DOCTOR OF PHILOSOPHY

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a Wild Fruit Species for Health and Livelihoods

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Conservation of *Mangifera sylvatica*: a Wild Fruit Species for Health and Livelihoods

A Thesis for a Double Degree of Doctor of Philosophy

by

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PRIFYSGOL BANGOR UNIVERSITY
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“Somewhere, something incredible is waiting to be known.”

— Carl Sagan

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Abstract

Many wild and underutilised plants contribute to food and nutrition. However, overexploitation due to ever increasing demand for wood products has frequently led to declines in populations of these species. Enhanced knowledge of the status of such species is necessary for livelihood security and conservation of these valuable species. The present study considers an underutilised and threatened species of Bangladesh, namely wild mango (*Mangifera sylvatica* Roxb.). Although this wild mango is one of the genetically closest species to the common mango (*Mangifera indica* L.) research is very limited and mostly focused on wood quality and phylogenetic relationships. Therefore, this study investigated the conservation potential of wild mango considering its contribution for food, nutrition and livelihoods. To do so, an assessment was made of the current and future distribution of the species, which is a crucial first step towards mitigation and management of future species losses or habitat shifts. The study characterized fruit quality by profiling morphological, nutritional and medicinal values. Finally, farmers' preferences, and the agroforestry potential of this unutilized native fruit species were explored. The study conveyed five key messages: 1. Wild mango may become extinct under future climate change scenarios so it is high time to start thinking about conservation initiatives. 2. Wild mango is a small sized mango with a large kernel in relation to other *Mangifera* species which provides significant nutritional and medicinal advantages that can contribute to nutrition and health of local people. 3. Wild mango fruit kernels producing a butter which has the potential to be used as a Cocoa Butter Alternative (CBA) thus providing new market potential. 4. Wild mango is a considered as a food for wildlife but local people are also appreciative of the taste and colour of the fruits and consume them. The unripe fruit is also sold to the pickle industry and can generate income during the fruiting season. 5. The crown architecture of wild mango is similar to other popular agroforestry species (*M. indica, Artocarpus heterophyllus* and *Acacia auriculiformis*). Therefore, urgent conservation initiatives are required to evaluate its potential as a new native agroforestry tree species. It is concluded that these attractive properties of wild mango could be promoted by a coalition of land use planners, climate change policy makers, government or non-government organizations, commercial breeder, potential investors (chocolate, butter, nutraceuticals, flavourings), food industries, food technologists, minister responsible for foreign direct

investment in Bangladesh and Bangladesh forestry department to promote more widespread cultivation of this wild fruit species to realise its full potential.

ABBREVIATIONS

AUC	Area Under Receiver Operator Curve
BFRI	Bangladesh Forest Research Institute
BHT	Butylated hydroxytoluene
CB	Cocoa Butter
CBA	Cocoa Butter Alternative
CBD	Cocoa Butter Deodorized
CBE	Cocoa Butter Equivalent
CBND	Cocoa Butter Non-Deodorized
CHTs	Chittagong Hill Tracts
CI	Competitive Index
CTG	Chittagong
CWR	Crop Wild Relatives
DBH	Diameter Breast Height
DMB	Domesticated Mango butter
DSC	Differential Scanning Calorimetry
ESI	Electro Spray Ionization
GC-MS	Gas Chromatography - Mass Spectrometry
HPLC	High Pressure Liquid Chromatography
PAR	Photosynthetically Active Radiation
R/FR	Red/ Far-Red
RCP	Representative Concentration Pathway
SC-CO_2	Supercritical CO_2
SDM	Species Distribution Model
SYL	Sylhet
VIF	Variance Inflation Factor
WMB	Wild Mango Butter

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Chapter 1. Introduction

1.1 Background

Wild forest crops provide a large proportion of global food needs; hundreds of species are utilised at a local level and contribute substantially to household food and livelihood security. Moreover, many wild plant species are also important, both nutritionally and culturally (Kaimowitz and Sheil, 2007). These wild forest crops are also known as Crop Wild Relatives (CWR) and are closely related to domesticated crops under cultivation (Bioversity International, 2006). Their role in crop improvement is well established, dating back more than 60 years (Hajjar and Hodgkin, 2007). Wild crops can undoubtedly benefit modern agriculture, providing plant breeders with a broad pool of potentially useful genetic resources (Hajjar and Hodgkin, 2007). In addition, throughout the world, wild edible species make significant contributions in terms of food security, meeting nutritional and economic needs (Sundriyal and Sundriyal, 2001; Roshetko and Evans, 1997; Chin, 1985), which are a major concern for many tropical developing countries. With increasing climatic instability causing frequent agricultural crop failure, the role of wild crops in providing nutritional supplements to mankind is increasingly recognised (Maghembe, 1995). Many current human health problems are related to diet and here wild crops can play an influential role in mitigating such problems as many of them are rich in macro and micro nutrients (Leterme et al., 2006). Mwanjumwa (1982) highlights the potential of using wild fruits to meet the requirements of limiting micronutrients, Vitamin A and Vitamin C in the diets of rural populations and revealed that wild fruit species are high in dry matter, fibre and minerals. However, these wild crops are often threatened by environmental destruction, climate change, increasing population and land use changes. Considering the future, it is important to conserve them. Under these circumstances, the present study focuses on a locally threatened species, *Mangifera sylvatica* Roxb. Although, according to the IUCN red list, this species is in the least-concern category, the information on the species dates from 1998 and needs to be updated (World Conservation Monitoring Centre, 1998). However, according to the *Red Data Book* of Bangladesh, this is a threatened species in that country (Dutta et al., 2016; Baul et al., 2016; BFRI, 2013). *Mangifera sylvatica* Roxb. is currently threatened in India as well (Jiji, 2015). The species occurs mainly in the tropical humid forests, subtropical rainforests and tropical dry forests of the Indo-Malayan biogeographic region (Udvardy, 1975).

M. sylvatica is in the Anacardiaceae family. Local names include Uriam, Jangli Am, Banam, Baittaam and Chuchiam (in Bengali). The fruits are very rich in Vitamin C (Sundriyal et al., 1998). The ripe fruits are edible but do not match common mango fruits in terms of quality. Local people eat the fruit of this species and also use it for medicinal purposes. The fruits of *M. sylvatica* are very popular for jam, jelly and pickles (Sankaran et al., 2006; Sundriyal et al., 1998). It is also used to treat skin diseases in animals, whereby oil made from this mango is used as an insecticide and a parasiticide (Sharma and Joshi, 2004). It is believed that natural hybridisation has taken place between *Mangifera indica* and *Mangifera sylvatica* in Southeast Asia. A close relationship between *M. sylvatica* and *M. indica* has been clearly proven (Mukharjee, 1985). Examination of chromosome numbers and cytological features indicates that *M. indica* and *M. sylvatica* have the same chromosome number (n=20) (Nishiyama et al., 2006; Mukherjee, 1950; Mukharjee, 1957), indicating a close relationship between the two species. In addition, Mukherjee (1953) found that the karyotype of the cultivars of *M. indica* and *M. sylvatica* indicates a close relationship with a minor variation in the number of satellites and secondary constrictions, and the author concludes that it is difficult to find a definite relationship in their origins (Mukherjee, 1950). Therefore, the close genetic relationship of this species to *M. indica* indicates that these traits may be transferred to the common mango (*M. indica*) for commercial production in order to help fulfill nutritional needs.

1.2 Justification for the study

Despite substantial increases in food production, the people of Bangladesh are still poorly nourished. Many suffer from malnutrition and related diseases. Although the wild fruits derived from the forest areas are not consumed in great quantities compared to the main food staples, they do add diversity to diets, improve the palatability of staple foods and provide essential vitamins, minerals, proteins, carbohydrates, fats and calories inexpensively. Wild fruit trees offer vital insurance against malnutrition or famine during the seasonal food shortage and/or emergencies such as drought, flood and war (Hossain et al., 2011). However, these wild gene pools, especially those occurring in disturbed habitats, are under threat of genetic erosion and require immediate conservation to make use of their wider adaptability, tolerance/resistance to disease, insect-pests, yield, quality attributes and other biotic and abiotic traits.

In Bangladesh, there are 47 edible wild forest fruits available (Das, 1987). Among these, *M. sylvatica* is distributed in the Chittagong, Chittagong Hill Tracts (CHTs), Cox's Bazar and Sylhet regions of Bangladesh (Das, 1987; Khan et al., 2001). This is one of the most important species in the hilly region of Bangladesh. Alamgir and Al-Amin (2007) found that *M. sylvatica* has the second highest mean value in above-ground, below-ground and total organic carbon (2.78, 0.42 and 3.20 t/tree in Chittagong south forest division of Bangladesh. Moreover, they also state that, in considering total organic carbon, *M. sylvatica* has the fifth highest carbon storage (1.962 t/ha). Unfortunately, this species is declining at an alarming rate due to various factors. Dewan (2009) found that the Important Value Index (IVI) (i.e. IVI is a measure of relative abundance, relative frequency and relative density) of *M. sylvatica* in CHTs is very low (2.49) in comparison to other plant species. According to Dewan (2009), the reason behind the depletion is legal or illegal logging, as well as shifting cultivation with short rotation periods. In addition, forest fires used as a preparatory measure for shifting cultivation have a negative impact on the natural regeneration of this species. Moreover, in Bangladesh, *M. sylvatica* is mainly used in plywood (i.e. a panel consisting of an assembly of thin sheets of wood bonded together with an adhesive where the grain direction remains in alternate plies, usually at right angles) industry. Plywood is used for making tea chests, internal decoration of buses, aircraft, cabinet making (wardrobes, tables, dressers, etc.), house building (walls, ceilings, floors, doors, etc.) and sports goods. In Bangladesh, there are approximately 25 factories producing commercial plywood; the majority are located in Chittagong and the Chittagong Hill Tracts and one of the raw materials is *M. sylvatica*. Bangladesh Standards and Testing Institution recommends 16 timber species for plywood making and *M. sylvatica* is one of them (Sattar, 2006). Also, in Bangladesh, the young leaves of *M. sylvatica* are used as vegetables by the tribes in the CHTs (Jalil and Chowdhury, 2000). They also eat the fruit and use it for pickles. So, there is tremendous pressure on *M. sylvatica*, which has a negative impact on socio-environmental aspects. The effect will be severe on plywood industries and poor people including tribal groups who are dependent on forest resources. In Bangladesh, only the Bangladesh Forest Research Institute (BFRI) has undertaken an initiative to conserve the species. BFRI initiated two projects, entitled 'Ex-situ conservation of threatened forest tree species in different agro-ecological regions of Bangladesh' and 'Conservation of threatened plant species through domestication', which started in 2006–7 and 2003–4, respectively, and are still ongoing. Under these two projects, BFRI raised 17.5 ha and 0.20 ha of conservation plots from seed, together with eight and ten other threatened species as gene resource conservation plots (BFRI, 2013). This initiative is not sufficient to domesticate and conserve *M. sylvatica.* To ensure effective domestication, it is necessary to know the current distribution, the reasons for the decline of this species, the attractive features (diet-health relationship, nutritional composition), the potential land use (e.g. agroforestry) and practices for survival of the species, which will help to explore its potential for conservation and domestication to both the scientific community and the local community. Unfortunately, research for the conservation of *M. sylvatica* is scarce globally and, of course, in Bangladesh as well. Recently, in a global context, scientists undertook studies on different aspects of *M. sylvatica*, such as a PCR-based assessment of genetic diversity (Srivastava et al., 2007); genomic *in-situ* hybridisation (Nishiyama et al., 2006); phylogenetic relationships of Mangifera species (Yonemori et al., 2002, Eiadthong et al., 2000; Eiadthong et al., 1999); edibility (Sundriyal and Sundriyal, 2001); species distribution (Ahmed and Haque, 1993); bionomics aspects (De and Pande, 1988); and pollen morphology (Mondal et al., 1982), which indicate the popularity of, and interest in, this species. On the other hand, in Bangladesh, BFRI tried to domesticate this species through plantation establishment. The present study investigated the conservation value of *M. sylvatica* Roxb., a wild fruit species of Bangladesh that can contribute to the health and livelihoods of the people.

