals or intercropping potential. A study conducted by Behera *et al.* (2010) applied mycorrhiza fungi to the roots of young seedlings combined with an addition of fertilizer amendment with thicker stems and increased plant growth with higher biomass and yield as a result. An example of the breeding knowledge gap can be illustrated by Kaushik *et al.* (2007) who analysed seed traits of 24 *Jatropha* accessions by comparing phenotypic and genotypic variation and found significant differences in seed size and oil content of the seeds. Others findings revealed variability in phenotype to be higher than in genotype indicating the impact of environment to be greater than that of hereditary traits and that *Jatropha* mainly exists as a wild variety. A quick way forward is proposed by Mishra (2009) by applying a more qualitative analysis for initial selection for an improved variety by using paired comparison based on a limited amount of traits. Here trees were evaluated on ‘n’



criteria where individual trees are scored as better (1), equal (0) or worse (-1) than the other. A weight is added to each trait defining importance (e.g. seed yield =8, tree height = 1) and all traits are summed yielding a total score.

Once the first breeding selection has been made from wild- to improved varieties, a start can be made on the more in depth testing and selecting of the acquired base gene pool. Important here is to state that accessions used for breeding trials should be planted in several locations to account for differing climatic conditions and to enable scouting for a productive strain that can fit a multitude of climates (W.M.J. Achten, Maes, Aerts, et al. 2010). This type of cultivar development is the central theme of this study with the objective of assessing the performance of *Jatropha* accessions for the central-Javanese climate and making a selection to use as input for (the continuation of) future breeding programs. More specifically, six *Jatropha* accessions grown in a monoculture setup and planted in two locations on central-Java, Indonesia are compared on the basis of non-destructive aboveground measurements (allometry) and harvested yields. The accessions are part of a long-term multi location trial and this study is part of a PhD program related to the “*Jatropha* Research and Knowledge Network” (JARAK) coordinated by Leiden University. JARAK consists of a multi-disciplinary team investigating *Jatropha* via social, economic and agronomic research.

### Main objective

Analysing accession performance on the basis of non-destructive aboveground measurements and yields and making a selection for future breeding programs.

### Sub questions

- How do the vegetative traits of accessions compare?
- How do accessions compare in terms of yield and productivity?
- Are there correlations linking the allometric and yield variables?
- Can a relation be identified linking the groups of branch orders?
- Can harvest be predicted based on found allometric relations?

## Methodology

All work conducted for this thesis research was divided into three periods. a) The general setup, research site scouting and adjustment of the research proposal, b) the field work and data collection done in central-Java, Indonesia and c) Data analysis and typing report done in the Netherlands.

### Research sites

The research area consisted of two sites located in central-Java, Indonesia around the town of Wonosari in the Gunungkidul regency. Geographically, Gunungkidul is located between 7°46'8°09' South latitude and 110°21'-110°50' East longitude. Sites were labelled as the Seed Garden (SG) located in Paliyan district and the Breeding Trial (BT) located in Wonosari district (Figure 1).

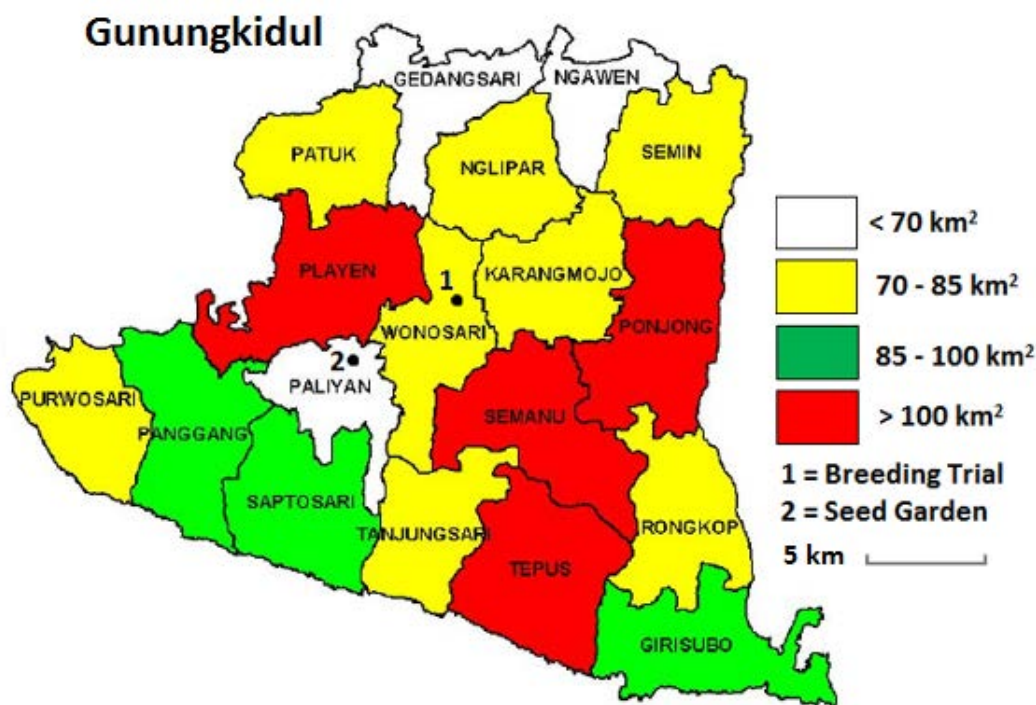


Figure 1 Topography of Gunungkidul province with location of research site with scale (BPS-KG 2012).

Soil types were defined by using a range of soil maps provided by the FAO. Here it was found that the breeding trial soil contains Red Mediterranean soils and Lithosols (calcareous materials) and that the seed garden soil is made up of a combination of soil complexes, including regosols (volcanic ashes), lithosols and andosols (Food and Agriculture Organization 2014). A note here is that although information comes from a reliable source, proper soil analysis will be needed to determine local soil types with certainty as soil composition tends to fluctuate.

The Food and Agriculture Organization (2014) classifies *Jatropha* cultivation in central-Java as a rain fed upland agricultural system emphasizing the importance of a steady water input. The central-

Table 2 Precipitation values of research areas and for Gunungkidul province in mm t<sup>-1</sup>. (BPS-KG, 2012)

Rainfall (mm) per district in 2011													
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
<b>Paliyan</b>	395	512	307	222	148	-	-	-	-	20	146	433	2183
<b>Wonosari</b>	413	429	305	254	153	-	-	-	-	22	285	203	2064
Rainfall (mm) average Gunungkidul 2006-2011													
<b>Gunungkidul</b>	246	296	248	184	114	44	33	30	106	93	178	301	1873

Javanese climate is defined as tropical with a high relative humidity of 70-90% and an average annual temperature of 28°C with only little fluctuation throughout the year (BPS-KG 2012). Seasonal rainfall varies greatly and is based around the monsoon with a dry season from June to October and a rainy season from November to May (Table 2). According to W. M. J. Achten et al. (2010) primary growing conditions of *Jatropha* have been defined as the climate variables for the locations where *Jatropha* is naturally distributed. For optimal crop production the variables need to be in the following range: Temperature  $T_{\text{mean}} = 23.4\text{--}26.2$ ,  $T_{\text{min}} = 14.4\text{--}19.4$  and  $T_{\text{max}} = 31.5\text{--}34.0$  all in °C, annual precipitation between 1207 and 2001 mm year<sup>-1</sup> and the length of the growing season (LGS) between 6-9 months. Important to note is that *Jatropha* can flower multiple times during a growing season, but requires a steady input of water to maintain its inflorescence (Raju & Ezradanam 2002). When comparing the optimal primary growing conditions to the ones found for central-Java, one can see that temperature, precipitation values and LGS show overlap indicating an opportunity for physic nut production.

### The accessions

The *Jatropha* accessions used in both research sites originate from conventional breeding experiments done in Indonesia executed by Argo Inovasi. Here the variety improvement protocol is collecting germplasm, selecting suitable candidates for the growth of an improved population and breeding with individuals belonging to this population to obtain more developed generations of progeny which are tested in multi-location trials. Main selection criteria used are the average number of capsules per shrub and the yield potential in tonnes per hectare. Different locations were added to reflect upon performance of accessions under varying environment. The accessions all have a code containing used start population, location and generation (Table1). The breeding trial accessions are third generation and belong to a current breeding program, due to intellectual property rights the names of these accessions have been replaced by letters A through E. The accession from the seed garden is IP1A and will be denoted as F.

**Table 1 Accession coding (Pers.Comm. Dr. Rully Dyah Purwati and Juliana Tjeuw)**

Coding used by Agro Inovasi	
IP <sub>x</sub>	Improved population where $x = x^{\text{th}}$ generation
P	Pahuwon, representing a wet climate (west-Java)
M	Muktiharjo, representing a moderate climate (central-Java)
A	Asembagus, representing a dry climate (east-Java)
IP3A	3 <sup>rd</sup> generation accession selected in the dry climate of east-Java
Coding used in study	
A <sub>x</sub> to F <sub>x</sub>	Accessions where $x = x^{\text{th}}$ repetition
A <sub>x</sub> #	# = tree number
A <sub>2</sub> 5	Tree number 5 of the second repetition of accession A

### The Breeding Trial

The breeding trial is part of a long-term multi-location experiment which started December 14<sup>th</sup> 2010 on multiple islands in Indonesia, the goal of which is to find a specific accession which responds best to local climate. The Wonosari trial consists of three fields in which 12 third generation accessions of *Jatropha* were planted and monitored where each accession was represented by three 64m<sup>2</sup> square plots of 16 trees. The ground itself was provided by a (teak tree) nursery owned by local government. Due to lack of funding, one of the repetitions (repetition 3 of Figure 2) was replaced by teak trees in 2012 and in the beginning of 2013, maintenance on all fields was halted. Out of the remaining two repetitions, several plots were unsuitable for research due to poor tree health. In Figure 2 a more detailed scheme of the experimental setup is given. During initial scouting of the BT a detailed census was set up which can be found in Figure 17 of appendix A. There was terracing in the field causing

erosion and the total surface area of the BT was estimated using a Garmin eTrex 20 handheld GPS and was found to be 0,1623 ha with a GPS accuracy of  $\pm 5$  meters. Workers were hired three times in the December to February period to clear all (used) plots of weeds.

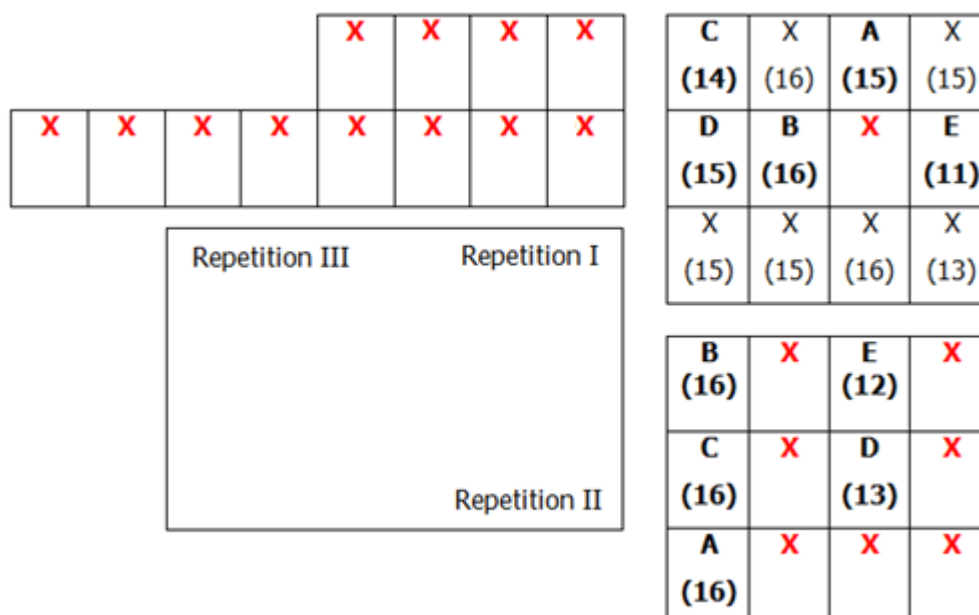


Figure 2 Schematic layout of the breeding trial containing 3 repetitions of 12 accessions. Each plot contains 16 trees with a 4x4 grid setup and a surface area of 64m<sup>2</sup> (Pers.comm Dr. Rully Dyah Purwati). Plots marked X were left out due to poor plot conditions and tree health. Plots marked X contain accessions left out due to lack of repetitions. Plots marked A through E were included in experiment and in all cases the number between brackets ( ) represents the number of healthy trees present per plot.