1.3 Aims and objectives of the study

The overall aim of this study is to assess the conservation value of *M. sylvatica* (a wild fruit species), by identifying and quantifying chemical (e.g. nutritional composition), socio-economic (e.g. willingness to conserve, factors responsible for depletion of this species, etc.) and physical (e.g. identification of agroforestry suitability of the species) parameters that can influence the consumption and conservation of this species. To fulfill the overall aim of the study, the following specific objectives need to be fulfilled:

Objective 1: To determine the current and future distribution to identify areas of high conservation value under climate change for *M. sylvatica*.

Specific questions:

Q1. *What is the current distribution of* M. sylvatica *in Bangladesh?*

Q2. *Which bioclimatic variables most influence the predicted distribution of* M. sylvatica*?*

Q3. *To what extent is the potential distribution of this species expected to change under projected future climate changes?*

Q4. *Will there be an elevation shift of this species due to climate change?*

Objective 2: To characterise the fruit quality of *M. sylvatica* and to determine variations in fruit quality with site.

Specific questions:

Q1. *How great is the variation in fruit size, pulp and kernel weights between trees?*

Q2. *What are the nutritional and medicinal properties of* M. sylvatica *fruit?*

Q3. *Are there any differences in fruit quality due to site variation?*

Q4. *Is there any potential in the fruit for the production of agroforestry tree products to enhance smallholders' livelihoods and income?*

Objective 3: To assess the potential of *M. sylvatica* butter as a cocoa butter alternative (CBA).

Specific questions:

Q1: *What is the fatty acid composition of* M. sylvatica *butter?*

Q2: *What is the triglyceride composition of* M. sylvatica *butter?*

Q3: *What are the physical properties of* M. sylvatica *butter?*

Q4: *What is the melting profile of* M. sylvatica *butter?*

Q5: *How similar is* M. sylvatica *butter to cocoa butter and* M. indica *butter?*

Objective 4: To identify factors causing barriers for conservation of *M. sylvatica* and potential to be integrated as an agroforestry species.

Specific questions:

***Q1:** What are the issues creating barriers to the conservation of the species?*

***Q2:** What are the present uses and attractive features of* M. sylvatica*?*

***Q3:** How suitable is* M. sylvatica *as an agroforestry species?*

1.4 Thesis structure

Introduction (**Chapter 1**), demonstrates the importance of wild forest crops for health, food security and livelihood. Additionally, a detailed description of the conservation importance of *M. sylvatica* is highlighted. In **Chapter 2**, a short review of the existing literature on *M. sylvatica* is presented to find the research gaps. In **Chapter 3**, a widely used species distribution model (SDM), MaxEnt, is used to investigate the impact of climate change on the future habitat suitability. In **Chapter 4,** morphological, nutritional and medicinal traits of wild mango are investigated to promote increased use and cultivar development. In **Chapter 5,** to promote the domestication and commercialisation of the species, investigation on the potential of *M. sylvatica* kernel as a cocoa butter alternative (CBA) is carried out. CBA has great potential for the food and cosmetic industries. In **Chapter 6**, the farmer's perception of the use of this species and barriers to its domestication are determined. Investigation of the wild mango's integration suitability as an agroforestry species and its compatibility with other domesticated agroforestry tree species was conducted through tree architecture. In **Chapter 7**, a brief discussion on the overall potential of this species for domestication and conservation is written based on the present study. Finally, some limitations of the current research and suggestions for future research are made.

1.5 Authorship

Four chapters in this thesis have been prepared as manuscripts for peer-reviewed journals. Since the papers have multiple authors, the pronoun 'we' rather than 'I' is used throughout the body of the thesis. However, the inputs from my co-authors were limited to supervisory support and advice, provision of data and commenting on drafts. At the time of submission, Chapter 5 has been accepted for publication. Chapter 3 has been accepted with major revision. Chapters 4 and 6 are ready for submission. The contribution of the co-authors to each chapter is given below.

Chapter 3: This chapter has been accepted with major revision for publication as:

Akhter, S., McDonald, M. A., van Breugel, P., Sohel, S., Kjær, E. D. 2017. Habitat distribution modelling to identify areas of high conservation value under climate change for Mangifera sylvatica Roxb. of Bangladesh. Land Use Policy 60, 223–232

I conceived the idea and design with Prof. Morag McDonald (principal supervisor), Paulo van Breugel (PhD student, Department of Geosciences and Natural Resources Management, Copenhagen University, Denmark) and Shawkat Sohel (PhD student, Tropical Forestry Group, School of Agriculture and Food Science, University of Queensland, Australia). I collected the field data. I analysed the data in collaboration with Paulo van Breugel and Shawkat Sohel. Later, the whole manuscript was written by me and edited by Morag A. McDonald, Erik Dahl Kjær, Paulo van Breugel and Shawkat Sohel.

Chapter 4: This chapter will be submitted for publication as:

Akhter, S., McDonald, M. A., Kjær, E.D., Jashimuddin, M., Marriott, R. 2016. Morphological nutritional and medicinal traits of wild mango (*M. sylvatica* Roxb.): Implications for increased use and options for cultivar development. This will be submitted in Journal from Elsevier/Sciencedirect in 2016.

I conceived the idea and design with Prof. Morag A. McDonald (principal supervisor), Prof. Ray Marriott. I collected the field data with the help of Prof. Mohammed Jasimuddin, Institute of Forestry and Environmental Science, University of Chittagong, Bangladesh. I analysed the data with the help of Prof. Ray Marriott. Later, the whole manuscript was written by me and edited by Morag A. McDonald and Prof. Erik Dahl Kjær. I analysed and wrote the paper with advice from all co-authors.

Chapter 5: This chapter is published as:

Akhter, S., McDonald, M. A., Marriott, R. 2016. *M. sylvatica* (wild mango): a new cocoa butter alternative. *Scientific Report*, 6, 32050; doi: 10.1038/srep32050 (Nature group journal).

Sayma Akhter, Morag McDonald and Ray Marriott planned the research. Sayma Akhter collected samples, conducted the laboratory experiments, analysed the data and prepared the manuscript drafts. Ray Marriott assisted with the laboratory experiments. Morag A. McDonald reviewed and edited the manuscripts.

Chapter 6: This chapter will be submitted for publication as:

Akhter, S., McDonald, M. A., Jashimuddin, M. 2016. Agroforestry potential of a wild mango (*M. sylvatica* Roxb.). This will be submitted Journal from Elsevier/Sciencedirect in 2016.

Sayma Akhter, Morag A. McDonald and Mohammed Jashimuddin planned the research. Sayma Akhter collected the data, analysed the data and prepared the manuscript drafts. Mohammed Jashimuddin assisted with the field data collection. Morag A. McDonald reviewed and edited the manuscripts.

Chapter 2. Towards conservation of *M. sylvatica* of Bangladesh: a review

2.1 Introduction

At the threshold of the twenty-first century, more than 800 million people, 20% of the developing world's population, is food insecure, lacking economic and physical access to the food required to lead healthy and productive lives (FAO, 1996). Around 34%, 185 million, pre-school children in developing countries are seriously underweight for their age (UN ACC: SCN, 1992). Millions of people are at risk of nutrient deficiencies (Bread for the World Institute, 1996). Feeding millions of undernourished people depends not only on increased productivity of the limited number of domesticated crops in the modern world but also the use of underutilised wild species (Farooq and Azam, 2002). The latter and their natural products make significant contributions to mankind and are often a means of survival for millions of poor rural households (Belcher et al., 2005; Fisher, 2004; Scherr et al., 2004). Wild foods, including wild fruits, are important as a diet supplement, or as a coping mechanism in times of food shortage and famine, and provide an important safety net for the rural poor (Guinand and Dechassa, 2000; Mojeremane and Tshwenyane, 2004; Getachew et al., 2005; Redzic, 2007; McSweeny, 2004; Takasaki et al., 2004). Furthermore, wild foods may contribute to diet diversity (FAO, 2005). They can also serve as dietary replacements for populations that normally consume unhealthy foods (Redzic, 2007). Wild fruits provide vitamins, flavourings and compounds of nutritional, health such as alkaloids, essential oils and phenolics. Apart from their value in assuring food and nutritional security, the income potential from these trees is also enormous in an impoverished economy (Maghembe et al., 1998).

In Bangladesh, rates of malnutrition are among the highest in the world. More than 54% of preschool-age children, equivalent to more than 9.5 million children, are stunted, and 56% are underweight. Malnutrition among women is also extremely prevalent in Bangladesh. More than 50% of women suffer from chronic energy deficiency (FAO, 1999a). This scenario, however, can be improved through better dietary management. Here, wild fruits can play a significant role. The estimated total fruit availability per person per day of 155g is much higher than the current consumption of 34g per person per day in Bangladesh (FAO, 2007). However, this gap can be minimised, especially by the people who live in and around the forest, from the wild resources of Bangladesh. Bangladesh abounds with a large variety of tropical and sub-tropical wild fruits. These fruits have both food and market value, and different delicious foods such as jam, jelly, squash, pickle and juice can be produced from them. An estimation showed that Bangladesh spent more than 3 million Euro in 2014 on fruit imports, whereas, a large number of wild fruits are available and are grown with very little care and maintenance (Saha, 1997). Das (1987) reported the status and distribution of 47 edible fruit species from Bangladesh forests. However, a number of wild fruit species are becoming extinct and endangered due to deforestation and habitat destruction (Hossain and Uddin, 2005). *M. sylvatica* is one such species, which is now threatened due to overexploitation and habitat destruction (BFRI, 2013; Uddin and Hassan, 2010). Hence, it is important to conserve this wild fruit species. Unfortunately, the importance of this wild mango (*M. sylvatica*) as a food supplement and means of survival during times of drought and famine has largely been overlooked by the research and development community. Moreover, to make the conservation action successful, species-specific information is required. The present study, therefore, seeks to appraise the ecology, botany, conservation importance and research gaps regarding *M. sylvatica*, which will provide baseline information to underpin successful conservation efforts in Bangladesh.

2.2 Botanical description

The scientific name of this wild mango is *Mangifera sylvatica* Roxb., which belongs to the Anacardiaceae family. The species has various common names in Bangladesh such as Uriam (Chittagong), Jangli-am (Sylhet), Kosh-am (Bengali), Garey-am (Chakma), Laksmi-am (Sylhet), Bon am and Baitta am (Chittagong). *M. sylvatica* are long-lived (100 years) evergreen trees that can reach heights of 15–30m with a straight bole 15m long and 3m in girth at 300–1,300m altitudes. The tree height is between 6–20m in Bangladesh (Baul et al., 2016; Mamun and Hossain, 2011) and up to 25m in India (Paul, 2013). Fruits are ovate, elliptical and variable in size and with a thin skin (Baul et al., 2016). *M. sylvatica* has an umbrella-like canopy (Figure 2.1), which covers a wide area at the mature stage. Variability in canopy shape and openness influences various ecological factors, e.g. light penetration, nutrient uptake and competition with other plants. The flowers are pinkish-white in glabrous panicles of more than 30cm long. The leaves are lenceolate, narrower and longer than leaves of *M. indica*. The bark is ashy-grey or silvery (Figure 2.1), irregularly cracked and exuding a whitish gum. The bole is straight and cylindrical (Das and Alam, 2001). The wood of this species is light (specific gravity 0.54), moderately hard, straight and medium textured. It saws with ease and machines to a smooth surface; it is easily worked by hand tools (Council of Scientific and Industrial Research, 1962). The colour of the sapwood and heartwood is not well defined, the wood is light brown with dark-brown streaks. Growth rings are defined due to a dense fibre zone and bands of parenchyma delimiting growth rings. The wood is indented by the nail, straight and medium textured (Sattar and Akhtaruzzaman 1997). The seed germination rate of *M. sylvatica* is 60–75% compared to 60–90% for *M. indica*. *M. sylvatica* is known as the mother of all mangos (http://natureproducts.net/Forest_Products/Trees/Mangifera_sylvatica.html) and also it is believed that there is a close relationship between *M. sylvatica* and *M. indica* because of their similar chromosome number (Mukherjee, 1950 and 1957; Nishiyama et al., 2006).

Figure 2.1: a) *M. sylvatica* fruit; b) canopy; c) bole; d) seedlings; e) leaf and seed

2.3 Distribution and ecological requirements

M. sylvatica mainly occurs in tropical humid forests, subtropical rainforests and tropical dry forests of the Indo-Malayan biogeographic region (Udvardy, 1975). Specifically, it is found in Bangladesh, India, Nepal, Myanmar, Thailand, China and Cambodia. In Bangladesh, *M. sylvatica* is distributed in the hilly areas such as Chittagong, Cox's Bazar, Chittagong Hill Tracts (CHTs) and Sylhet (Baul et al. 2016). It generally grows in areas where rainfall ranges at an average of 2,600–3,000mm/year and a mean annual temperature of 22–30°C. *M. sylvatica* grows in the hilly areas of Bangladesh where the soil type is known as 'Brown Hill Soils'. The Brown Hill Soils are spread in the hilly regions and vary from brown sandy loam to clay loam (FAO, 1999b). According to FAO, the soil type known as Dystric Cambisol is suitable for the growth of *M. sylvatica* (Table 2.1).

Table 2.1: Soil physical and chemical properties

Soil properties	Value	Soil properties	Value
sand % topsoil	32.7	sand % subsoil	29.8
silt % topsoil	30.3	silt % subsoil	37.6
clay % topsoil	37.1	clay % subsoil	32.3
pH water topsoil	4.9	pH water subsoil	5.3
OC % topsoil	3.28	OC % subsoil	0.87
N % topsoil	0.23	N % subsoil	0.05
BD topsoil	0.8	BD subsoil	0.9
C/N topsoil	12	C/N subsoil	15

Source: FAO soil database

2.4 Flowering, fruiting and propagation

M. sylvatica flowering occurs during the winter between January and March. Some varieties develop all of their flowers within ten days after the first bud opens, whereas others may take several weeks or even months. The latter types have a better chance of setting fruit because of the longer period available for pollination and for overcoming unfavourable weather conditions (Mukherjee, 1953). It is interesting to note that all the wild forms of *M. sylvatica* and *M. indica* flowers per panicle range from 1,000 to 6,000 (Mukherjee, 1953). The absolute temperature needed for floral induction varies among varieties and climates, but night temperatures between 8°C and 15°C with day temperatures around 20°C are typically needed (Bally, 2006). Young trees will produce fruit between 7 and 8 years of age. Initially, hundreds of fruits can be set on each flowering inflorescence. The tree naturally thins the crop by shedding fruit throughout the fruit-development period. The main fruiting time is between May and June. The propagation method for *M. sylvatica* is by seed and is very similar to *M. indica*.

2.5 Uses

M. sylvatica has fruits that are eaten by the local people and also used for medicinal purposes (Sarmah, 2012). The fruits of *M. sylvatica* are very popular for jam, jelly and pickles (Sankaran et al., 2006; Sundriyal et al., 1998). In Rangamati Sadar Thana in Bangladesh, 5.76% of people use this species as a fruit (Miah, 2012). Moreover, this species is a food source for the Hoolock gibbon, which is one of the endangered wildlife species of Bangladesh (Muzaffar et al., 2007) and also consumed by Rhesus macaques (Sengupta and Radhakrishna, 2015). The young leaves of *M. sylvatica* are cooked as vegetables and used as pickles by the tribes in the CHTs, Bangladesh (Chowdhury and Miah, 2003; Jalil and Chowdhury, 2000). It is also used to treat skin diseases in animals, whereby the oil made from this mango is used as an insecticide and parasiticide (Sharma and Joshi 2004). One to two whole ripe fruits are daily used orally as laxatives in Sikkim, India (Chanda et al., 2007). The wood is used for teachest plywood and match boxes. The green fruit is pleasantly aromatic, more so than the domesticated mango, and can be made into chutney, pickles, etc. However, there is much research scope in the fruit of this species (Das and Alam, 2001). The wood is used for the same purpose as mango wood and in the market it is commonly found mixed with *M. indica*. It yields strong and fairly ornamental plywood (Council of Scientific and Industrial Research, 1962).

2.6 Research and conservation interventions in Bangladesh

In Bangladesh, a few initiatives have been taken by two organisations, namely the Bangladesh Forest Research Institute (BFRI) and the Bangladesh Forest Department. The BFRI initiated two projects, entitled ‘Ex-situ conservation of threatened forest tree species in different agro-ecological regions of Bangladesh’ in 2006–7 and another project entitled ‘Conservation of threatened plant species through domestication' in 2003–4. Under these two projects, the BFRI raised 17.5 ha and 0.2 ha of conservation plots from seed together with eight and ten other threatened species as gene resource conservation plots (BFRI, 2013). The Forest Department also undertook a plantation programme in Cox's Bazar South Forest Division. In 2012, they planted 1,000 seedlings and raised 1,500 seedlings in the nursery to plant in June 2013. Recently, in 2014, the Arannayk Foundation supported the Institute of Forestry and Environmental Sciences, Chittagong University (IFESCU) in conserving and restoring threatened species of Bangladesh that can be used as the source of seeds and research for students in the future (Arannayk Foundation, 2016). However, these initiatives are not sufficient to conserve *M. sylvatica* in the current extreme situation. To ensure effective domestication, species-specific information is required such as why the species is declining, what are its attractive features (nutritional composition), what are the potential land use (e.g. agroforestry) practices for survival of the species that will help to explore its potential for conservation and domestication. Unfortunately, conservation research on this species is scarce globally and even nationally. However, between 1950 and 2010 researchers and scientists increased their focus on the *Mangifera* genus (Figure 2.2), such as PCR-based assessment of genetic diversity (Srivastava et al., 2007), genomic *in situ* hybridisation (Nishiyama et al., 2006), phylogenetic relationships of *Mangifera* species (Yonemori et al., 2002; Eiadthong et al., 2000; Eiadthong et al., 1999), edibility (Sundriyal and Sundriyal, 2001), species distribution (Ahmed and Haque, 1993), the bionomics aspect (De and Pande, 1988) and pollen morphology (Mondal et al., 1982), which indicates the popularity of this species (Table 2.2). The present study investigates the conservation value of a threatened species *M. sylvatica* Roxb. a wild fruit species of Bangladesh that can contribute to the health and livelihoods of the people.

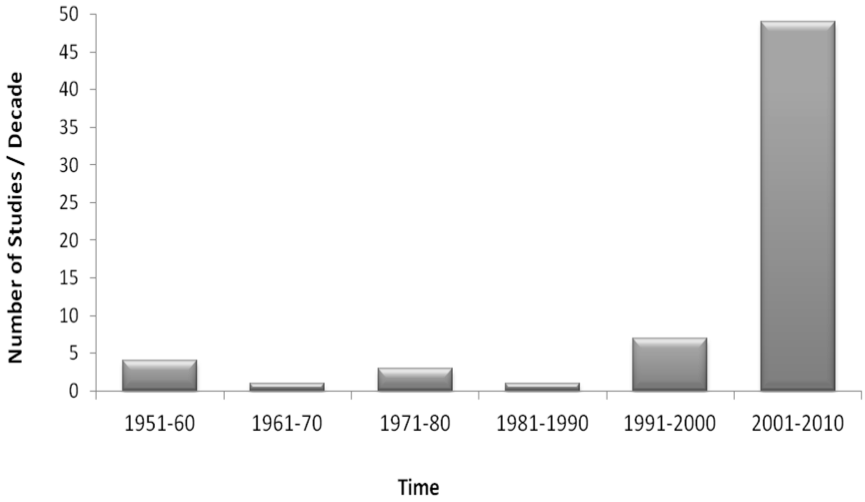


Figure 2.2: Popularity of *M. sylvatica* over time

Table 2.2: Global research on *M. sylvatica*

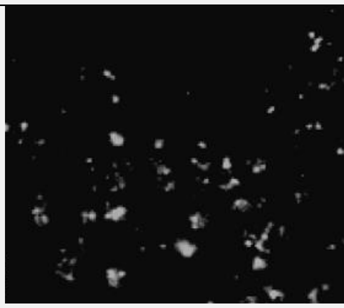
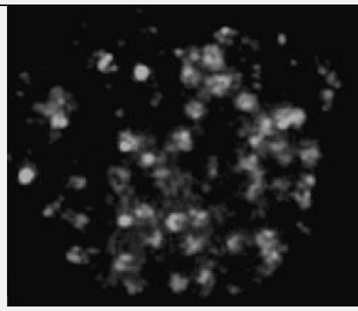
Research	**Country**	**Reference**
Carbon stock	Bangladesh	Ullah and Al-Amin, 2012; Alamgir and Al-Amin, 2007
Distribution	Bangladesh, India	Choudhury, 2015; Selvan, 2014; Zaman et al., 2010
Fruit yield	Bangladesh	Hossain and Uddin, 2005
General chemical analysis	India	Sundriyal and Sundriyal, 2004
Phylogenetic study	Thailand, India	Nishiyama et al., 2006; Krishna and Singh, 2007
Regeneration study	Bangladesh, India	Baul 2016; Deb and Sundriyal, 2007
Status and use	India, Malaysia, Indonesia, Bangladesh, China, Myanmar, Nepal	Uddin and Hassan, 2010; Sarmah, 2010; Miah, 2012; Baul 2016
Wood anatomy	India	Gupta and Agarwal, 2008

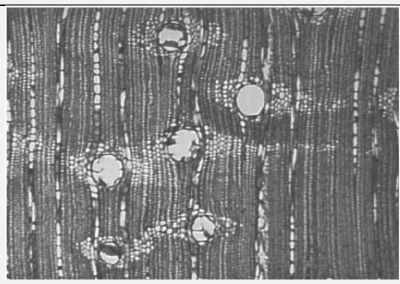
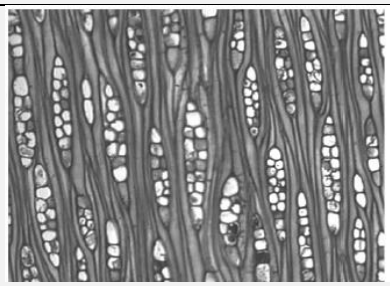
2.7 Closeness of *M. sylvatica* and *M. indica*

Mangifera sylvatica is known as the mother of all mangos (http://natureproducts.net/Forest_Products/Trees/Mangifera_sylvatica.html) and also it is believed that there is a close relationship exists between *M. sylvatica* and *M. indica* because of their similar chromosome number (Mukherjee, 1950; 1957). This might be helpful to popularize and domesticate *M. sylvatica.* Agroforestry could be a potential sector for conservation of wild forest fruits. 22.6% *M. indica* species are planted in agroforestry of Bangladesh (Hasan et al., 2008) and due to closeness of *M sylvatica* with *M. indica* there is a potentiality of introducing this species as agroforestry species in Bangladesh. Moreover the traits of wild mango can be used to improve the production, quality of domesticated mango. Physical differences and similarities of *M. sylvatica* and *M. indica* are given below (Table 2.3).