### The Seed Garden

The Seed garden, located in the Paliyan district, is made up of multiple large fields with a combined total surface area of  $\pm 13$ ha where two first generation accessions of *Jatropha* are grown. It is government-owned, setup in the end of 2006, managed until the end of 2010 and was setup with the idea to get a better understanding of the growth of *Jatropha* in relation to yield. When scouting the area it became apparent that large areas were unusable including all of the IP1M accession and a large part of the IP1A's (see part about accessions). The research site consists of two fields divided by a road and the census can be found in Figure 18 appendix A. Note that it differs from the BT census because trees in the SG were planted without proper structure, other crops were planted in close vicinity and all trees belong to one accession. Here a small amount of (hand-picked) trees were selected to reduce possible inconsistencies and an expansion was made in determining the surface area per tree. Surface area per tree was divided into SG-T, *Jatropha* trees only and SG-OC, which includes other crops (Table 9 appendix A). After selecting the right fields and trees, the total used surface area of the SG was estimated using a Garmin eTrex 20 handheld GPS and was found to be 1,658 ha for field 1 and 1,226 ha for field 2 (Figure 18 appendix A).



Figure 3 Picture of the breeding trial, 02-12-2013

## Fieldwork measurements

The fieldwork methodology used for this study consists of short, relatively simple measures all stated below. A similar methodology was used for both research sites. Table 10 in Appendix D gives the summary file of all gathered data.

### Tree height (mm), TH

Tree height was measured identifying the highest tree point including the leaves. It is measured using a bamboo stick marked with 2cm intervals and given in mm. A spirit level was attached to the bamboo to assure perpendicular positioning.

### Canopy height (cm), CH

Canopy height was defined as the level where the tree starts to crown. Some cases proved difficult to assess due to irregular growing patterns and were determined by a “rule of thumb” of 75% of all leaves being above this point. CH is measured in a similar way as TH and also given in cm.

### Canopy span (cm), CS

Canopy span was determined by stepping back 2m and finding the largest length span between two branches located oppositely from each other with leaves included. The bamboo stick was used to mark where these points were on the ground and the distance between these points was measured using a tape measure. Important to note here is that canopy span is measured only to be used in the total canopy volume calculation given below and contains a measure of length.

### Canopy shape (circle or ellipse), CSh

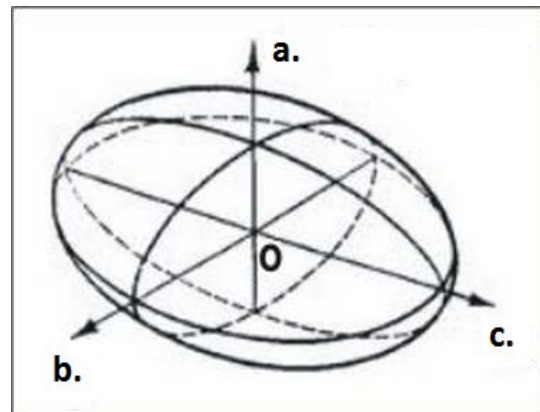
Canopy shape was found by observing the general shape of the tree and stating whether the overall shape was considered to be circular or ellipsoidal.

Above measures were used to estimate the total canopy volume (TCV). The calculation is based on geometry and depends on canopy shape (Figure 4 and Equation 1).

**Equation 1 Total canopy volume calculation for a circle or an ellipsoidal canopy shape** (Web formulas 2015).

$$TCV_{sphere} = \frac{4}{3}\pi ab^2 = \frac{4}{3}\pi \left(\frac{1}{2}(TH - CH) * \frac{1}{4}CS^2\right)$$

$$TCV_{ellipsoid} = \frac{4}{3}\pi abc = \frac{4}{3}\pi \left(\frac{1}{2}(TH - CH) * \frac{1}{8}CS^2\right)$$



**Figure 4 Total canopy volume of tree.**

a.  $\frac{1}{2}$  (tree height – canopy height)

b.  $\frac{1}{2}$  canopy span for sphere,  $\frac{1}{4}$  canopy span for ellipsoid

c.  $\frac{1}{2}$  canopy span for sphere,  $\frac{1}{2}$  canopy span for ellipsoid

For the sphere, b is equal to c and expressed as  $(\frac{1}{2}CS)^2$  or  $\frac{1}{4}CS^2$ . In the ellipsoid b represents only half of C and is expressed  $\frac{1}{4}CS$  which, combined with c as  $\frac{1}{2}CS$  makes  $\frac{1}{8}CS^2$ .

### Stem diameter (mm), SD

Surface around the stem of the tree was cleared and stem diameter was measured  $\pm 10$ cm above the ground using callipers. Measured twice, the second being done perpendicularly to the first to correct for deviating stem shapes and SD is given as the average of the two in mm.

### Surface area per tree ( $m^2$ tree<sup>-1</sup>), PD<sup>-1</sup>

Surface area per tree was determined by measuring the distance between a tree and all (North, South, East and West) of its neighbours. For the seed garden a distinction was made where other crops were either included (SG-OC) or excluded (SG-T) as neighbours. Equation 2, which holds for all PD<sup>-1</sup>; BT-PD<sup>-1</sup>, SG-OC and SG-T, is given below. Throughout the document, surface area per tree has



been expressed as m<sup>2</sup> per tree since this was in line with the definition used for the LAI-calculation (APPENDIX C).

**Equation 2 Surface area per tree calculation.**

*surface area per tree* =  $(\frac{1}{4}((N + S) * (E + W)))$  Where PD<sup>-1</sup> is given in m<sup>2</sup> per tree.

**Harvest (g)**

Harvest was collected several times throughout the growing season. For each tree and for every collection the following protocol was maintained:

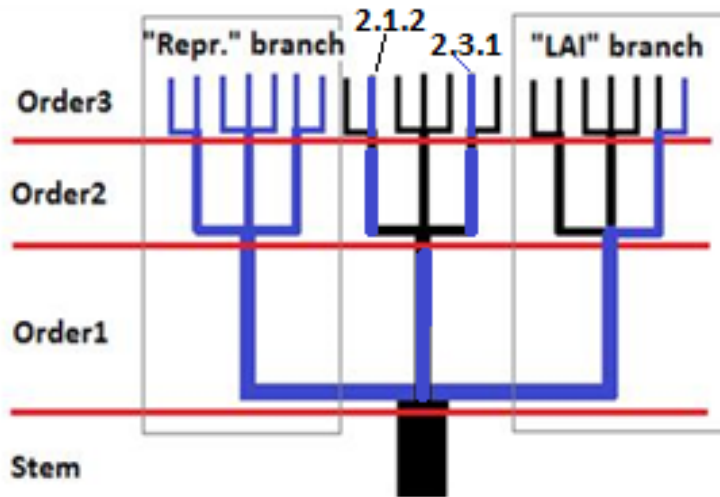
- Collect all (ripe) capsules that have turned yellow/brown
- Collect all capsules fallen on the ground
- Remove capsule stem
- Count number of capsules
- Determine total weight of all capsules (in g using 0,00g precision scale)
- Select number of capsules (maximum 10) that represent harvested batch best and
  - Determine individual capsule weight (in g using 0,00g precision scale)
  - Count number of seeds per capsule
  - Determine seed weight (in g using 0,00g precision scale)

The choice for capsules representing the batch best was based on picking the average shape, size and ripeness out of the total harvested batch per plot.

**Representative branches and branching orders**

For several measurements a classification system has been used making distinction between separate branch parts possible. This is important because it can give insight into what tree part contributes most to tree growth or production. It is common practice with e.g. fruiting trees where production is increased via pruning under correct pruning-intervals. First the amount of 1<sup>st</sup> order branches was observed and noted, then a part of these were identified as representative branches. Determining the right amount was done qualitatively and with the aim of representing each tree as best as possible while trying to reduce the amount of time spent per tree. Generated data was scaled up to parameter per tree by multiplying the average representative branch (or branch part) by the total amount of first order branches found on the tree. Identified representative branches were marked using red coloured rope.

Each representative branch was divided into branching orders as seen in Figure 5, every determined branch part was marked in the data log and numbered on tree itself with permanent marker to maintain overview. For each branch part the following measures were conducted:



**Figure 5 Schematic overview of tree branch classification.** The representative branch includes one 1st order branch with all attached higher orders. The LAI branch contains one 1st order branch and includes only one branch part of each higher order. The LAI branch contains one 1<sup>st</sup> order branch and includes only one branch part of each higher order. Individual branch parts will be identified by following the tree structure; 2.1.2 represents a 3<sup>rd</sup> order branch part and is the second 3<sup>rd</sup> order of the first 2<sup>nd</sup> order of the second 1<sup>st</sup> order.

Branch stem diameter (mm), b, t

Branch stem diameter was measured in a similar fashion as with tree SD except for the distinction made between bottom (b) - and top (t) diameters.

Branch length (mm), L

Branch length (L) was determined from branch base to tip or new branch part following the curves of the branch using a tape measure.

Branch length and diameter were used to calculate the branch volume. The volume will most likely be overestimated because the branch tips are long and thin whereas the geometry gives equal weights to the bottom and top diameter. By averaging out the found volumes of each branch part for each branching order and adding them up, an estimate can be given for the volume of a representative branch. Multiplying this number by the amount of 1<sup>st</sup> orders found on the tree yields the total volume per tree or the total accumulated biomass volume (TAB). Consequently, a fraction of how much each order adds to TAB can be calculated via Equation 3.

**Equation 3 Biomass fraction calculation.**

$$Fr. x O = \frac{\text{Volume of } x O}{\text{Total volume of whole average branch}} \quad \text{Where } x O \text{ is the total volume of the } x^{\text{st}} \text{ order branches.}$$

Disease (mm), D

If disease or rot was present, the length of the diseased branch part was measured in mm. If the entire part was diseased, the original branch length was used (Figure 6a). Disease was then expressed as a percentage of total tree length indicating how healthy a tree is and is calculated via Equation 4.

**Equation 4 Disease estimation calculation**

$$D = \frac{\frac{1}{n} \sum_{i=1}^n (x_1 \dots x_n)}{\frac{1}{n} \sum_{i=1}^n (y_1 \dots y_n)} * 100 \quad \text{Where } x \text{ is length of diseased branch part, } y \text{ is total branch length and } D \text{ in } \%.$$



#### Pruning, P

If a branch part was pruned it was marked with a P and the remaining branch length (so from base to prune) was measured (Figure 6b).

#### Capsule bundles, CB

When branches contained capsules, CB was noted with the amount of capsules present in bundle as a number. CB 4 represents one CB with 4 capsules; CB1 CB4 means 5 capsules on a branch spread over two bundles (Figure 6c).

In a similar way to determining the fraction of biomass distribution, productivity per branching order is calculated. A conversion factor was used to get from amount of capsule to seed dry weight and the fraction of productivity was calculated via Equation 5.

#### Equation 5 Productivity fraction calculation

$$Fr. x O = \frac{SDW \text{ of } x O}{Total \text{ SDW of whole average branch}}$$

Where x O is the average of the x<sup>st</sup> order branch.

#### Aborted flower bundles, AF

If any aborted flower bundles were observed, it was marked as AF (Figure 6d).

#### LAI branches

In order to get an estimate of Leaf Area Index per tree, a LAI branch is identified (Figure 5). Identified LAI branches were marked using blue coloured rope and on each branch the following is determined.

#### Total branch length (mm), tBL

Measured from base 1<sup>st</sup> order to tip highest order excluding leaves and given in mm.

#### Leafy branch length (mm), lBL

Measured from branch tip to the point where a bald patch of ±10cm is found, given in mm.

#### Number of leaves (#), #L

This is determined by counting to the total number of leaves on entire LAI branch.

#### Leaf dimension (mm), L and W

Leaf dimension is given by first identifying three representative leaves and then measuring the length from petiole to leaf tip and width between two leaf tips perpendicularly oriented to leaf petiole. Both are given in mm.

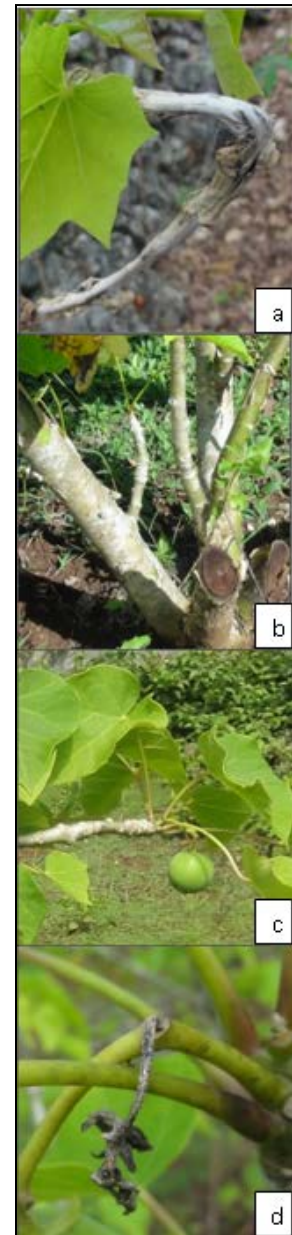


Figure 6 Conditions of branch parts: a Diseased (D), b Pruning (P), c Capsule bundle (CB) and d Aborted Flower (AF)

Above measures were used to estimate LAI, it is calculated using the non-destructive Leaf Area Index estimation protocol designed for *Jatropha* trees by Wageningen UR Plant Research International (WUR-PRI). The procedure is rather extensive and is given in Appendix C. The formula given in line J of the protocol was gotten from a study conducted by Jongschaap et al. (2007). LAI is given in m<sup>2</sup> leaf per m<sup>2</sup>.

### Laboratory measurements

In addition to the field work, lab work was done to determine the seed dry weight for all different accessions. At the agricultural department of the Universitas Muhammadiyah Yogyakarta (UMY) samples of 4-6 capsules per accession were made and dried in an oven set at 103°C for ±18 hours. An analytical scale was used to determine fresh and dry weight of both seed and capsule in grams. Samples had been frozen to reduce moisture loss on the 1,5 hour drive from Wonosari to Yogyakarta.

### Materials

All used materials and equipment for the above described measurements are given below in Table 3.

**Table 3 Materials used for conducting measurements.**

Materials	Used for	Unit
Handheld GPS	Determining total surface area	ha
Coloured rope	Marking relevant branches	-
Permanent markers	Marking branching orders	-
Callipers	Stem and branch diameters	mm
Bamboo with 2cm intervals and attached spirit level	Tree and canopy height and canopy span	cm
Tape measure	Branch lengths and canopy span	mm
Precision scale	Capsule and Seed weight	g
Oven	Seed dry weight determination	-

### Data analysis

All abovementioned measurements were entered into a template Excel file (Table 10 Appendix D) which processes all raw data into a summary file containing parameters of interest per tree, plot average and accession. This template was developed after all fieldwork was done simultaneously to the data entering. Because raw data was noted per branch part a classification was made per group of branch orders per tree so fractions of total biomass and productivity per branch order could be obtained. This was done in a similar fashion to the determination of total accumulated biomass volume. Due to timeframe and weather conditions, the intervals and frequency of the harvest moments varied per plot causing inconsistencies when comparing the total harvests per accession. Therefore harvest was calculated in two different ways to increase accuracy of analysis. The *Actual Harvest* is based on the *weight* of all harvested capsules and expressed in grams plot<sup>-1</sup>. Here the difference between weighing moments had significant impact since capsules were wet or dry, or blown in from the borders of another plot by a storm. Note: Because a number of trees did not have any capsules, the parameter was expressed per plot and each tree was given 1/n<sup>th</sup> of total plot harvest where n is the number of live trees in the Excel file. This causes the standard error to be smaller since it is only based on the difference between the repetitions and not the trees.

In addition to actual harvest values, a simple harvest prediction model was generated using gathered data. The *Model Harvest* is based on the *number* of capsules found on the tree during harvest measurements. Capsules per averaged representative branch were multiplied by the number of 1<sup>st</sup> orders to get capsules per tree, this number was multiplied with the average capsule weight and expressed in grams tree<sup>-1</sup>. Difficulty here was that the number of capsules per tree differed with the Actual Harvest findings.

Both parameters were also expressed in grams seed dry weight hectare<sup>-1</sup> by multiplying the amount of capsules with average number of seeds capsule<sup>-1</sup> and the average seed dry weight accession<sup>-1</sup>

gotten from the laboratory measurements. Both were multiplied by the number of live trees to get predicted harvest per plot or per accession.

Further data review consisted of statistical testing with post-hoc analysis executed in GenStat 15<sup>th</sup> edition statistical software. Here a one- or two way ANOVA was done combined with a LSD-test to identify significant differences. The choice for these statistics were based on personal communication with Msc. J. Tjeuw and [afstudeerbegeleider.nl](mailto:afstudeerbegeleider.nl) with a consultation on SPSS/Genstat computer software. All parameters averages were tested per plot, accession (1-way) or both (2-way). GenStat was also used to obtain summary statistics including number of observations, minimum, maximum and average values, standard deviation and standard error of mean. A summary of the statistical tests is given in table 11 Appendix E.

Within the results and discussion, the output of the ANOVA tests are given as ( $F = a, p = b$ ) where the  $F$ -value depicts the used inputs for the test and the  $p$ -value represents the confidence interval. The  $p$ -value should be smaller than 0,05 for the test to result in significant differences.

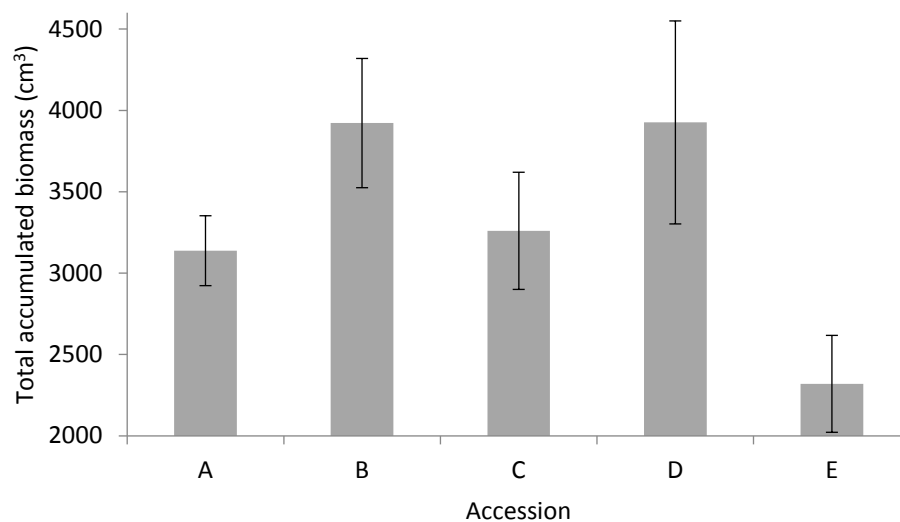
## Results

All accessions have been screened for nine parameters looking for significant differences between accessions, plots or both. Initial data analysis showed significant differences between accessions for four out of nine investigated parameters and are given below. Additional study revealed true difference between first and second plot when accession was discarded from analysis and was found for five out of nine parameters. Note that trees from the breeding trial differ in several properties (tree age, planting density, repetitions and generation) in respect to the seed garden trees so no comparison will be made. Furthermore no significant and/or relevant results were gathered from the data collected from the seed garden since it showed high variability and will only be handled shortly. In Table 4 an overview can be found of studied trees per accession. The raw data used for setting up graphs and figures can be found in Table 10 Appendix D.

**Table 4 Number of live trees per plot and total per accession given as n. The total number of analyzed trees and plots are 144 and 10 for the breeding trial and 45 and 3 for the seed garden, respectively.**

Accession	Plot 1	Plot 2	Plot 3	n
A	16	15	-	31
B	16	16	-	32
C	16	14	-	30
D	15	13	-	28
E	11	12	-	23
F	15	15	15	45

## Vegetative parameters



**Figure 7 Total accumulated biomass volume given per accession with standard error of mean and n=144.**

Figure 7 shows the distribution of the total accumulated biomass volume per accession. Significant differences were found between accession averages ( $F=2,5$   $p=0,045$ ) where accession E performs worse than B, C and D. A showed no significance with others and no other relations were found.

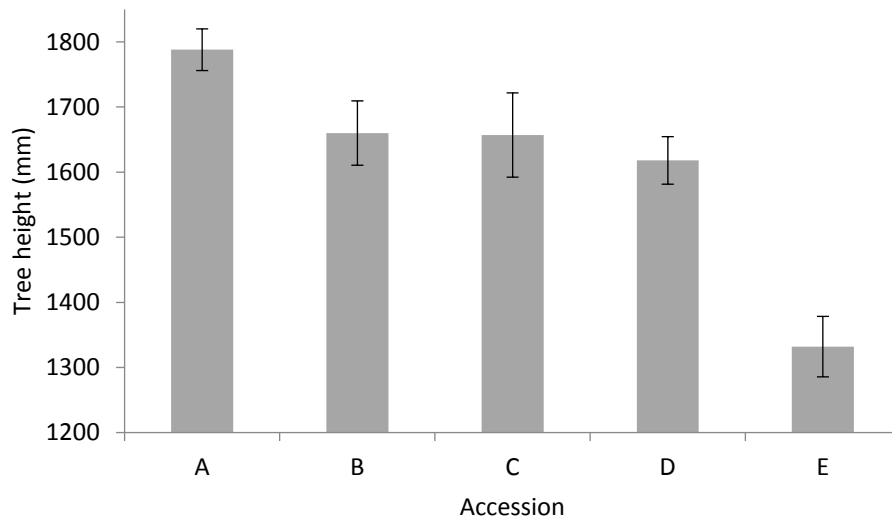


Figure 8 Tree height given per accession with standard error of mean and n=144.

In figure 8, division of tree height per accession is distributed. Accession E has the lowest score when compared to all other accessions ( $F=11,75$   $p<0,001$ ). No other differences were found.

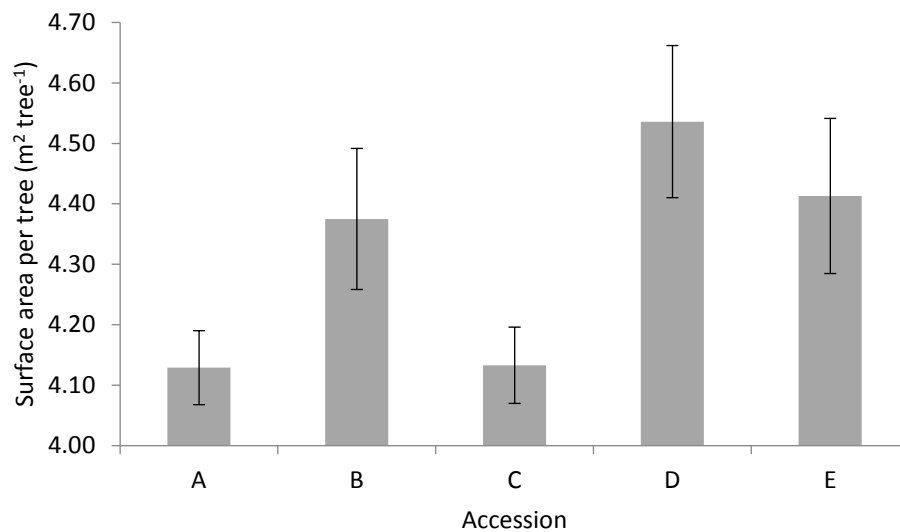


Figure 9 Surface area per tree given per accession with standard error of mean and n=144.

Figure 9 shows significant differences found in Surface area per tree per accession. Here the only observation is that A and C differ from D and E ( $F=3,83$   $p=0,006$ ). Also note that  $PD^{-1}$  is expressed as  $m^2$  per tree and can be transformed to planting density by dividing one by  $PD^{-1}$ . E.g.  $PD^{-1} = 4,4 m^2$  per tree and  $PD = 0,23$  tree per  $m^2$ .

Table 5 Relevant averaged vegetative parameters given per repetition using 1-way ANOVA.

	Plot -1	Plot -2	ANOVA and post-hoc l.s.d. at 5%
Stem diameter (mm)	62.01	57.79	$F=5.46, p=0.021$
Leaf area index ( $\text{m}^2 \text{ leaf m}^{-2}$ )	0,38	0.24	$F=12.56, p<0.001$
Total accumulated biomass volume ( $\text{cm}^3$ )	3780	2928	$F=6.10, p=0.015$
Surface area per tree ( $\text{m}^2 \text{ tree}^{-1}$ )	4.41	4.20	$F=6.81, p=0.010$
Total canopy volume ( $\text{m}^3$ )	0.77	0.51	$F=10.68, p=0.001$

Shifting the focus from factor accessions to repetition only (so plot -1 or -2) resulted in significant differences in five vegetative parameters which are stated in Table 5. Values of Stem Diameter are given with an accuracy of a hundredth of a mm. Although the accuracy of this measure can be questioned, this was what was gotten from the digital callipers. Note that found averages of the 1<sup>st</sup> repetition are all higher when compared to the 2<sup>nd</sup>.