Table 2.3: Comparison of M. sylvatica and M. indica to highlight their Physical differences and similarities

Tree Characteristics	*Mangifera sylvatica*	*Mangifera indica*
Common Names		
Name	Uriam, Banam, Baittaam, Chuchiam, Laksmi-am , Jangli-am	Am, Ingsara, Thakachu, Jegachu, Bochu
Botany		
Tree size	Large evergreen tree, upto 45 m	Medium to large evergreen tree, 15–30 m long
Bark	Ashy grey or silvery	Brown or ashy grey
Leaves	Lanceolate, narrower and longer than the leaves of indica, lateral veins are curved	Oblong-lenciplate, 12 to 20 cm long, glossy green avobe, lateral veins are about 20 paris, Petiole about 2.5 cm long
Flowering	January to March	December and April
Fruiting season	May to June	April to July
Flower	Pinkish white in glabrous panicles of more 30 cm long	Inerect pubescent panicle

Fruit	Drupe 7 to 10 cm long, obliquely tapering acuminately slightly compressed	Drupe, ovoid or globose, laterally compressed
Fruit color	Yellowish	Yellow to orange
Taste	Sweet and sour when ripe	Sweet when ripe and sour when green
Propagation	Seed	Seed and vegetative part
Chromosome Number	2n (n= 20)	2n (n= 20)
Chromosomes of *M. indica* following GISH using FITC-labeled genomic DNA probe. White fluorescence indicates hybridization signal to the probe		
Seed Germination	65-75%	60-90%
Environmental Preference		
Altitude	300-1300 m	0-1200 m
Temperature	22-30^oC	19-35^oC
Average rain	2600-3000 mm	500-2500 mm
Soil	Brown hill soil, optimum P^H range is 4.9 to 5.3	Well drained soil with P^H 5.5 - 7.5 and fairly tolerant to alkalinity,
Distribution		
Natural Range	Tropical humid forests, subtropical rainforests / woodlands and tropical dry forests / woodland of the Indo-Malayan biogeographic realm Specially found in India, Bangladesh, Thailand, Cambodia and Myanmar.	Tropical Asia, Borneo, Java, Sumatra and the Malay Peninsula.
Local range	Chittagong, Cox's Bazar, Chittagong hill tracts and Sylhet.	Throughout the country

Wood Characteristics (Anatomical)		
Inter Vessel pit diameter (µm)	8-13	7-12
Ray width (µm)	1-2	1-2
Ray height (µm)	428	252
Vessel member length (µm)	471	443
Vessel diameter (µm)	182	176
Fibre length (µm)	936	963
Fibre diameter (µm)	13-16	14-18
Wood color	Light brown with dark brown streaks and pinkish cast	Pale yellowish brown to Reddish brown and darkening on exposure
Wood anatomy		

Source: Mukherjee, 1953; Mukherjee, 1985; Bally, 2006; Gupta and Agarwal, 2008; Nishiyama et al., 2006.

2.8 Research gap and conclusion

A good number of wild forest species produce edible fruits, playing an important role in people's diet by providing vitamins, macro and micronutrients, herbal medicines, and contributing to the livelihoods of people. However, these wild plant resources are threatened by habitat destruction and overexploitation. This creates an alarming situation. In Bangladesh, *M. sylvatica* is a threatened species currently facing similar circumstances. The longer the delay, the greater will be the danger of losing these valuable genetic resources. We may have to pay an extremely high price to bring them back, if even possible, for their future use in the country to develop new varieties of crops, horticultural, forestry and medicinal plants. Documentation and information on *M. sylvatica* is poor in Bangladesh. This situation could well be averted through demonstrating its proven value and potential. Information on nutritional and medicinal values, proper utilisation and conservation values are yet to be included in scientific studies. Furthermore, the needs for simultaneous research on the direct and indirect beneficial effects of *M. sylvatica* (income, food, wood, shade, soil conservation) are essential. A lack of awareness about the current and future potential distribution and use is a major limitation in conservation. This information could help set priorities for collection, conservation and utilisation of this species. Unfortunately, only a limited number of studies have been conducted, mostly to determine the wood's anatomical features and the genetic relationship between *M. indica* and *M. sylvatica*. Information on most aspects related to domestication and conservation of *M. sylvatica* is scarce. It must be acknowledged that, in spite of being a native species, no research has been conducted on the suitability of the species for quick and efficient domestication and conservation compared with other woody species, which is a big research gap in this area. In addition, domestication through agroforestry practice is a wonderful approach whereby *M. sylvatica* can also become a suitable species for integration in different agroforestry systems, providing a powerful means of livelihood and nutritional benefit together with sustainable development. Government and Non-governmental Organisations can play an important role in popularisation and conservation of *M. sylvatica* in the hilly regions of Bangladesh. Identification and documentation of the potential of this species and the reasons for its depletion are urgently needed to popularise the wild fruit as deforestation accelerates the loss of this valuable genetic resource.

Chapter 3. Habitat distribution modelling to identify areas of high conservation value under climate change for *M. sylvatica* Roxb. of Bangladesh[1]

Abstract

The impact of climate change on ecosystems, especially at the species level, is already being observed across the world. To assess potential future climate change effects on species, scientists often use species distribution modelling (SDM). The estimation of likely changes in the distribution of species under future climate conditions is a crucial first step towards the mitigation and management of future species losses or habitat shifts. Considering this, the aim of the present study is to predict the effect of climate change on a valuable threatened tree species, *M. sylvatica* Roxb., of Bangladesh using MaxEnt. The current potential distribution as by the model suggests that around 5% of the study area is highly suitable wild mango habitat, with between 6% and 11% being moderately suitable. Under the RCP 4.5 scenario, the net decrease in suitable habitat is predicted to be 7% by 2070. Under the RCP 8.5 scenario, the model predicts that the total area suitable for mango will reduce by 12% by 2050, disappearing altogether by 2070. Therefore, urgent measures are required for the conservation of *M. sylvatica* in Bangladesh. The application of the species distribution model may provide policymakers and conservationists with a useful tool for the prediction of future distribution (at both local and regional scales) of poorly known species with high preservation concerns. The approach used in this study can provide a rapid assessment of the future conservation status of other important forest tree species in Bangladesh to improve our understanding of the vulnerability under changing climates.

[1]Akhter, S., McDonald, M. A., van Breugel, P., Sohel, S., Kjær, E. D. 2017. Habitat distribution modelling to identify areas of high conservation value under climate change for *Mangifera sylvatica* Roxb. of Bangladesh. *Land Use Policy* 60, 223–232

3.1 Introduction

Global climate change is occurring at an unprecedented rate. Average temperatures have increased by 0.85^{o}C in the last century and are predicted to continue to increase by a minimum of 0.3^{o}C–1.7^{o}C to a maximum of 2.6^{o}C–4.8^{o}C by 2100 (IPCC, 2013). There are known effects of this change on ecosystems, species and biodiversity (IPCC, 2015; CBD, 2010; Hansen et al., 2001). Effects include changes in phenology, habitat range shifts and extinction risk in one-fifth of plant species (Brummitt and Bachman, 2010; Lenoir et al., 2008; Fischlin et al., 2007; Hickling et al., 2006, Parmesan, 2003). Hence, it is important to understand the impacts of climate change on the future habitat distribution and suitability of species. Bangladesh is a biodiversity hotspot zone because of its geographical and climatic traits. It supports species numbers of at least 5,000 angiosperms, five gymnosperms, 113 mammals, 628 birds, 126 reptiles, 22 amphibians, 708 fish, 2,493 insects, 66 corals, 168 algae and 15 crabs (MoEF, 2001; IUCN, 2000; Ahmed and Ali, 1996; Khan, 1991). This rich biodiversity is highly vulnerable to climate change. According to the GOB (2005), the average increase in temperature would be between 1.4^{o}C and 2.4^{o}C; predicted increases in rainfall are 6% and 10% for the two projection years 2050 and 2100, respectively. The consequences of climate change are already felt in Bangladesh. There has been an increase in the frequency and intensity of floods, cyclones and droughts. Together with a rise in sea levels, these pose an increasing threat to the livelihoods of people (Ahmed, 2006). Unfortunately, very few studies have been conducted yet on the impact of climate change on forest ecosystems and biodiversity in Bangladesh (Alamgir et al., 2015; Sohel et al., 2016). Yet, for conservation planning, knowledge of species' potential distribution under current and future climates is imperative. For example, species re-introduction is a common strategy for recovery of depleted species as well as restoration of degraded habitats and ecosystems (Polak and Saltz, 2011; Rodríguez-Salinas et al., 2010; Nazeri et al., 2010; Ren et al., 2009). To ensure the long-term success of re-introduction strategies, a detailed knowledge of the current distribution of the species is required as well as its potential distribution under future climates (Adhikari et al., 2012). Here, species distribution modelling (SDM) is an effective tool to predict potential distribution and habitat suitability of target species, which can be used to design proper conservation plans.

In Bangladesh, there are 59 edible wild forest fruits available (Das, 1986), an important one of which is the wild mango species, *M. sylvatica*. According to IUCN, the species is in the category of 'lower risk/least concern'. However, this status is based on the data from 1998, and therefore needs updating (World Conservation Monitoring Centre, 1998). The species is threatened in Bangladesh (BFRI, 2013; Dutta et al., 2016) and India (Jiji, 2015). *M. sylvatica* is one of the most important species in the hilly region of Bangladesh, not only for its fruits but also because of its role in carbon storage. Alamgir and Al-Amin (2007) found that *M. sylvatica* has the second highest mean value in above-ground, below-ground and total organic carbon (2.78, 0.42 and 3.20 t/tree) in the CHTs of Bangladesh. Moreover, they also state that *M. sylvatica* has the fifth highest total organic carbon storage (2.00 t/ha) among the species available in hilly regions. Unfortunately, this species is declining at an alarming rate due to various factors. Dewan (2009) found that the important Value Index (IVI) of *M. sylvatica* in the CHTs is very low in comparison to other plant species. According to Dewan (2009), the reason behind the depletion is legal or illegal logging as well as shifting cultivation. Forest fires associated with clearance for shifting cultivation have a further negative impact on the natural regeneration of this species. Moreover, in Bangladesh, this species is used mainly in the plywood industries for making tea chests, internal decoration of buses, aircraft, cabinet making, house building and sports goods. In Bangladesh, there are approximately 25 factories producing commercial plywood; the majority are located in Chittagong and the CHTs and one of the major raw materials used is *M. sylvatica*. The Bangladesh Standards and Testing Institution recommends 16 timber species for plywood production and *M. sylvatica* is one of them (Sattar, 2006). In Bangladesh, the young leaves of *M. sylvatica* are used as vegetables and the fruits are consumed (fresh and processed) by the tribes in the CHTs (Jalil and Chowdhury, 2000). Therefore, a decline in the *M. sylvatica* population will have a negative impact on the socio-environmental aspect. Bangladesh Forest Research Institute (BFRI) has undertaken initiatives to conserve the species. It has initiated two projects: 'Ex-situ conservation of threatened forest tree species in different agro-ecological regions of Bangladesh' and 'Conservation of threatened plant species through domestication', which started in 2006–7 and 2003–4, respectively. Under these two projects, the BFRI raised 17.5 ha and 0.2 ha of conservation plots from seed, together with eight and ten other threatened species as gene banks (BFRI 2013). Over time, *M. sylvatica*'s habitat has shrunk due to an ever-increasing demand on land for cultivation, industry, human habitation, over exploitation, illegal logging and encroachment. An important first step in the conservation of this valuable species is to provide information on its potential habitat extent for re-introduction and restoration. Without this, it is difficult to plan and implement strategies for species conservation, including re-introduction in areas where it has disappeared. The present study aims to fill this gap by modelling the potential distribution under current climate conditions and to use these models to assess the potential effects of projected climate changes on the future habitat suitability and distribution of this species. We thereby considered three research questions. First, which bioclimatic variables most influence the predicted distribution of *M. sylvatica*? Second, to what extent is the potential distribution of this species expected to change under projected future climate changes? Finally, will there be an elevation shift of this species due to climate change?