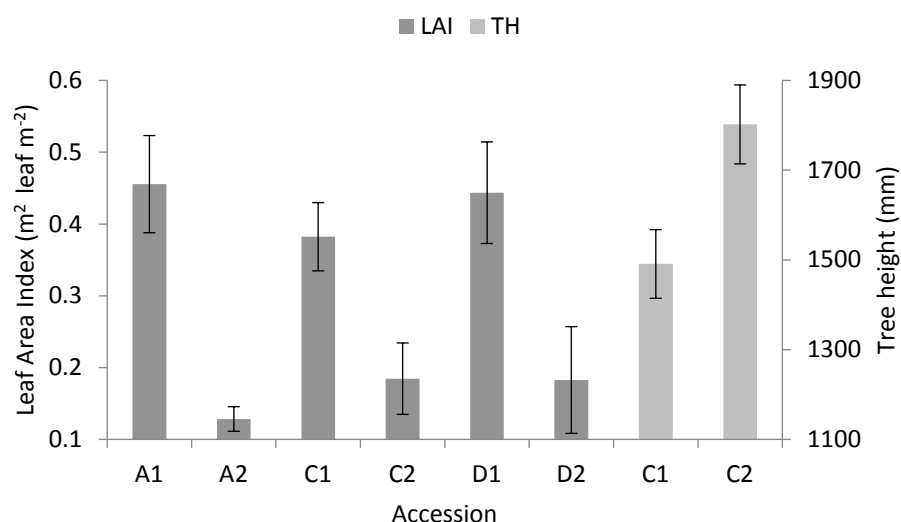


Figure 10 Leaf area index (n=79) and tree height (n=30) given per plot with standard error of mean.

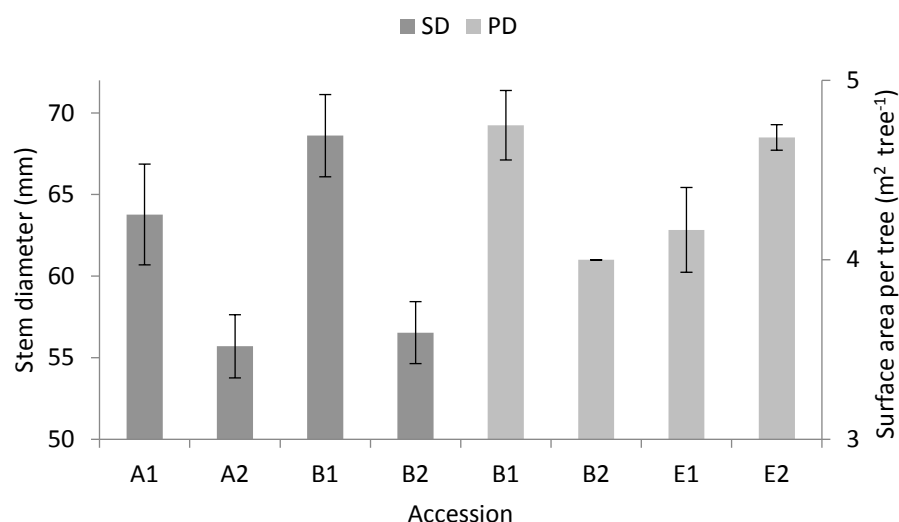


Figure 11 Stem diameter (n=63) and Surface area per tree (n=55) given per plot with standard error of mean.

The results of a 2-way ANOVA including both repetition and accession as treatment factors are given in figures 10 and 11. Here the focus is put on variation found within accessions only since comparing all individual plots goes beyond the scope of this research. Significant differences were found in four vegetative parameters. For LAI accessions A, C and D show difference where plots belonging to the first repetition score higher. For TH C2 scores higher than C1. With SD accessions A and B show variation where the first repetition scores higher and with PD B1 scores higher than B2 and E2 scores higher than E1.

## Harvest

From Table 6 it can be gathered that accession A provides the highest average yield of 195.14 kg fresh capsule weight with stems removed  $\text{ha}^{-1}$  and E scores lowest with 33.59. Furthermore accessions A, B and D show little fluctuation within the two methods of determining yields whereas C and E fluctuate. When these findings are compared to Table 7 it can be seen that the accessions perform in accordance with previous measurements. Note; It is unknown whether values from Table 7 are given as yield per season or year and whether or not they are based on multiple measurements. Although certain patterns exist between values from Tables 6 and 7, no comparison can be made.

**Table 6 Overview of modelled and actual yield per accession given in kg fresh capsule weight with stems removed per  $\text{ha}$ . Model yield is based on the number of capsules found through harvest measurements and multiplied by the average capsule weight. Actual yield is based on the actual weights from when the capsules were harvested (n=144).**

Accession	Model yield	Actual yield	Average yield
A	193.80	196.47	195.14
B	152.33	149.17	150.75
C	201.04	164.89	182.97
D	64.41	55.15	59.78
E	23.24	43.94	33.59

**Table 7 Documented yield for the breeding trial up to December 2011 given in kg fresh weight  $\text{ha}^{-1}$  (Pers. Comm. Dr. Ibu Rully).**

Accession	Documented yield
A	570.67
B	385.41
C	492.43
D	215.22
E	280.06



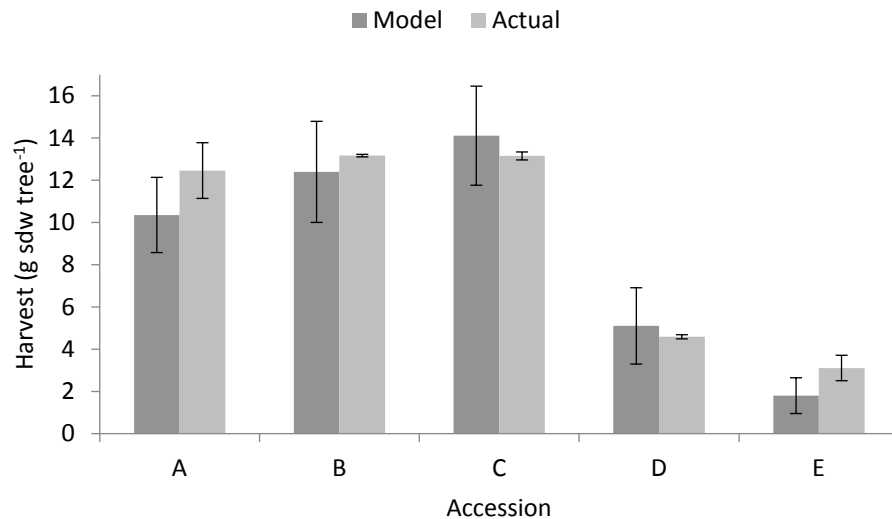


Figure 12 Model and actual yield given per accession and expressed in grams seed dry weight per tree<sup>-1</sup> with n =144.

To further comment on the productivity of an accession, the harvest of kg fresh weight ha<sup>-1</sup> was translated to grams seed dry weight tree<sup>-1</sup> (Figure 12). The difference between the two being the addition of a conversion factor derived from the laboratory experiment converting fresh to dry weight. When looking at the model yield in seed dry weight per tree, it shows that accession E scores lower than A through C and accession D scores lower than accessions B and C ( $F=6.1$   $p<0,001$ ). Within the actual yield values, the significant difference can be expanded to by stating that accessions D and E both differ from A, B and C ( $F=51.04$ ,  $p<0,001$ ).

### Correlations and allometry

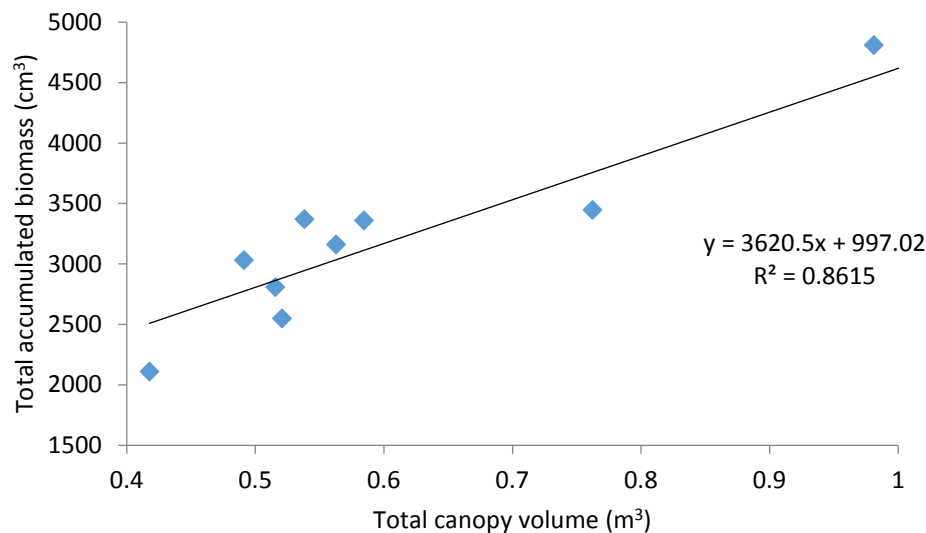


Figure 13 Correlation between measured total canopy volume and allometrically determined total accumulated biomass volume based on breeding trial plot averages (n=10).

Out of the nine vegetative parameters only one correlation was found between total accumulated biomass volume and total canopy volume with an  $R^2$  of 0.86 (Figure 13).

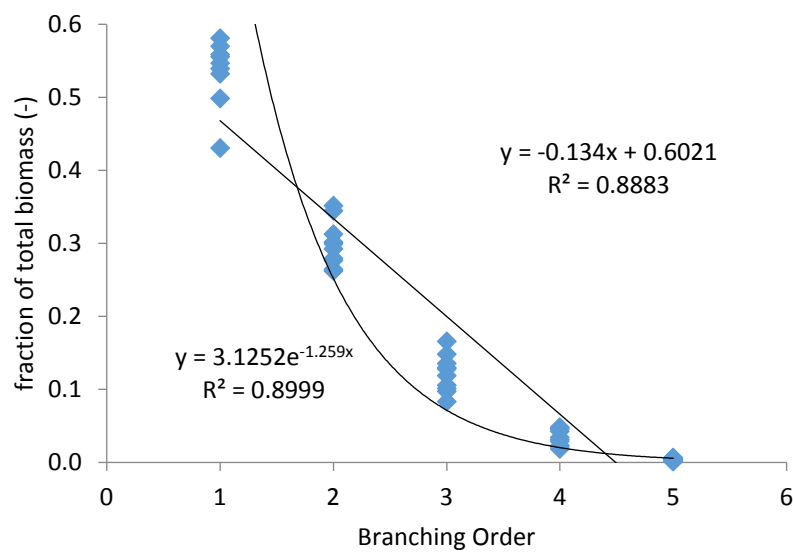


Figure 14 The fraction of total accumulated biomass volume each branching order group adds to the total accumulated biomass volume given per plot (n=50).

When looking at the biomass partitioning per branching order groups (Figure 14) the initial results hinted at a fixed pattern existing between all groups of branching orders. Further investigation of this relation through correlation gives both a linear and exponential trend line with an  $R^2 = 0.90$  and  $0.89$  respectively. In a similar fashion to Figure 14 the branching orders were compared to fraction of total harvest (productivity) but no relations were found.

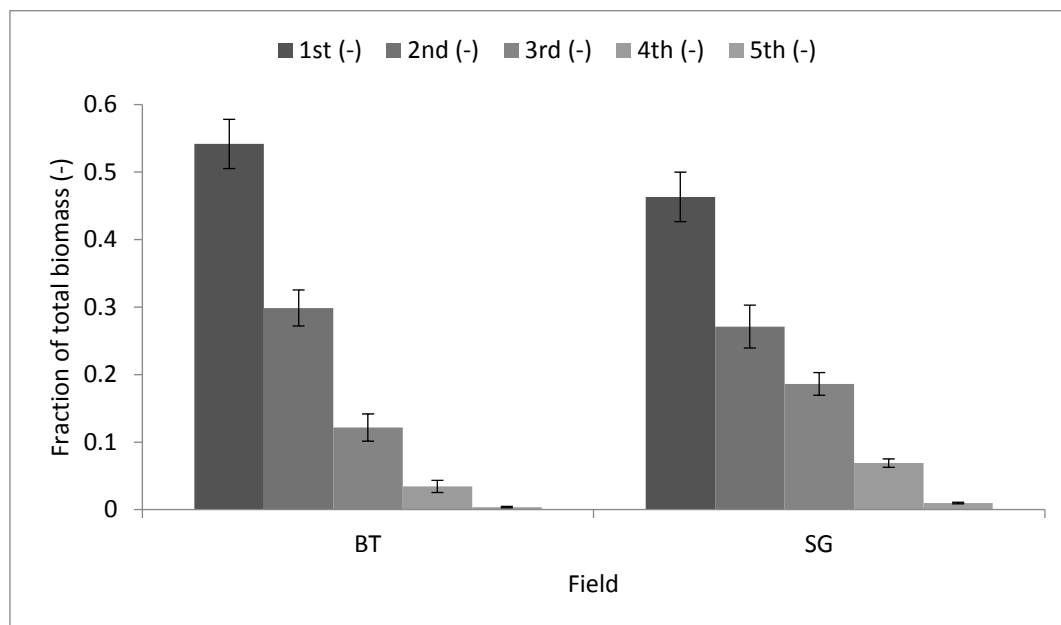


Figure 15 Distribution of biomass per branching order given for the breeding trial (n=144) and seed garden (n=45).