3.2 Materials and methods

3.2.1 Study area

The study area (Figure 3.1) is located in the eastern part of Bangladesh, in Sylhet, Chittagong, the Chittagong Hill Tracts (CHTs) and Cox's Bazar. The natural vegetation in this region is hill forest (evergreen to semi-evergreen forest) and is considered one of the most biodiversity-rich hotspots in the country (Sohel et al., 2015; Akhter et al., 2013; Sohel et al., 2010; Biswas and Choudhury, 2007; Mukul et al., 2014). The mean annual temperature ranges from 22^{o} to 25^{o}C. The mean annual humidity ranges from 75% to 83%. In the last decades, there has been a trend of increasing mean annual temperature and rainfall from 1989 to 2009 in the north-eastern regions of Bangladesh (Akhter et al., 2013), including our study area.

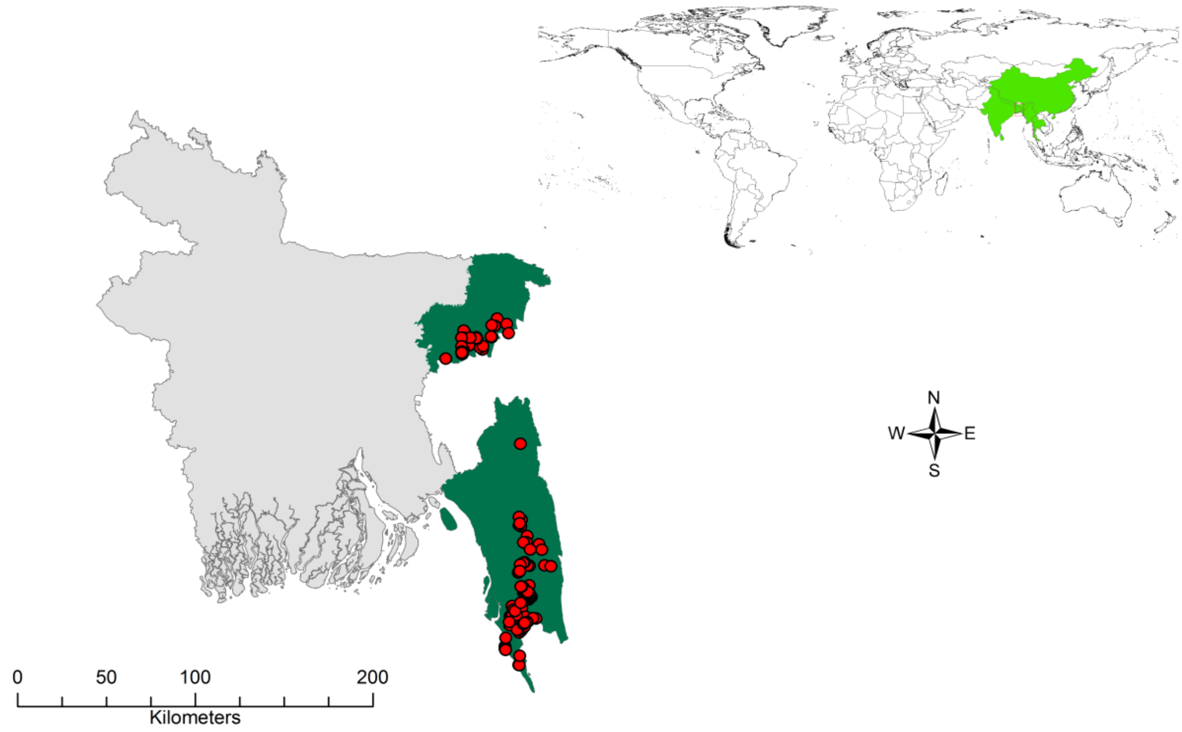


Figure 3.1 Global and local (Bangladesh) distribution of wild mango (*M. sylvatica*). Local distribution based on individuals sampled during field survey in 2014 (red dots). Light-green colours represent the global distribution of wild mango (Udvardy, 1975)

3.2.2 Studied species

The investigated species for this study is *M. sylvatica* Roxb, a wild mango species, which belongs to the Anacardiaceae family. The species has several different local names in Bangladesh and we will henceforward refer to this species as Uriam. Uriam is mainly distributed in the tropical forests and subtropical rainforests of the Indo-Malayan biogeographic region (Udvardy, 1975), in India, Bangladesh, Thailand, Cambodia, Nepal, China and Myanmar (Figure 3.1). In Bangladesh, the species is distributed in the hilly areas such as Chittagong, Cox's Bazar, the Chittagong Hill Tracts and Sylhet (Figure 3.1). The species prefers 'Brown Hill Soils', which are found in the hilly regions and vary from brown sandy loam to clay loam (FRA, 2000). A recent study showed that the kernel of this species can be used as a cocoa butter alternative (Akhter et al., 2016) and has the potential to be incorporated into small-scale forestry programmes (Baul et al., 2016).

3.2.3 *M. sylvatica* occurrence data dollection

As the species is locally threatened, to gain information on the species location, key informant interviews were conducted. Key informants were considered based on: a) their familiarity with the forest area, and b) an adequate background on the status of *M. sylvatica* in the study area. Selected respondents included: local forest department officials, forest product collectors, and indigenous group leaders living within and outside of the forest area. 253 trees (134 trees from Cox's Bazar, 95 trees from Sylhet, 23 trees from Chittagong and 1 tree in Banderban) were located in the study area and their positions recorded using Global Positioning System coordinates (Figure 3.1).

3.2.4 Spatial modelling

MaxEnt (version 3.3.3k) was used to model the distribution of the species under present climate conditions and project the potential distribution under future climates. MaxEnt is a widely used species distribution model that can be used to predict the probable distribution of a species based on biophysical and bioclimatic parameters (Phillips et al., 2006). It needs only presence data points (Phillips et al., 2006) and performs relatively well with small sample sizes compared to other modelling methods (Kumar and Stohlgren, 2009; Wisz et al., 2008; Pearson et al., 2007; Papers and Gaubert, 2007; Elith et al., 2006; Weber, 2011). MaxEnt generates an estimate of habitat suitability for the species that varies from 0 (lowest suitability) to 1 (highest suitability). The strong attributes of MaxEnt are: i) it has strong mathematical definition; ii) it gives a continuous probabilistic output; iii) it can handle both continuous and categorical environmental data; iv) it can investigate variable importance; v) it has the capacity to handle low sample sizes; and vi) it has simplicity for model interpretation (Elith et al., 2011; Pearson et al., 2007; Phillips et al., 2006).

Models were created using the default parameters and based on present climate conditions. As calibration data, we used a 75% random selection of the presence points and 10,000 random selected background points, and the other 25% presence points were used to evaluate the model's goodness-of-fit using the Area Under the Receiving Operator Curve (AUC) as the evaluation statistic. The area under the curve (AUC) was used to assess the accuracy of the model. Generally, AUC values range from 0 to 1. A high AUC value indicates that the model is performing well. The output from the model gave AUC values ranging from 0 to 1, where 0 indicates a lower probability of distribution and 1 indicates a higher probability or suitability of distribution. To validate the model robustness, we executed 10 replicated model runs for the species. To find out which variables were most important, the jackknife procedure of the MaxEnt model was used. Jackknife is a statistical estimator of a parameter obtained by systematically leaving out each observation from a dataset, calculating the estimate of the importance and then finding the average of those calculations. We used the average of 10 model runs to create potential distribution maps under current conditions. We used the same approach, but using the future bioclimatic data as input, to create potential distribution maps under projected future climate conditions. We did this for all four combinations of the two RCPs (RCP 4.5 and RCP 8.5) and years (2050 and 2070). The 0 to 1 suitability values of the resulting species suitability distribution maps were aggregated into four classes of potential habitats following the method by Yang et al. (2013), viz. 'high potential' (> 0.6), 'good potential' (0.4–0.6), 'moderate potential' (0.2–0.4) and 'least potential' (< 0.2).

Table 3.1: Environmental variables used in the study

Code	Climatic variables	Unit
BIO 1	Annual mean temperature	°C
BIO 2	**Mean diurnal range (mean of monthly max. and min. temp.)**	**°C**
BIO 3	**Isothermality ((Bio2/Bio7) × 100)**	
BIO 4	Temperature seasonality (standard deviation × 100)	C of V
BIO 5	**Maximum temperature of warmest month**	**°C**
BIO 6	Minimum temperature of coldest month	°C
BIO 7	Temperature annual range (Bio5–Bio6)	°C
BIO 8	Mean temperature of wettest quarter	°C
BIO 9	Mean temperature of driest quarter	°C
BIO 10	**Mean temperature of warmest quarter**	**°C**
BIO 11	Mean temperature of coldest quarter	°C
BIO 12	Annual precipitation	mm
BIO 13	Precipitation of wettest period	mm

BIO 14	**Precipitation of driest period**	**mm**
BIO 15	**Precipitation seasonality (CV)**	**C of V**
BIO 16	Precipitation of wettest quarter	mm
BIO 17	Precipitation of driest quarter	mm
BIO 18	**Precipitation of warmest quarter**	**mm**
BIO 19	**Precipitation of coldest quarter**	**mm**

N.B. Variables with bold were used for the final model run. The other variables were removed because of high Value (Supplementary Table 3.1).

3.2.5 Bioclimatic variables

We used 19 climatic variables (Table 3.1) as potential predictors of *M. sylvatica*'s habitat distribution. Bioclimatic variables (Hijmans et al., 2005) are biologically more meaningful to define the eco-physiological tolerances of a species than simple rainfall or temperature (Graham and Hijmans, 2006). They are, therefore, frequently used in modelling species distributions (Yang et al., 2013; Garcia et al., 2013; Adhikari et al., 2012). Data layers for these bioclimatic variables were downloaded from the WorldClim database, with 30 arc seconds (~1 km) spatial resolution. To project the future distribution and habitat suitability due to climate change, we used projected climatic data for 2050 and 2070 based on the IPCC fifth assessment report available from www.worldclim.com. Among four future greenhouse gas concentration trajectories (also known as representative concentration pathways or RCPs) two GHG concentration trajectories, RCP 4.5 and RCP 8.5, were considered for the two time periods. RCP 4.5 represents a stable scenario where GHG will be stabilised due to green technologies and the radiative force will reach up to 4.5 W/m^2 by 2100 (Wise et al., 2009; Clarke et al., 2007; Smith and Wigley, 2006; Guisan and Thuiller, 2005). RCP 8.5 represents a relatively extreme scenario where GHG will continuously increase throughout 2100, at which time radiative force will reach 8.5 W/m^2 (Meinshausen et al., 2011; Riahi et al., 2007). A widely used global circulation model, HadGEM2-CC (Hadley Global Environment Model 2 Carbon Cycle) was used in this study because of its well-known acceptability (Shrestha and Bawa, 2014). This model has been used to perform all of CMIP5 (Coupled Model Inter Comparison Project Phase 5) and was also used by the IPCC in its fifth Assessment Report (Shrestha and Bawa, 2014).

The bioclimatic variables and the species presence data were extracted and prepared using ArcGIS 10. To minimise multi-collinearity, we used a step-wise variance inflation factor (Graham, 2003) selection procedure, whereby we first computed the VIF using all bioclim variables. The variable with the highest VIF was removed and VIF was computed for the remaining variables. This was repeated until the VIF was below 10 for all remaining variables. The remaining eight bioclimatic variables (Table 3.1, Supplementary Table 3.2) were used in our model.

3.3 Results

3.3.1 Model evaluation

The model calibration test for *M. sylvatica* yielded satisfactory results (AUC_{train} = 0.92 ± 0.002 and AUC_{test} = 0.89±0.02). This indicates that the bioclimatic variables used for the model explained the predicted distribution very well. Amongst the input variables, mean diurnal range (BIO 2) contributed 29.2% and precipitation seasonality (BIO 15) contributed 22%. BIO 2, BIO 15, BIO 14 and BIO 18 together contributed 83% to the model. Maximum temperature of warmest month (BIO 5) is the lowest contributed climatic variable. The jackknife test showed that mean diurnal range (BIO 2) has the highest training gain when considered alone (Figure 3.2).

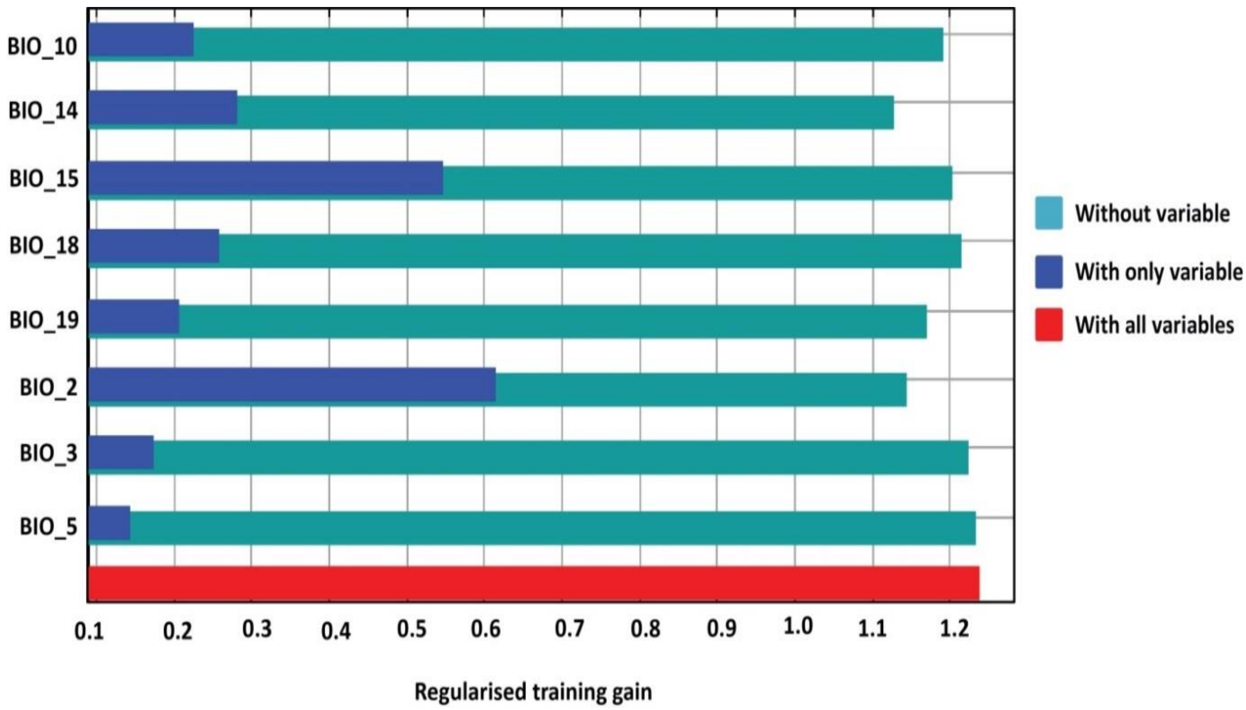


Figure 3.2: The Jackknife test for evaluating the relative importance of environmental variables

3.3.2 Species response and habitat suitability under present and future climatic conditions

Response curves showed changes in the logistic prediction when each predictor variable changed by keeping all other variables at their average sample value. Responses of the species to the changes of bioclimatic variables are shown in Figure 3.3. The response curves showed that the distribution of *M. sylvatica* is highly controlled by both temperature and precipitation. The probability of presence is high when mean diurnal temperature (BIO 2) is above 10^{o}C. The probabilities of increasing the presence of the studied species increased with increased temperature and were found to be highest between 32 ^{o}C and 33^{o}C. The mean temperature of the warmest quarter (BIO 10) provides the mean temperatures during the warmest three months of the year, which can be useful for examining how such environmental factors may affect species seasonal distributions. The species, however, started to decline when the temperature exceeded more than 28^{o}C during the warmest month. The maximum temperature of warmest month (BIO 5) is useful when examining whether species distributions are affected by warm temperature anomalies throughout the year. Precipitation seasonality (BIO 15) provides a percentage of precipitation variability where larger percentages represent greater variability of precipitation. The results showed that the study species was able to tolerate higher variability (ranges from 98% to 115%) of rain. Precipitation of the coldest quarter (BIO 19) provides total precipitation during the coldest three months of the year. The study species showed a higher probability of presence in the winter season when rainfall ranges between 30mm to 36mm (Figure 3.3). Precipitation of the warmest quarter (BIO 18) provides total precipitation during the warmest three months of the year and the model showed that this species prefers moderately high amounts of rain in the range of 800–1200 mm.

The predicted current distribution of *M. sylvatica* under current climatic conditions is shown in Figure 3.4. Based on our model, about 4.58% of the study area is ‘highly suitable’ for wild mango habitat, followed by ‘good potential’ at 6.53% and ‘moderate potential’ at 10.71%, while 78.19% of the area is ‘least suitable’ or ‘unsuitable’ for this species (Table 3.2). Current suitable habitats for *M. sylvatica* were predicted in the north-eastern and south-eastern parts of Bangladesh (Figure 3.4). The concentration of pixels with highly suitable, good potential and moderate potential is observed in the CHTs, Cox’s Bazar, and the Moulvibazar and Habiganj districts when the current predicted distribution habitat map is overlaid with the district map of Bangladesh.

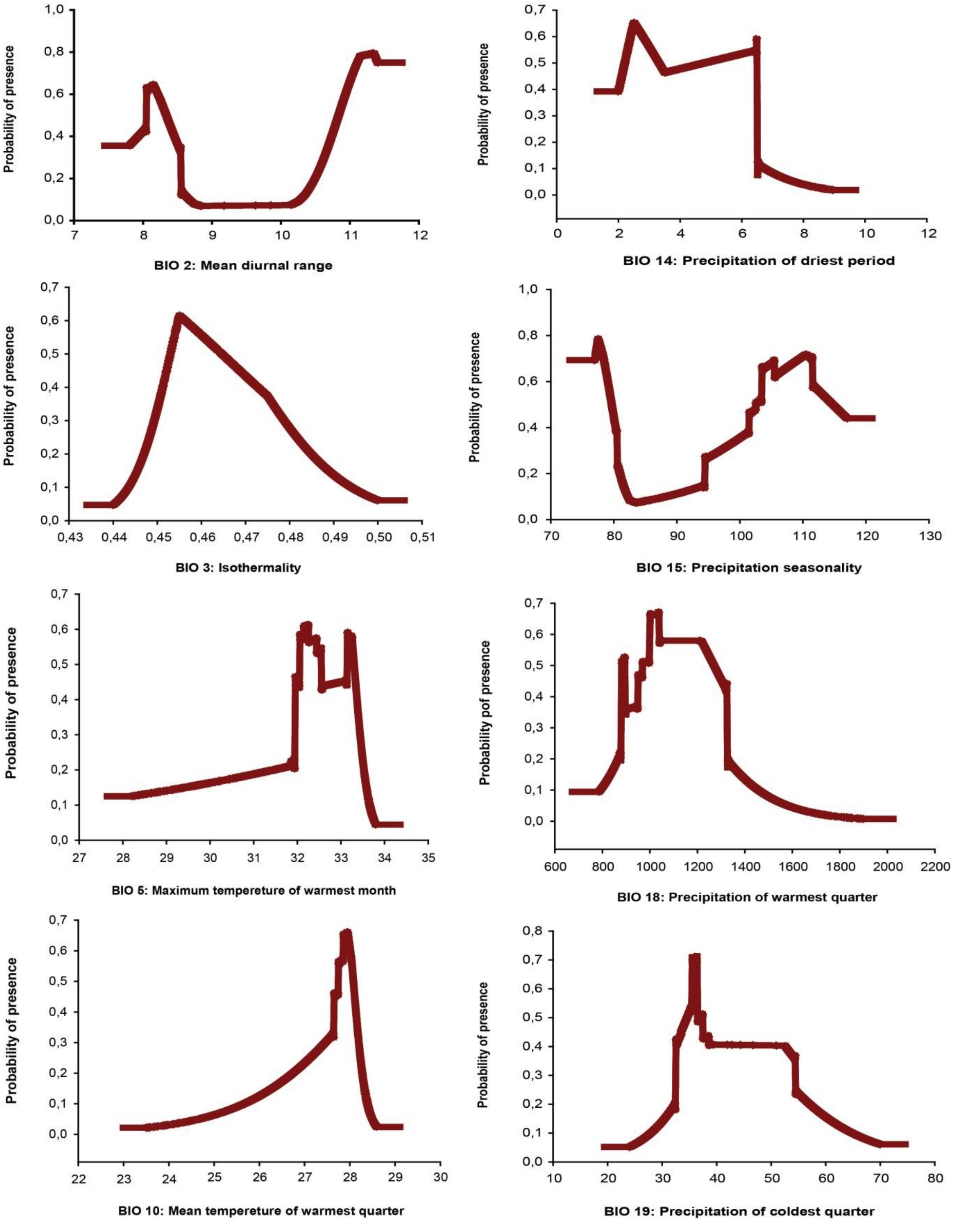


Figure 3.3: Response curves for the predictors of the MaxEnt model

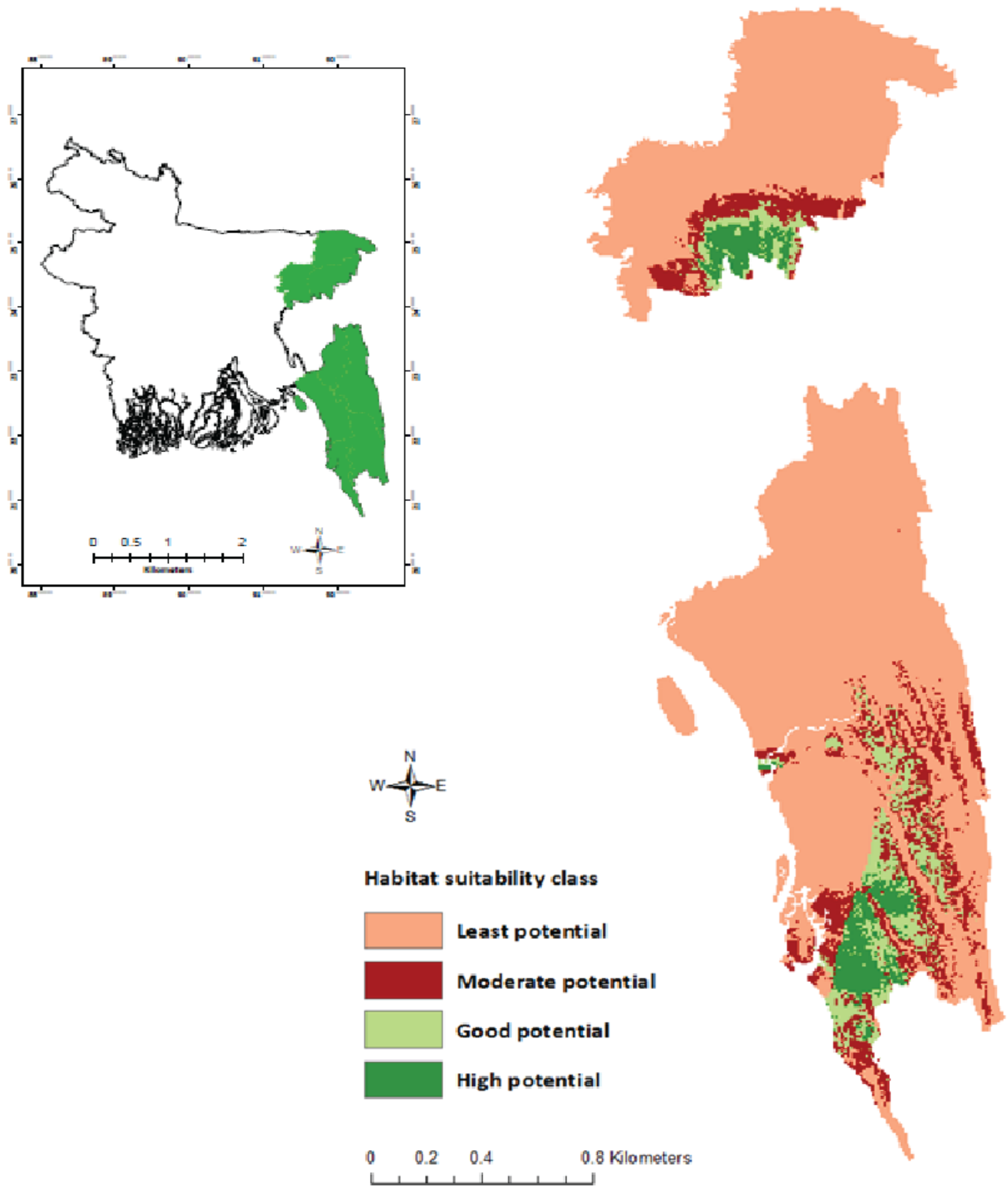


Figure 3.4: Predicted current potential distribution of *M. sylvatica* under current bioclimatic conditions. Green colours in the map of Bangladesh at the top left indicate the position of the study area