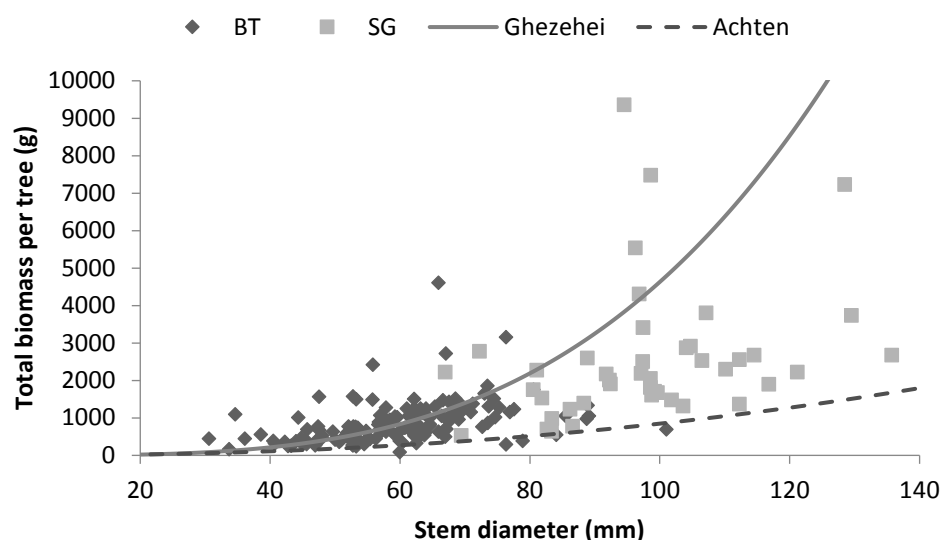
Figure 15 shows the difference in contribution to total accumulated biomass volume between breeding trial and seed garden trees for the first five branching orders. Note that due to differing circumstances between the two fields no direct comparison can be made.

Table 8 shows a comparison of gathered data with the predictions of two allometric equations. The equation from Ghezehei et al. describes the gathered data best for the younger, smaller trees found

**Table 8 Comparison of accession averages versus the allometric equations from (W.M.J. Achten, Maes, Reubens, et al. 2010) and (Ghezehei et al. 2009). SD = Stem Diameter, TAB = Total accumulated biomass volume, B = biomass. (n<sub>BT</sub> =144, n<sub>SG</sub> =45)**

Accessions	Experiment			Achten et al.		Ghezehei et al.	
	SD	TAB	B	B	Accuracy	B	Accuracy
	(mm)	(cm <sup>3</sup> )	(g)	(g)	(%)	(g)	(%)
<b>BT</b>							
A	59.74	3128.00	813.28	395.85	48.67	822.58	98.86
B	62.57	3921.75	1019.66	440.96	43.25	960.97	94.24
C	61.46	3267.05	849.43	422.83	49.78	904.57	93.51
D	60.35	3888.80	1011.09	405.37	40.09	851.25	84.19
E	53.81	2329.66	605.71	310.37	51.24	579.39	95.65
					<b>46.61</b>		<b>93.29</b>
<b>SG</b>							
F	100.09	10037.90	2609.85	1316.10	50.43	4643.96	22.06

in the breeding trial with an average accuracy of 93.3%. For the seed garden however, accuracy drops to 22.1%. The equation gotten from Achten et al. shows a more homogeneous distribution, but with lower accuracy with a breeding trial average of 46.6% and a seed garden average of 50.4%. To make this comparison possible, a constant for wood density of 0.26 g cm<sup>3</sup> was used to convert from volume to weight (W.M.J. Achten, Maes, Reubens, et al. 2010). Accuracy was calculated as  $((B_{study} / B_{experiment}) * 100)$ . If  $B_{study} > B_{experiment}$  then  $(100 - (B_{study} / B_{experiment}))$ .



**Figure 16 A comparison of breeding trial (n=144) and seed garden trees (n=45) with allometric equations that use stem diameter to predict woody biomass. Where “Achten” is expressed with  $B = 0.029 D^{2.328}$  and “Ghezehei” as  $B = 0.000907 D^{3.354}$ . BT = breeding trial, SG = seed garden, B = Woody Biomass and D = Stem Diameter.**

The predictive strength of both allometric equations are illustrated in Figure 16 and are plotted against an XY-scatter of all available data points. Here it can be seen that values from the breeding trial show somewhat of an overlap whereas the seed garden has high variability.

## Discussion

Below all the above mentioned results are discussed. As an addition to this analysis an extra section was added commenting on the reliability of the data and its linked shortcomings encountered during research.

### Vegetative parameters

When Figures 7, 8 and 9 are combined several statements can be made. First of all accession E performs poorest when compared to the others. It has a higher surface area per tree in comparison to A and C while it scores lower on both tree height (where  $E < A$  through D) and TAB (where  $E < B, C, D$ ). One would expect that a higher surface area per tree causes less competition resulting in higher plant growth. From the same figures it can be argued that accession A shows deviating plant architecture producing tall and thin trees when compared to others. A shows a low surface area per tree which could cause the lower TAB (no significant difference in TAB between A and E), but simultaneously A shows the highest value for tree height. Because the high TH-value for A is insignificant when compared to C through D, this point is open to debate. A critical note is that  $PD^{-1}$ -values for both D and E are high while they show similar or lower other stats (for example TAB  $D = A$  and TAB  $E < C$ ). This suggests either that there are other forces exerting a stronger effect on plant growth than surface area per tree, or that the differences in  $PD^{-1}$  are too small ( $\Delta_{max} = 2.48 \text{ tree m}^{-2}$ ) for any noteworthy influence.

The results of shifting the factor of analysis from accession to plot are shown in Table 5. Here it becomes apparent that accessions belonging the plot -1 have overall higher values for five vegetative parameters than plot -2 suggesting anomalies in the field. When looking at the breeding trial layout (Figure 2) and census (Figure 17 Appendix A), tree health is lower and mortality rates are higher in the plot -2 part of the field. Figures 10 and 11 show the extended analysis including both plot and accession via a 2-way ANOVA. Note that only the significant differences between plots of the same accession are given. These figures support the claim made with table 5 that the first repetition of plots score higher than the second. Tree height plot C and surface area per tree plot E contradict this statement, but in this case  $PD^{-1}$  can be discarded since a) it is not influenced by external factors and b) no interaction was found between  $PD^{-1}$  (accession B and E) and LAI (A, C and D) which should be the case since the  $PD^{-1}$  is included in the LAI calculation. The other inconsistency can be explained by the location of the plots in the field (Figure 2). Both plots are located on the far left of the field where the negative impact on plant growth is considered to be less. Plot C1 is located in the top left of the field where two trees had died and a more severe weed problem was present than in plot C2 (Figure 17 Appendix A) causing more competition and thus reduced plant growth resulting in the tree height difference.

### Harvest

Table 6 reveals large differences in harvest values where the yield of accession E is about  $1/6^{\text{th}}$  of that of A. Interesting to see is that when accessions are ranked on average yield, a pattern similar to harvest measurements done three years earlier can be seen where D and E score relatively low compared to A, B, C and A scoring highest (Table 6 and 7). This only gives an indication because the methodology used for obtaining values from table 7 is unknown and there can only be speculation as to the amount of harvest moments, the sample size or the number of repetitions. Figure 12 further confirms original findings where significant differences in harvest can be observed between accessions A, B, C and D, E. Although the found variation is large, performance of all accessions appears below standard when compared to literature where values of seed dry weight range from 0.1 to 4.5  $\text{kg tree}^{-1}$  as opposed to 0.002 to 0.014  $\text{kg tree}^{-1}$  (Iiyama et al. 2012). An argument that needs to be taken into account is that only part of the harvest was collected. *Jatropha* has the potential to flower multiple times in the 6 to 9 month growing season where in this study it was only collected for 2 months. If harvest values were extrapolated to the full length of the season, values

can still be considered poor when compared to other, similar studies which results from inadequate input and management causing poor plot and tree health.

When comparing both methodologies used for determining harvest, it can be stated that the “model” variant gives a good estimate. In spite of being based on capsule-holding branch parts and seed and capsule weight averages, found yield values are in line with other findings (Table 6 and 7 and Figure 12). Increasing the number of observations per growing season could be done so that both under- and overestimations can be reduced (e.g. accession A or E in Figure 12). Harvest determination via branch parts could hold potential in expanding harvest prediction models because the allometry of the location of capsules on the tree is included in the calculation. Unfortunately no correlation was found linking productivity to the branching order groups, but once a similar experiment with healthy trees is conducted this could prove to be interesting. In a similar way to Figure 14, equations can be developed that take into account tree age and health and estimates harvest per tree, per tree part and per ha. This level of analysis could provide insight in *Jatropha* management practices. An example here would be that the prediction shows accession X having highest productivity on branch parts belonging to the 3<sup>rd</sup> order and that 5<sup>th</sup> and higher orders add nothing. This way the pruning tactics could be altered in such a way that each tree gets as many 3<sup>rd</sup> order branches as possible. The addition of tree age into the equation could also be interesting where an older tree favours a certain branching order group as seen in Figure 15.

### Correlations and allometry

From Figure 13 it can be seen that a relation exists between total accumulated biomass volume and total canopy volume which holds some implications. When executing field work, determining TCV is a rather quick-and-dirty method whereas TAB proved to be quite labour-intensive. Depending on the goals of the research a choice can be made to skip TAB-measurements and derive it by using the TCV in combination with the correlated equation (Figure 13). Important when doing this is that boundaries are determined within which the equation gives an accurate prediction since it will only hold for a certain range of TCV's. When the analysis focuses more in-depth on individual trees the TAB would be better to determine since it can be linked to other parameters via the branching orders.

The correlations found in Figure 14 show a relation that could be described as higher branching order groups contributing about half of what its predecessors to total biomass. This can also be written as  $fr\ i_n = \frac{1}{2} fr\ i_{n+1}$  where  $i_n$  is branching order group (n) and  $i_{n+1}$  is a higher group of branching orders (n+1). Although the comparison made between the harvest and allometric relations proved to be inconclusive, the fact that a significant distribution of biomass per branching order group exists could provide a basis for the expansion of existing *Jatropha* allometry models to incorporate productivity.

The knowledge gap here that needs to be overcome is a) finding the mechanism responsible for fruit-set allocation linking branching orders to an amount of capsules and b) linking this mechanism to the correlation of Figure 13. Once these have been done the TAB, fraction of biomass per BO and the number of representative branches per tree can determine how many branches there are for each BO. This in combination with an estimate of how many capsules on average each BO holds could provide a solid basis for harvest prediction. Achieving this would again require a set of accurate harvest and vegetative measurements done on healthy and well maintained *Jatropha* trees.

Another point that needs to be taken into account is that patterns found are prone to deviating age and/or accession. Figure 15 shows that the difference between two consecutive branching orders is smaller for the seed garden than for the breeding trial. Seeing as the second contains trees of an older age, this would hint at a) the total difference between branching orders gets smaller with age and b) the higher branching orders start to contribute more to total biomass as the tree ages. This aspect is not included in the above determined pattern and needs to be when comparing *Jatropha* trees of deviating ages and/or accession. In a similar fashion, the estimated  $fr\ i_n = \frac{1}{2} fr\ i_{n+1}$  ratio needs to be adjusted for trees with deviating age when being incorporated into models for harvest or allometric prediction.

Other studies investigating allometric relations for *Jatropha* found stem diameter to be a good indicator in successfully predicting certain vegetative traits. An experiment conducted by Achten *et al.* (2010) investigated possible patterns that exist in the growth of *Jatropha* seedlings and distribution of biomass by scouting for allometric relations. Here an equation was found successfully predicting aboveground dry biomass (B) of the seedlings based on stem diameter (D) with equation  $B = 0.029 D^{2.33}$  with  $R^2 = 0.89$ . Another study conducted in a similar fashion found a relation between aboveground dry biomass and stem diameter described with  $B = 0.000907 D^{3.354}$  with an  $R^2 = 0.91$  (Ghezehei *et al.* 2009). Both equations were tested and compared with gathered data (Table 8). Some critical notes here are *a)* in both papers a distinction is made between biomass gotten from stem and branches and biomass gotten from foliage since values are based on destructive measurements. In this study, total accumulated biomass volume is based on non-destructive measurements only and additional biomass added from foliage is excluded which suggests and underestimation. *b)* In both papers other relations (stem diameter versus LAI and crown depth versus aboveground dry biomass) were found and mathematically described. Unfortunately none of these other relationships could be reproduced due to the inconsistencies in the data. *c)* In this study, total accumulated biomass volume is based on branch averages with estimated volume and given in unit volume  $\text{cm}^3$ . In the papers, biomass is expressed as a weight based on a density of dry grams  $\text{cm}^3$ . To overcome this a conversion factor was used but this has not been verified for the trees used for this study. In Figure 16 all data points were plotted against the two predictions. From both Table 8 and Figure 16 it can be gathered that Achten *et al.* shows a constant underestimation whereas Ghezehei *et al.* shows both under- and overestimations indicating that either the trees used for this study show a deviating size or the equations lack accuracy. A study done in Malawi assessed both equations with similar methodology and ended up with a consistent overestimation of up to 55% (Makungwa *et al.* 2013) suggesting that the equations are consistent and that the trees used for our study deviate from the norm. However, this gives only an indication because the studies were conducted on different continents with varying climatic conditions and should be assessed individually.