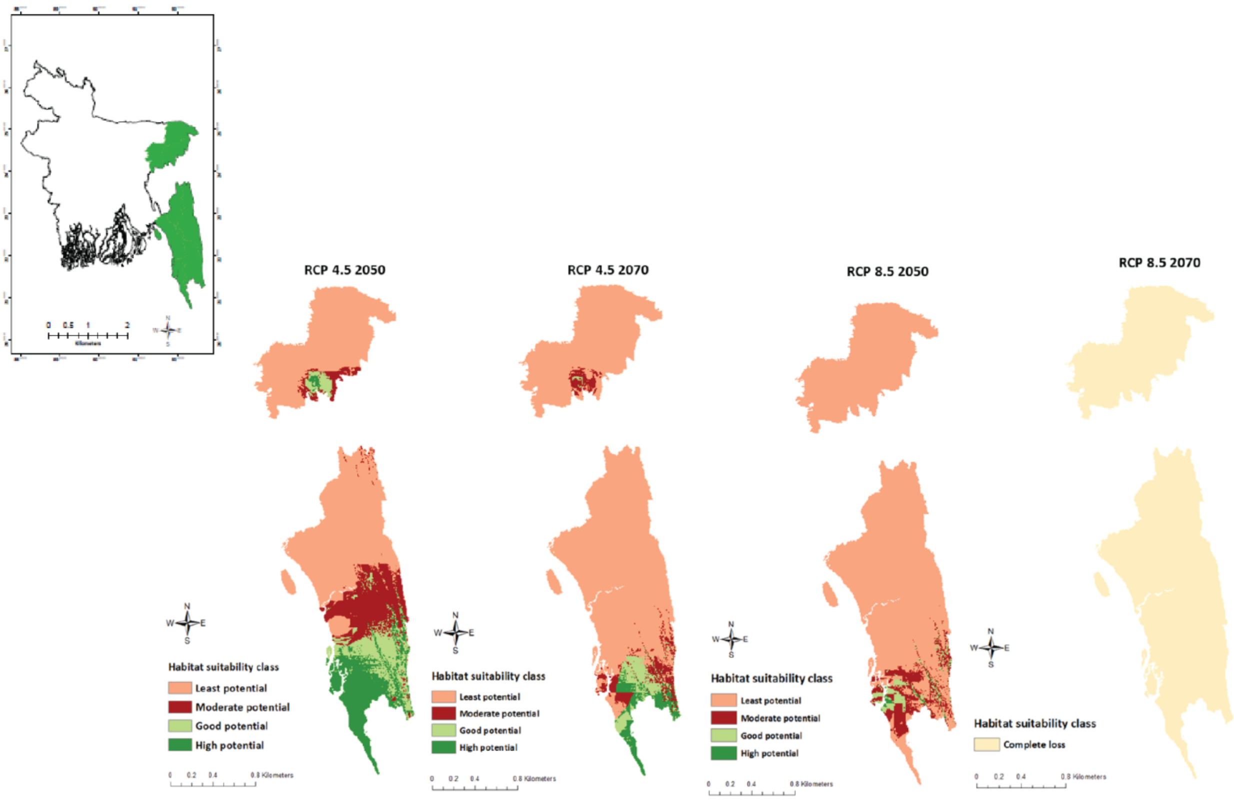


Figure 3.5: Future distribution of *M. sylvatica* under climate change using the IPCC scenario. Green colours in the map of Bangladesh at the top left indicate the position of the study area

According to the results of the MaxEnt modelling, the predicted future ranges of habitat are likely to be negatively affected by future climate. The predicted distribution and habitat change of wild mango in future climate scenarios are shown in Figure 3.4 and Table 3.2. The maximum expansion (16.05% addition to the current potential suitable area considering high, good and moderately suitable areas) would occur under RCP 4.5 by 2050, whereas the total loss of habitat was predicted for the year 2070 for RCP 8.5. Most of the expansion was observed in the CHTs and Cox's Bazar and in RCP 4.5 during 2050. In RCP 4.5 during 2070, a 7.82% reduction of suitable habitat was observed compared to the present distribution. However, a decline in habitat was observed for RCP 8.5 during both 2050 and 2070, and in 2070 the species will be completely lost. Wild mango habitat will be completely lost from the north-eastern part of the country under all scenarios.

Table 3.2: Changes in habitat class of *M. sylvatica* under current and future climate scenarios

Suitability class	Area (km^2)								
	Current	RCP 4.5 2050	Area change	RCP 4.5 2070	Area change	RCP 8.5 2050	Area change	RCP 8.5 2070	Area change
Least potential	28116	22347	-5769	30929	2813	32603	4487	0	-28116
Moderate potential	3851	5280	1429	1971	-1880	2363	-1488	0	-3851
Good potential	2347	3584	1237	1637	-710	877	-1470	0	-2347
High potential	1646	4749	3103	1423	-223	117	-1529	0	-1646

3.4 Discussion and conclusion

This study is the first attempt to look into the impact of climate change on the distribution and range of suitable habitat of a threatened wild mango species of Bangladesh. The study findings show that the distribution of the threatened wild mango species is partly controlled by precipitation-related bioclimatic variables (Figure 3.2 and Figure 3.3), with the most important explanatory variables being mean diurnal temperature, precipitation seasonality, precipitation of the warmest quarter and precipitation of the driest period. Especially under the RCP 8.5 scenario, these factors are projected to show considerable changes, causing large parts of the current distribution area to become unsuitable by 2050, while the whole study region is projected to become unsuitable by 2070. It should be noted that RCP 8.5 represents a more extreme scenario. On the other hand, rainfall patterns are expected to become more erratic in many parts of the world, while extended dry spells may become more frequent; factors that we did not include in our models. Recent climate data analysis for the period

1961–2010 shows that temperature is increasing slowly and the total amount of annual precipitation is increasing along with increasing trends in consecutive dry days (CDD) (Islam et al., 2014). In light of the potential importance of rainfall patterns for *M. sylvatica*, these extreme climate events in future may cause more stress to the existing populations and prevent expansion to areas that would be considered suitable when considering average conditions only.

Given that a number of bioclimatic variables, highly correlated to currently used variables, were excluded from our analysis, more analyses are needed to determine the sensitivity of the species considering more environmental and climatic variables. It is important to notice that we only employed one GCM and two RCP scenarios. Among the two RCP scenarios, RCP4.5 is more important in terms of business as it deals with green technologies. For a more reliable assessment of future changes, we need to consider a wider range of GCMs and all four RCP scenarios (RCP 2.5, RCP 4.5, RCP 6.0, RCP 8.5) for better understanding of the vulnerability and sensitivity of the studied species to climate change. Moreover, although our models were based on climate data only, we were able to capture the observed distribution well, but the need remains to consider other biophysical factors, such as soil characteristics, land cover and geology (Alamgir et al., 2015) and aspect data on hill sideto obtain more reliable and relevant predictions of where the species will be able to grow in the next decades. Such data with higher resolution is currently scantily available for Bangladesh. For example, new global data sets such as the http://soilgrids.org/ are being developed, but currently still have a limited thematic (wide confidence limits) and spatial accuracy (Hengl et al., 2014). In the case of land cover data, the land use classification of freely available global-scale data from http://www.eea.europa.eu/data-and-maps/data/global-land-cover-250m does not cover the detailed land use of the study area. However, better resolution satellite images were not analysed in this study due to funding limitations. Moreover, the addition of regional/local climate change models may give considerably more reliable predictions of climate change effects. Addition to this, the MaxEnt model can't deal with pollinators which is an important factor that influence on species distribution. However, in light of the limitations of only one GCM and two RCP scenarios (RCP 4.5 and RCP 8.5), and as regional models were not used, our study provides a preliminary assessment of the effects of climate change on the species, and is useful to highlight the most vulnerable areas.

The predicted potential distribution of *M. sylvatica* was largely in agreement with what we know about the distribution of this species, with the species' major habitats to be the forests in the CHTs, Cox's Bazar, and the Molovibazar and Habigang districts. Our results confirm that future climate changes may result in a major decline in the potential distribution of this species, especially under the scenario in which GHG will continue to increase at the current pace throughout 2100. In contrast, under the stable climate change scenario (RCP 4.5), the potential distribution of the species may even show a modest increase in the south-eastern part of our study area by 2070. Restoration of depleted populations may, therefore, be an option in these parts of the country if humanity manages to limit the increase in radiative force to less than 4.5 W/m^2.

The future for the species is considerably bleaker in the north-east. Our model results suggest that, under both climate change scenarios, conditions are likely to become unsuitable for the species. Other studies in different locations also show both reduction, expansion and complete habitat loss due to climate change (Barrett et al., 2013; Molloy et al., 2013; Dullinger et al., 2012; Dawson et al., 2011; Loarie et al., 2008; Bakkenes et al., 2002). *M. sylvatica* seems to have shifted upwards little, which may have also caused the local extinction of wild mango from north-eastern regions under all climate scenarios. These results might be a useful guide for government and NGOs responsible for the conservation and management of forest resources. There is still a major knowledge and research gap about how forests respond to climate change at local scales and what adaptation strategies can be taken in such contexts (Kaeslin et al., 2012). Here, Species Distribution Modelling (SDM) can be a helpful tool that can provide information on the effect of climate change on species' geographic distribution and habitat suitability. This information can facilitate better management and conservation of those species, especially for threatened and rare species. Protection of threatened species' habitat is an important step. Here, protected areas can play a significant role. Apart from declaring protected areas, landscape connectivity between non-protected areas should also be taken into account (Hannah et al. 2002). Additionally, the active participation of the local, forest-dependent people is fundamental, especially in developing countries, as successful forest conservation is not possible without their active participation (Sohel et al., 2010). To engage the local people in the decision-making process of conserving forests, a participatory approach or co-management system should be introduced (Mukul et al., 2012). Apart from this, establishing non-timber forest product facilities in buffer zones around the protected areas will help reduce the pressure on the protected areas for forest-based livelihood activities (Mukul et al., 2016). The government of Bangladesh has prioritised the above mentioned issues, so most of the protected areas of Bangladesh are now under a co-management system. Finally, an awareness programme should also be prioritised to address climate change issues and protect the valuable forest resources of Bangladesh for future generations (Sohel et al., 2016). From the present study, the area identified from the predicted potential current distribution can be used for the re-introduction of wild mango trees. This will help restoration of the forest as well as improve the overall conservation status of the species. This pioneer study can act as an early warning for climate change effects on valuable threatened tree species of the forest ecosystems of Bangladesh. Taking this into consideration, conservation managers could start to think of adapting policy that can reduce the extinction risk of this valuable species. Information generated here will benefit commercial users such as timber, pickle and butter producer; wildlife and ecosystem services officers in government or NGOs; Land use planners concerned with climate change; commercial breeder; climate change policy makers in Bangladesh. The method and approach used in this research can be applied to other forest tree species of Bangladesh to see how they respond to climate change impact in order to better inform conservation decisions.