## Other

Data analysis showed that LAI values for all evaluated trees were unrealistically low. For comparison, a study conducted in Madagascar relating LAI and biomass partitioning to variable water supply used both destructive measurements and a hemiview (which is considered a reliable tool in estimating the LAI of trees and bushes) to determine LAI and values ranged from 1.2 to 6.1 with 75% of the cases above 3 and  $n=250$  (Rajaona *et al.* 2010). This large difference indicates an error present in either the used LAI-methodology, the wrongful choosing of representative (LAI) branches or that an inappropriate time was chosen to collect the LAI data which is most likely. This because the LAI measurements were done when the trees were producing fruit and due to the lack of fertilizer, the trees started shedding their leaves resulting in smaller and less leaves than usual. To correct for this phenomenon, leaf scars found on branches were included in the leaf count and leaf sizes were measured multiple times but with no success.

## Data reliability and shortcomings

### Data reliability

Although all data used for this research has been obtained through accurate observations and measurements, the quality of certain parts can be questioned. Below, several aspects are given describing the cause for this doubt in reliability. In general, the lack of maintenance and management of both sites plays an important role in data reliability. The lack of fertilizer and pesticide application caused disease, shedding of leaves and irregular fruiting patterns. This mismanagement had started around the end of 2010 leaving many trees perished. If this were not the case the sample size of plots would increase adding to data accuracy and more accessions could be included in the study (Figure 2). Also, several trees were pruned with the aim of creating a path

through a field instead of optimizing tree productivity. Cases were found where an estimated 1/3 of a tree was cut away which lead to an irregular tree shape and biomass division thereby further degrading the accuracy of plot data. Finally, the absence of pesticide caused some local weed problems. Although weeds found in and around the plots were trimmed three times during the scope of the fieldwork, they will undeniably have had influence on individual tree performance due to competition for sustenance.

For the breeding trial plots the following anomalies can be added:

- Terracing placed throughout the BT has different spacing when comparing the East and West side of the field. This results in fluctuating surface area per tree of 4 to 4.75 in plot average while the experimental setup states a solid PD=4 for each individual tree adding to uncertainty of the determined LAI.
- The BT census (Figure 17 Appendix A) shows that there is terracing present in the field and that the top left corner contains dead, severely diseased and dwarfed trees. This area strongly deviated from the rest of the BT and can be caused by erosion washing away the fertile soil causing nutrient shortage, a local soil or air borne pathogens causing disease or severe weed problem competing for nourishment. (Pers.comm. Juliana Tjeuw)
- Along all the terraces, rows of mammoth grass were planted causing competition for nutrients. Some plots were between terraces while others overlapped. The mammoth grass itself was frequently harvested by locals who would damage the *Jatropha* trees or brush of capsules.

For the seed garden the following anomalies can be added:

- Because large areas of the field were barren, several trees ended up with a large surface area per tree of up to 16m<sup>2</sup> per tree. Even when corrected for other crops growing in close range, certain PD<sup>-1</sup>s remain high (9.6) making the LAI estimation unreliable since PD<sup>-1</sup> plays an important role in the LAI-calculation protocol (Appendix C).
- During measurements, local farmers started to cultivate crops on small plots of land scattered throughout the SG. Because this happened gradually every trip to SG was a surprise, some trees had to be dropped from the experiment and caused great fluctuations in multiple variables. The clearest example was tree number 4 which had been intercropped with peanut in an early stage and fertilizer. This tree had a measured total harvest of 1124 grams which, when compared to the total SG harvest of 4689.7grams, makes up ±24% of harvest while being only ±2% of the total population.
- During the period when there was still maintenance (until end of 2010), any tree that died was replanted making it impossible to determine tree age thereby adding to the uncertainty of the SG-dataset.

#### *Shortcomings*

- The measurement of flower bundles was originally set up in a similar way as was done for the capsule bundles. So observing, counting and using similar coding; FB(3) would mean one flower bundle holding three flowers. This has only been partly covered and excluded from analysis.
- During fieldwork branching orders were counted and marked up to the 7<sup>th</sup> order. After looking at the data, it appeared that < 1 % of both fr. biomass and productivity was added by the 5<sup>th</sup> and higher orders. Therefore, only orders 1<sup>st</sup> to 5<sup>th</sup> were taken into account during analysis.
- When observing the branching orders it was in some cases difficult to determine the number of 1<sup>st</sup> orders because trees showed deviating branching patterns from the stem. In some cases the branching already started underground. Here, an assumption was made that all branch parts sticking out of the soil were counted as a 1<sup>st</sup> order although this can be subject to debate.



- Actual values of % diseased tree will most likely be underestimated. This because certain branches containing rot had fallen from the tree and could not be included in the measurements.
- Out of the different accessions, variety E performed poorest when it comes to production. This can be seen from the yield values found in E1. E2 shows much higher values (total harvest of 14.1 vs 425.7 grams). This difference can be explained by the fact that in the first week of measurements, our Headquarters (tent, equipment, lunch, etc.) was located next to the E2 field. During first harvest moment, 22 capsules were counted for E2 tree #3 while there were only two capsules on the dwarfed tree. It is highly likely that capsules from other plots/trees that were not included in the experiment were gathered by one of the workers and put together underneath tree #3 which would explain the large difference.
- The variables Pruned(P), Flower bundles (FB), Aborted flowers (AF) were measured but not used due to an incomplete or unreliable experimental situation.
- On January 11<sup>th</sup> 2014, a heavy rain and thunder storm shook capsules off the trees. At this point in time, the first series of harvest measurements had not been completed for all plots and the number of capsules per tree deviates from reality.
- In and around all plots, local farmers had planted mammoth grass to feed their livestock. These were harvested multiple times per week and once a big bale was collected they would exit the field via the shortest possible route brushing against branches which caused additional capsules to fall.
- Due to limited timeframe and fear of losing yield data, the teams were assigned to observe, count and harvest the capsules from the tree. Since there was only one scale present, some harvested capsules were measured one to two days later during which the capsules had already shed some of their water weight which further distorts the measurement.

## Concluding remarks

Out of all monitored accessions A and C are the best candidates to be used in the continuation of breeding programs for the central-Javanese climate due to trends found in resource allocation favouring production efficiency. Accessions B, D and E can be discarded with reasonable certainty while F remains unknown. Further specification will require additional research with increased sample size and repetition since a) no significant differences were found between the two primary contenders and b) the variation between repetitions of the same accessions was high. Additional relations found can be used as a stepping stone to expand harvest predictive- and allometric models increasing accuracy and level of detail.

In order to achieve a more in depth understanding of the interaction between *Jatropha*'s vegetative- and generative stage, the focus needs to be put on scouting for ratios and parameters which can be used to strengthen harvest prediction. An example would be to look at how production is distributed between the representative branches and branching order groups. This could provide useful insights in *Jatropha* location-allocation mechanisms e.g. all capsules on one of the three branches versus an even distribution or fruit set branching order group 3 > group 4. Once this has been determined, a pruning strategy for *Jatropha* can be set up which is already common practice in other (fruiting) production trees.

Similarly the level of analysis needs to be scaled down from per accession or per plot to per tree or perhaps to per branching order. An example here would be deriving the LAI branch length from the data file, checking how this compares to the measured LAI branch and repeating the LAI-protocol. A start of this has been made in the produced excel templates where all data is inserted tree<sup>-1</sup> or tree part<sup>-1</sup>.

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- Personal communication with Dr. Ibu Rully (Dec 2013- Mar 2014); combination of Q/A and emails with documents and a presentation as attachments.
- Personal communication with Juliana Tjeuw (Oct 2013 – Mar 2014); initial setup documents, Q/A and explanation of methodology
- Forestry department of Wonosari, Gunungkidul (Dec 2013); meeting and agricultural province report.
- Interviews:
- Pak Heri (caretaker of breeding trial)
  - Pak Sunardi (head of breeding trial)

- Pak Sukardi (head of seed garden and member of Gunungkidul forestry department)
- Kanigoro and Mongol farmers from Gunungkidul (21 farmers interviewed when scouting for usable *Jatropha* fields)

## Appendix A Census maps

Figure 17 Census breeding trial, terracing  $\pm 1\text{m}$  height difference.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
X	X	X	X	X	H+	H+	H+	H+	H+	X	X	X	H+	H+	H+	H+	X
W	X	X	X	X	X	H+	H+	H+	H+	H+	X	H+	H+	H+	H+	H+	W
V	X	X	H	X	H	X	X	H	H	H	H	H	H	H	H	H	V
U	H	X	H	X	H	H	H	H	H	H	H	H	H	H	H	H	U
T	H	D80	X	H	H	H	H	D40	X	X	X	X	H	H	H	H	T
S	X	H	X	H	H	D25	H	H	H	H	H	H	H	H	H	H	S terrace
R	X	H	H	H	H	H	H	H	H	X	H	H	H	H	H	H	R
Q	X	X	D90	X	D50	X	X	X	X	H	X	H	H	H	H	H	Q
P	X	D90	X	X	X	X	X	X	X	H	H	D70	H	H	H	H	P
O	X	H	D90	D90	D90	H	D80	H	H	X	X	X	H	H	D5	H	O
N	H	H	X	H	H	H	D90	H	H	X	X	X	D10	H	H	H	N
M	X	H	X	D50	H	H	D80	H	H	H	X	H	H	H	D5	H	M terrace
L	H	X	D10	H	H	H	H	H	H	P50	H	H	H	D20	H	X	L
K	P50	H	H	D5	D10	H	D5	H	H	D25	H	H	D20	H	D10	H	K
J	P50	H	D5	H	H	H	H	H	D5	D5	H	H	D10	H	H	H	J
I	H	H	H	D25	H	D10	H	D5	H	D5	H	H	H	D5	D10	H	I
H	H	H	H	H	X	D5	H	D5	D5	H	D10	H	H	H	H	H	H
G	X	H	H	D95	H	X	H	H	H	H	H	H	H	H	H	X	G terrace
F	D95	H	H	P25	D5	H	X	D10	H	H	H	H	H	H	H	H	F
E	X	H	X	H	H	H	D5	H	D5	H	H	H	D10	D5	H	H	E
D	H	H	D40	D5	H	H	H	H	H	H	H	H	H	H	H	D95	D
C	H	H	D90	D20	H	H	H	H	H	P25	H	H	D5	H	H	H	C terrace
B	H	H	H	D40	H	H	H	H	H	P25	H	H	H	H	H	H	B
A	H	H	H	H	H	H	H	P50	H	H	H	H	H	H	H	H	A
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	

### Legenda

- H Healthy/normal
- X Dead/Cutdown
- D Diseased (with estimated %)
- P Pruned (with estimated %)
- Dwarf
- H+ Severe dwarf
- A lot of weeds
- Severe weeds
- Completely overgrown
- Dwarf + Weed
- Used plots





Figure 18 Census Seed garden

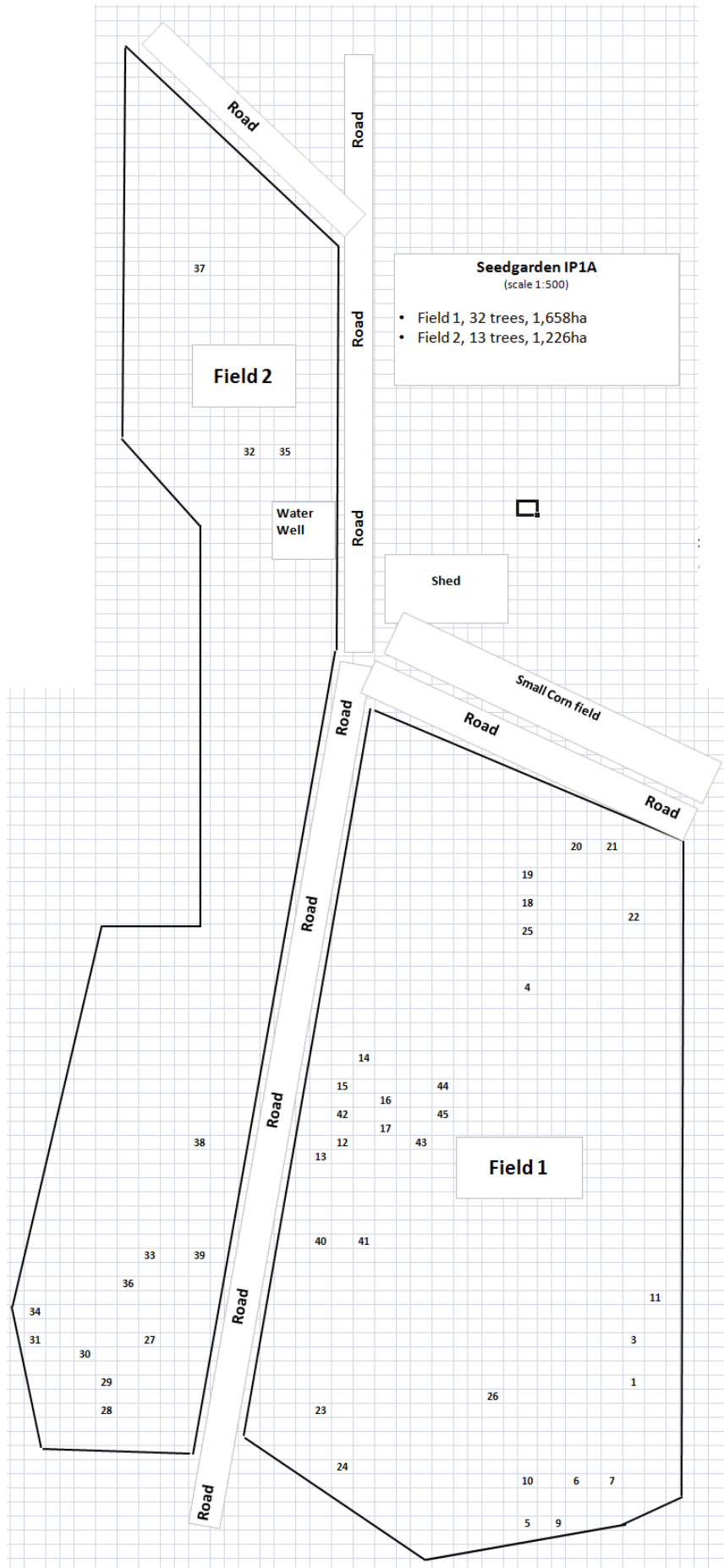


Table 9 Overview of Surface area per tree in Seed Garden expressed in m<sup>2</sup> tree<sup>-1</sup>.

Tree #	Allo. #	TH	# O1	Seed Garden - Tree				SG-T	Seed Garden – Other Crop				Crops				SG-OC	Comment
S		<1980		N	W	S	E		N	W	S	E	N	W	S	E		
1	1	1860	3	4,0	1,5	2,0	2,0	5,3	1,0	1,5	2,0	2,0	CT	-	-	-	2,6	Cashew tree to N ±1m TH
2	2	1900	5	4,0	4,0	4,0	2,0	12,0	4,0	4,0	1,5	1,0	X	X	TT	g	6,9	Teak tree to N, ±2,5m TH
3	4	1763	3	4,0	4,0	2,0	4,0	12,0	2,0	1,5	1,0	1,0	c	c,g	c	c,g	1,9	Ploughed field, peanuts and cassava
4	5	1980	5	2,0	2,5	2,5	4,0	7,3	2,0	1,0	2,5	1,0	-	g	-	c,g	2,3	Neighbour 9 to E
5	6	1760	3	4,0	3,0	4,0	3,0	12,0	1,0	1,0	4,0	2,0	g,TT Dw	g	X	g	3,8	Ploughed field, peanuts. Teak tree to N, ±3m, Neighbour 7 to E
6	10	1900	2	2,0	4,0	4,0	4,0	12,0	1,0	1,5	4,0	1,0	CT	g	DT	g	3,1	Cashew tree to N ±1m TH
7	11	1700	2	4,0	2,5	4,0	2,5	10,0	1,5	2,5	4,0	1,0	CT	-	X	g	4,8	Cashew tree to N ±5m TH
8	17	1940	4	4,0	4,0	3,0	3,0	12,3	4,0	3,0	3,0	1,5	X	g	-	g	7,9	Neighbour 43 to S
9	22	1980	3	2,0	4,0	4,0	4,0	12,0	1,0	1,0	1,0	4,0	Dw T	c	c	DT	2,5	
10	24	1690	2	4,0	3,5	4,0	4,0	15,0	4,0	3,5	2,0	1,5	X	-	TT	g	7,5	Teak tree to S, ±2m TH
11	28	1820	5	1,5	4,0	2,0	4,0	7,0	1,5	1,0	2,0	1,0	-	g	-	g	1,8	
12	29	1720	6	2,0	3,5	4,0	4,0	11,3	2,0	1,0	4,0	1,0	-	g	X	g	3,0	Neighbour 27 to N, neighbour 30 to W
13	32	1880	3					4,0									4,0	Tree uprooted during experiment
14	35	1450	4	4,0	1,5	2,0	3,0	6,8	4,0	1,5	2,0	3,0	-	-	-	-	6,8	
15	37	1320	2	4,0	2,0	4,0	4,0	12,0	3,0	1,0	1,0	1,5	TT	g	g	g	2,5	Teak tree to N, ±3m TH
M		1980-2300		N	W	S	E		N	W	S	E	N	W	S	E		
1	3	2140	3	4,0	4,0	4,0	4,0	16,0	2,5	2,5	1,5	1,0	g	TT	g	TT	3,5	Teak tree to W, ±2m TH, Teak tree to E ±3m TH
2	13	2080	2	1,5	2,0	2,0	2,0	3,5	1,5	2,0	2,0	1,0	-	-	-	g	2,6	Ploughed
3	14	1990	4	3,5	4,0	4,0	3,0	13,1	3,5	2,0	4,0	2,5	-	g	X	g	8,4	Ploughed
4	16	2080	3	4,0	2,0	4,0	4,0	12,0	1,5	2,0	2,5	1,0	CT	-	TT	g	3,0	Cashew tree to N ±1m TH. Neighbour 15 to W. Teak tree to S ±4m TH
5	21	1980	4	4,0	2,0	2,0	4,0	9,0	1,0	2,0	1,0	1,5	c,g	-	c	g	1,8	Neighbour 20 to W
6	23	1980	5	4,0	4,0	4,0	4,0	16,0	2,0	1,0	4,0	2,0	TT	g	DT	c	4,5	Teak tree to N, ±2m TH
7	27	2190	4	4,0	2,0	2,0	3,0	7,5	2,0	2,0	2,0	3,0	g	-	-	-	5,0	
8	33	2040	4	4,0	3,5	3,0	4,0	13,1	4,0	1,5	3,0	4,0	X	TT	-	DT	9,6	Teak tree to SW, ±4m TH
9	34	2160	8	2,0	4,0	2,0	3,0	7,0	2,0	1,5	2,0	1,5	-	g	-	g	3,0	
10	36	2080	3	4,0	4,0	4,0	2,0	12,0	4,0	1,0	1,5	2,0	-	g	g	-	4,1	
11	40	2070	2	2,5	2,5	4,0	2,5	8,1	2,5	1,0	1,5	1,0	-	g	g	g	2,0	
12	41	2020	3	4,0	2,0	2,0	4,0	9,0	4,0	2,0	1,0	2,0	-	-	g	g	5,0	
13	42	2100	3	4,0	3,0	2,0		4,5	0,5	1,5	2,0		TT	g	-		0,9	Teak tree to N, ±1m TH. Neighbour 12 to S
14	43	2000	3	3,0	2,0	3,0	4,0	9,0	3,0	2,0	3,0	3,0	-	-	-	g	7,5	Neighbour 17 to N
15	45	1990	4	2,5	3,0	4,0	2,5	8,9	2,5	0,3	1,0	2,5	-	g	g	-	2,4	Neighbour 14 to W
L		>2300		N	W	S	E		N	W	S	E	N	W	S	E		
1	7	2530	3	4,0	3,0	4,0	4,0	14,0	1,5	1,0	4,0	1,5	g	g	-	g	3,4	Ploughed field, peanuts Neighbour 6 to W
2	8	2440	6	4,0	3,0	2,0	4,0	10,5	4,0	1,0	2,0	1,0	-	g	-	g	3,0	Ploughed field, peanuts
3	9	2440	5	2,0	3,0	4,0	2,5	8,3	2,0	3,0	4,0	1,0	-	-	-	g	6,0	Ploughed field, peanuts. Neighbour 5 to W
4	12	2600	3	2,0	4,0	4,0	4,0	12,0	2,0	2,0	2,0	2,0	-	g	TT	g	4,0	Neighbour 42 to N, Teak tree to S ±5m TH
5	15	2240	4	2,0	3,5	3,5	2,0	7,6	2,0	3,5	3,5	2,0	-	-	-	2,0	4,8	Ploughed field, peanuts. Neighbour 16 to E
6	18	2500	4	2,0	4,0	2,0	3,0	7,0	2,0	1,0	2,0	2,5	-	g	-	c	3,5	
7	19	2340	3	1,5	4,0	2,0	2,5	5,7	1,5	1,5	1,0	2,5	-	TT	g	-	2,5	Teak tree to W ±2m TH
8	20	2390	4	4,0	2,0	2,0	2,0	6,0	1,0	1,5	2,0	2,0	g,c	c	-	-	2,6	Neighbour 21 to E
9	25	2300	5	2,0	3,0	2,0	4,0	7,0	0,5	1,5	2,0	3,0	TT	g	-	c	2,8	Teak tree to W ±1m TH
10	26	2480	4	2,0	3,0	2,0	3,0	6,0	2,0	3,0	2,0	1,5	-	-	-	g	4,5	
11	30	2590	11	2,0	4,0	2,0	3,5	7,5	2,0	1,0	2,0	1,0	-	g	-	g	2,0	Neighbour 29 to E
12	31	2490	6	1,5	3,0	2,0	4,0	6,1	0,5	1,5	2,0	1,5	g	g	-	g	1,9	
13	38	2600	4	4,0	2,0	3,5	4,0	11,3	1,0	2,0	3,5	2,0	g	-	-	TT	4,5	Teak tree to E ±1m TH
14	39	2850	5	4,0	4,0	3,0	4,0	14,0	4,0	4,0	3,0	1,0	X	DT	-	R	8,8	Ploughed field, peanuts
15	44	2300	4	2,5	2,0	3,5	4,0	9,0	2,5	2,0	3,5	1,0	-	-	-	c,g	4,5	

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**Figure 19 Layout of template Excel file.** Colors correspond to summary file (Fig.1) L = leaf length, W = Leaf width, #BO = Number of branch orders, AVG BL = Average branch length per branch order, D = disease, CB = Capsule bundle, #C = number of capsules, FB = Flower bundles, Length = total length of all branch orders, Volume = total volume of all branch orders, Vol (%) = percentage of total volume per branch order, SDW = Seed dry weight, #S / C = Number of seeds per capsule, Total Actual = total actual harvest per tree, Total Model = total harvest per tree based on Excel calculations.

## Appendix C LAI Calculation protocol

Protocol Non-destructive Leaf Area Index (LAI) estimations Wageningen UR - Plant Research International		
	Measurement (with an example)	
	Calculated field	
a	Identify the jatropa tree Write down identification codes and observation date	
b	Establish the plant density of the jatropa stand	
c	[m2 tree-1]	4,00
d	Identify a representative branch	
e	Count the number of leaves on the representative branch	17,00
f	Measure the length of the part with leaves of the representative branch	90,50
g	Identify a representative leaf in the mid of the leaf- section of the branch	
h	Measure width of the leaf between outer tips	17,00
i	Measure length of the leaf tip to the start of the petiole	24,50
j	Estimate Leaf Area of the leaf; $LA = 0.84 * (h * i) ^ 0.99$	329,38
k	Estimate Leaf Area of representative Branch; $LAB = e * j$	5599,47
Precise LAI assessment		
l	Count the total number of branches	20,00
m	Measure/estimate each branch length and give average value	89,40
n	Measure/estimate each branch length that has leaves and give average:	69,67
o	Calculate total branch length with leaves: $l * n$	1393,40
p	Calculate LA per tree as: $LAT = (o / f) * k / 10000$	8,62
q	Calculate Leaf Area Index as: $LAI = p / c$	2,16

LAI Calculation															
Field_ID	Plot	Tree#	c	e	f	h	i	j	k	l1	m	n	o	p1	q1
			Plant density	Number of Leaves on representative branch	Length of branch part with leaves	Leaf width	Leaf length	Leaf area of Leaf	Leaf Area of representative branch	Total number of branches	Average branch length of all branches	Average branch length with leaves of all branches	Total branch length with leaves	Leaf Area per tree	Leaf Area Index
			[m2 tree-1]	[#]	[cm]	[cm]	[cm]	[cm2]	[cm2]	[#]	[cm]	[cm]	[cm]	[m2]	[m2 m-2]
BT	A1	1	4,0	27,0	30,0	7,8	6,9	43	1174	6,73	181,93	44,12	297	1,16	0,29
	A1	2	4,0	31,0	21,7	8,5	6,4	44	1366	5,26	173,14	33,43	176	1,11	0,28
	A1	3	4,0	29,0	26,0	7,3	6,7	39	1141	4,47	124,76	21,89	98	0,43	0,11
	A1	4	4,0	41,0	18,0	5,7	4,7	22	904	6,03	176,85	18,95	114	0,57	0,14
	A1	5	4,0	56,0	67,0	6,0	7,7	37	2083	5,68	158,65	62,16	353	1,10	0,27

Appendix D Excel summary file.

Table 10 Excel summary file where numbers in bold represent plot averages and Colors correspond to Excel template (Fig.19) SD = Stem diameter, SEM=Standard error of mean, TH = tree height, PD<sup>-1</sup> = Surface area per tree, D = disease, TCV = total canopy volume, LAI = Leaf area index, TAB = total accumulated biomass volume, 1<sup>st</sup> tm 7<sup>th</sup> in blue = contribution to biomass per branching order, model total = total modelled harvest, model SDW = modelled seed dry weight harvest, Actual total = actual total harvest, Actual SDW = Actual seed dry weight harvest, 1<sup>st</sup> tm 7<sup>th</sup> orange = contribution to yield per branching order. Number of observations (n) is given in Table 4.

	SD	SEM	TH	SEM	PD <sup>-1</sup>	SEM	D	SEM	TCV	SEM	LAI	SEM	TAB	SEM	1st	SEM	2nd	SEM	3rd	SEM	4th	SEM	5th	SEM	6th	SEM	7th	SEM
	(mm)		(mm)		(m2 tree-1)		(%)		(m3)		m2 leaf m-2)		(cm3)		(-)		(-)		(-)		(-)		(-)		(-)		(-)	
A1	63,78	3,09	1740,00	37,57	4,00	0,00	0,61	0,35	0,76	0,12	0,46	0,07	3446,72	278,70	0,55	0,03	0,30	0,03	0,13	0,02	0,02	0,01	0,00	0,00	0,00	0,00	0,00	0,00
A2	55,70	1,94	1838,67	50,63	4,27	0,12	0,81	0,73	0,52	0,08	0,13	0,02	2809,28	316,73	0,56	0,04	0,26	0,03	0,13	0,02	0,05	0,01	0,00	0,00	0,00	0,00	0,00	0,00
B1	68,61	2,52	1655,00	77,40	4,75	0,19	1,98	0,79	0,98	0,21	0,36	0,05	4811,26	633,78	0,58	0,03	0,29	0,02	0,11	0,02	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00
B2	56,54	1,90	1665,63	63,86	4,00	0,00	1,51	1,02	0,49	0,07	0,38	0,06	3032,25	380,02	0,53	0,05	0,34	0,04	0,10	0,02	0,02	0,01	0,00	0,00	0,00	0,00	0,00	0,00
C1	64,52	3,55	1491,14	76,52	4,00	0,00	5,89	1,76	0,54	0,08	0,38	0,05	3372,42	414,01	0,54	0,02	0,26	0,02	0,15	0,02	0,04	0,01	0,01	0,00	0,00	0,00	0,00	0,00
C2	58,39	2,28	1801,63	87,97	4,25	0,11	2,28	1,93	0,56	0,11	0,18	0,05	3161,67	581,54	0,60	0,04	0,28	0,03	0,08	0,02	0,03	0,01	0,00	0,00	0,00	0,00	0,00	0,00
D1	57,31	1,72	1606,00	55,42	4,70	0,20	5,08	1,13	1,01	0,18	0,44	0,07	4416,08	1033,60	0,50	0,02	0,31	0,01	0,14	0,02	0,05	0,01	0,01	0,00	0,00	0,00	0,00	0,00
D2	63,40	3,59	1632,31	47,88	4,35	0,13	1,50	0,73	0,58	0,07	0,18	0,07	3361,51	629,98	0,56	0,05	0,30	0,05	0,10	0,02	0,03	0,01	0,01	0,00	0,00	0,00	0,00	0,00
E1	52,87	4,73	1408,18	63,11	4,68	0,24	2,14	0,77	0,52	0,15	0,19	0,04	2549,74	490,80	0,43	0,05	0,35	0,03	0,17	0,03	0,05	0,02	0,00	0,00	0,00	0,00	0,00	0,00
E2	54,75	3,86	1261,67	63,34	4,17	0,07	5,99	3,35	0,42	0,09	0,37	0,09	2109,59	361,25	0,57	0,05	0,28	0,03	0,12	0,03	0,03	0,02	0,00	0,00	0,00	0,00	0,00	0,00
F1 PD-T	91,68	48,38	1777,53	48,38	10,05	0,82	6,57	1,92	1,49	0,31	0,15	0,04	6711,49	744,47	0,51	0,03	0,29	0,02	0,14	0,02	0,04	0,01	0,01	0,00	0,00	0,00	0,00	0,00
F2 PD-T	99,64	126,79	2060,00	126,79	9,92	1,07	8,25	1,50	1,65	0,18	0,27	0,08	8344,62	1209,24	0,47	0,05	0,26	0,03	0,17	0,02	0,09	0,03	0,01	0,00	0,00	0,00	0,00	0,00
F3 PD-T	108,94	151,28	2472,67	151,28	8,79	0,85	3,63	1,18	3,82	0,73	0,31	0,08	15057,60	2292,47	0,40	0,04	0,26	0,03	0,24	0,03	0,08	0,02	0,02	0,01	0,00	0,00	0,00	0,00
F1 PD-OC	91,68	48,38	1777,53	48,38	4,08	0,55	6,57	1,92	1,49	0,31	0,01	0,00	6711,49	744,47	0,51	0,03	0,29	0,02	0,14	0,02	0,04	0,01	0,01	0,00	0,00	0,00	0,00	0,00
F2 PD-OC	99,64	126,79	2060,00	126,79	4,23	0,65	8,25	1,50	1,65	0,18	0,01	0,00	8344,62	1209,24	0,47	0,05	0,26	0,03	0,17	0,02	0,09	0,03	0,01	0,00	0,00	0,00	0,00	0,00
F3 PD-OC	108,94	151,28	2472,67	151,28	3,92	0,47	3,63	1,18	3,82	0,73	0,01	0,00	15057,60	2292,47	0,40	0,04	0,26	0,03	0,24	0,03	0,08	0,02	0,02	0,01	0,00	0,00	0,00	0,00
Accession	Model				Actual																							
	Total	SEM	SDW	SEM	Total (g)	SEM	SDW	SEM	1st	SEM	2nd	SEM	3rd	SEM	4th	SEM	5th	SEM	6th	SEM	7th	SEM						
	(g)		(g)		(g)		(g)		(-)		(-)		(-)		(-)		(-)		(-)									
	A1	1825,20	20,23	234,87	2,60	2022,30	0,00	311,30	0,00	0,03	0,02	0,71	0,08	0,22	0,06	0,03	0,02	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	
	A2	655,50	13,94	86,18	1,83	492,50	0,00	74,94	0,00	0,00	0,00	0,34	0,09	0,31	0,08	0,34	0,10	0,02	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	
	B1	1058,21	20,22	191,54	3,66	976,60	0,00	205,27	0,00	0,00	0,00	0,53	0,11	0,26	0,08	0,19	0,08	0,02	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	
	B2	1058,64	16,55	205,15	3,21	1087,00	0,00	216,03	0,00	0,00	0,00	0,52	0,09	0,41	0,08	0,07	0,04	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	
C1	1216,59	17,50	209,14	3,01	897,50	0,00	168,86	0,00	0,08	0,07	0,62	0,12	0,30	0,10	0,01	0,01	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	

C2	1256,90	21,17	214,15	3,61	1152,70	0,00	225,66	0,00	0,02	0,01	0,57	0,10	0,25	0,06	0,11	0,04	0,04	0,03	0,00	0,00	0,00	0,00
D1	576,64	17,86	98,54	3,05	348,80	0,00	61,59	0,00	0,00	0,00	0,71	0,11	0,25	0,05	0,04	0,02	0,00	0,00	0,00	0,00	0,00	0,00
D2	265,66	10,15	44,30	1,69	343,60	0,00	66,82	0,00	0,00	0,00	0,27	0,08	0,61	0,12	0,12	0,05	0,00	0,00	0,00	0,00	0,00	0,00
E1	14,10	0,86	1,76	0,11	14,10	0,00	1,76	0,00	0,00	0,00	0,50	0,09	0,50	0,09	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
E2	218,75	8,39	39,62	1,52	425,70	0,00	69,74	0,00	0,00	0,00	0,36	0,09	0,58	0,12	0,06	0,02	0,00	0,00	0,00	0,00	0,00	0,00
F1 PD-T	1860,86	49,19	225,47	5,96	1822,10	0,00	306,37	0,00	0,00	0,00	0,12	0,07	0,48	0,11	0,31	0,11	0,10	0,06	0,00	0,00	0,00	0,00
F2 PD-T	1731,66	20,70	256,40	3,08	986,70	4,11	164,51	0,00	0,00	0,00	0,09	0,04	0,54	0,09	0,22	0,05	0,15	0,07	0,00	0,00	0,00	0,00
F3 PD-T	3477,63	51,99	362,11	5,40	1881,00	7,58	231,39	0,00	0,00	0,00	0,06	0,03	0,45	0,09	0,32	0,07	0,17	0,06	0,01	0,01	0,00	0,00
F1 PD-OC	1860,86	49,19	225,47	5,96	1822,10	0,00	306,37	0,00	0,00	0,00	0,12	0,07	0,48	0,11	0,31	0,11	0,10	0,06	0,00	0,00	0,00	0,00
F2 PD-OC	1731,66	20,70	256,40	3,08	986,70	4,11	164,51	0,00	0,00	0,00	0,09	0,04	0,54	0,09	0,22	0,05	0,15	0,07	0,00	0,00	0,00	0,00
F3 PD-OC	3477,63	51,99	362,11	5,40	1881,00	7,58	231,39	0,00	0,00	0,00	0,06	0,03	0,45	0,09	0,32	0,07	0,17	0,06	0,01	0,01	0,00	0,00

SD = (SD1 + SD2)/2

TH = TH

PD<sup>-1</sup> = equation 2

D = equation 4

TCV = equation 1

LAI = LAI-protocol Appendix C

TAB = (Σ vol. representative branch)\* # 1<sup>st</sup> order branches

Fr. Biomass = equation 3

Model total = ((Σ # capsules per representative branch)\* # 1<sup>st</sup> order branches \* avg capsule weight)

→ Σ for all trees per plot

Model SDW = ((Σ # capsules per representative branch)\* # 1<sup>st</sup> order branches \* # seeds per capsule \* SDW)

→ Σ for all trees per plot

Actual total = Collected capsules per plot \* avg capsule weight

Actual SDW = Collected capsules per plot \* # seeds per capsule \* SDW

Fr. Harvest = equation 5

## Appendix E Summary statistical test results

**Table 11 Summary of the ANOVA test results with F-test showing the results of ANOVA and  $p$ -values showing significance when  $p < 0,05$ .**

Parameter	Accession only		Plot only		Accession and Plot	
	F-test	Significance	F-test	Significance	F-test	Significance
Stem Diameter	F=2,35, $p=0,058$	No	F=5,46, $p=0,021$	<b>Yes</b>	F=3,31, $p=0,013$	<b>Yes</b>
LAI	F=0,88, $p=0,48$	No	F=12,56, $p<0,001$	<b>Yes</b>	F=5.69, $p<0,001$	<b>Yes</b>
Disease percentage	F=2,26, $p=0,066$	No	F=0,85, $p=0,359$	No	F=2,11, $p=0,083$	No
Total accumulated biomass volume	F=2,50, $p=0,045$	<b>Yes</b>	F=6,1, $p=0,015$	<b>Yes</b>	F=0,63, $p=0,641$	No
Tree height	F=11,75, $p<0,001$	<b>Yes</b>	F=2,45, $p=0,12$	No	F=3,06, $p=0,019$	<b>Yes</b>
Surface area per tree	F=3,83, $p=0,006$	<b>Yes</b>	F=6,81, $p=0,010$	<b>Yes</b>	F=6,48, $p<0,001$	<b>Yes</b>
Total Canopy Volume	F=2,14, $p=0,079$	No	F=10,68, $p=0,001$	<b>Yes</b>	F=1,43, $p=0,226$	No
Harvest	F=6,10, $p<0,001$	<b>Yes</b>	F=1,29, $p=0,258$	No	F=1,29, $p=0,28$	No