Supplementary Table 3.1: Multi-collinearity test using VIF analysis among environmental variables

<table>
<tr><th>Variables</th><th>VIF Scores</th><td rowspan="9">Variables with VIF Score ≥ 10 were excluded for further analysis</td></tr>
<tr><td>bio_2</td><td>9.50</td></tr>
<tr><td>bio_3</td><td>4.52</td></tr>
<tr><td>bio_5</td><td>6.23</td></tr>
<tr><td>bio_10</td><td>4.26</td></tr>
<tr><td>bio_14</td><td>2.05</td></tr>
<tr><td>bio_15</td><td>6.75</td></tr>
<tr><td>bio_18</td><td>4.08</td></tr>
<tr><td>bio_19</td><td>6.07</td></tr>
</table>

Chapter 4. Morphological, nutritional and medicinal traits of wild mango (*M. sylvatica* Roxb.): implications for increased use and options for cultivar development[2]

Abstract

Mangifera sylvatica Roxb. is an underutilised and threatened wild fruit species found in Bangladesh, which is highly valued by local people as a source of fruit and is an important source of nutrition. As part of a feasibility study of the domestication and cultivar development potential of *M. sylvatica*, a preliminary study examined the morphological traits (fruit, kernel and pulp mass), nutritional profile (carbohydrate, sugar, pH, fat, protein, mineral and vitamins) and medicinal traits (total phenolic and phenolic profiling). The fruit of *M. sylvatica* is small (27.00g ± 7.03g) with a comparatively bigger kernel fruit (40% of its body weight). *M. sylvatica* fruit pulp has been proved to be a good source of carbohydrate, Vitamin C, sodium (Na) and potassium (K) and also has good medicinal properties (mangiferin and quercetin). The kernel is also a rich source of carbohydrate and has a good fatty acid profile (rich in stearic and oleic acids) consistent with cocoa butter, which indicates its potential to be used in the chocolate and confectionery industry. There is continuous variation in these traits, indicating opportunities for multiple trait cultivar development targeted at the food and pharmaceutical industries. The information generated in the study can be used as a stimulus to the process of domestication and to encourage widespread use of the species, which will ultimately help to conserve this wild underutilised fruit species.

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[2]Akhter, S., McDonald, M. A., Kjær E. D., Jashimuddin, M., Marriott, R. 2016. Morphological, Nutritional and Medicinal traits of wild mango (*Mangifera sylvatica* Roxb.): Implications for increased use and options for cultivar development. This will be submitted to *Journal from Sciencedirect/Elsevier* in 2016.

4.1 Introduction

Forests contribute to the food security and livelihoods of more than 1.6 billion people worldwide (FAO, 2016) by providing different timber and non-timber products (Tchoundjeu et al., 2010; Leakey et al., 2005b). Around 30% of the world's forests are used primarily for production of wood and non-wood forest products (Vinceti et al., 2013). Recently, forest tree improvement for non-timber forest products (fruits, nuts, resin, etc.) has been implemented from high-value multi-purpose trees (Tchoundjeu et al., 2010). However, trees that produce edible fruits are of special interest, because fruits trees can build a safety buffer and often can serve as important sources of vitamins, minerals and phytochemicals that improve human health conditions (Leakey, 1999). Intake of vegetables and fruits can thus lead to reduction of cardiovascular diseases (Hu, 2003; Ikram et al., 2009), certain cancers (Ikram et al., 2009; Riboli and Norat, 2003), immune system problems, arthritis, inflammation and brain dysfunction (Leong and Shui, 2002). The nutritional profiles of fruits from wild underutilised fruits in terms of micronutrients, fat, fibre and protein often make them an important supplement to staple foods (Leakey, 1999). Throughout the tropics there are many tree species presently used for the harvesting of fruits and other non-timber forest products at the local scale, but which are still not domesticated to the extent that they can provide products that can be traded beyond the local scale (Leakey and Tchoundjeu, 2001). For domestication of such multipurpose species, it is important to identify elite individuals in the wild or semi-domesticated population (Leakey and Page, 2006). Characterising phenotypic variation is a pre-requisite for cultivar development, which would benefit farmers through increasing productivity and product uniformity (Leakey et al., 2005b).

In Bangladesh, there are also several underutilised tree species such as *Borassus flabellifer*, and *Syzygium samaramgense* (Rahim et al., 2009), used for fruit collection, that have not undergone systematic improvement though development of varieties. Their nutritional value and potential for improvement though selections are typically poorly investigated, and many of these species may, therefore, represent underutilised species suitable to combat malnutrition. Currently, little attention has been given to increase the use of underutilised fruits to reduce malnutrition in Bangladesh (Rahim et al., 2009) and the important contribution of fruit trees to many farmers' livelihoods and nutrition is often not acknowledged in national reporting (Shajib et al., 2013). Many such indigenous fruit species are declining and there is an urgent need to conserve them (Shajib et al., 2013; Rahim et al., 2009). However, prioritising conservation efforts requires information on the positive attributes of such species, which is often lacking. Many of these species could hold the potential of being integrated into agroforestry systems since they are already used by local communities, but development of improved varieties through domestication may be required in order to make planting attractive. The first step in a domestication programme is the sourcing of germplasm through identification of elite individuals from the wild population (Leakey and Page, 2006). However, very few studies have been conducted on the selection and breeding of superior trees. Since selection of the mother tree is the first step towards cultivar development, studies of tree-to-tree variation in fruit traits, including phenotypic and nutritional characters, is important (Leakey et al., 2005b; Leakey and Page, 2006) and is, therefore, the topic of the present study.

In Bangladesh, there are 47 edible wild forest fruits available (Das, 1987), an important one being *M. sylvatica* Roxb. (wild mango), which is genetically very close to *Mangifera indica* (common mango). A close relationship between the two species has been reported by Mukherjee (1950, 1957) and Nishiyama et al. (2006), which indicates that *M. sylvatica* may have the potential to fulfil nutritional and livelihood needs. This species is declining at an alarming rate due to a variety of factors, including legal or illegal logging, shifting cultivation and forest fires associated with clearance for shifting cultivation, which have a negative impact on regeneration (Baul et al., 2016; Dewan, 2009). In Bangladesh, this species is one of 16 recommended species mainly used in the plywood industry (Sattar, 2006). So, overall, there is tremendous pressure on *M. sylvatica* and no conservation effort except for some minor initiatives by the Bangladesh Forest Research Institute (BFRI, 2013). Over time, the habitat of this species has shrunk due to an ever-increasing demand on land for cultivation, industry, human habitation, overexploitation, illegal logging and encroachment (Baul et al., 2016). Furthermore, natural hybridisation may take place between *M.indica* and *M. sylvatica* in Southeast Asia (Mukherjee, 1950; 1957), which may potentially erode the genetic base of *M. sylvatica* if *M. indica* varieties are planted abundantly in the landscape. Based on the above background, the objective of the present study is to quantify the phenotypic and nutritional value of the species with focus on phenotypic tree-to-tree variation and implications for identification of superior clones to be propagated and tested for cultivar development. The study was conducted in the hilly region of Bangladesh and more specifically addressed the following research questions.

1. How large are the variations in fruit size, pulp and kernel weights between trees?
2. What are the nutritional properties of *M. sylvatica* fruit and to what extent does this vary between individuals?
3. Is there variation in phenotypic and nutritional properties among different sites?
4. Is there any potential for the fruit to contribute to agroforestry systems to enhance smallholders' livelihoods and income?

4.2 Materials and methods

4.2.1 Study area

M. sylvatica is known as Jangli-am, Kosh-Am, Garey-Am, Lakkhi-Am, Bon Am, Baitta Am, Chuchi Am and Guti Am in Bangladesh, and its most common name is Uriam. The species occurs mainly in the tropical humid forests, subtropical rainforests/woodlands and tropical dry forests/woodland of the Indo-Malayan bio-geographic region (Udvardy, 1975). It is native to India, Bangladesh, Thailand, China, Cambodia and Myanmar (Figure 4.1). In Bangladesh, *M. sylvatica* is distributed in the hilly areas such as Chittagong, the Chittagong Hill Tracts (CHTs), Cox's Bazar and Sylhet (Figure 4.1). *M. sylvatica* prefers 'Brown Hill Soils' (brown hill soils are drained soils with a yellow-brown to strong-brown Dystric Cambisols) that are found in the hilly regions and vary from brown sandy loam to clay loam (FRA, 2000). For the present study, samples were collected from Chittagong, Cox's Bazar and Sylhet. The CHTs have the highest elevation compared to other sites. The species generally grows in areas where average rainfall ranges from 2,600–3,000 mm/year. In the Sylhet division, the rainfall range is high. The optimum growing temperature for *M. sylvatica* is 22°C –30°C and the temperature of Cox's Bazar is comparatively higher than Sylhet, Chittagong and the CHTs.

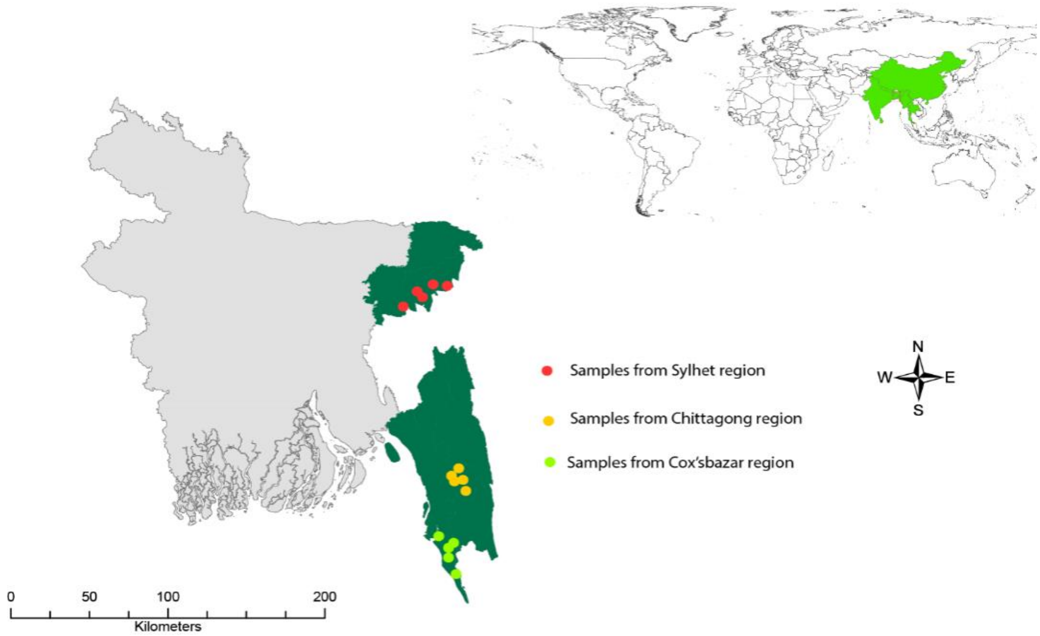


Figure 4.1: Study area map with sampling location and world distribution of *M. sylvatica*

4.2.2. Sample collection, preparation and analysis

Samples of fresh and ripe fruits were collected from three geographic locations (Cox's Bazar, Chittagong and Sylhet) in Bangladesh from April to May 2013. A total of fifteen trees from three sites were selected (five from each site). Ten fruits per tree were collected randomly throughout the canopy. The following fruit traits were assessed: fruit length, fruit width, fresh weight of fruit peel, kernel and pulpt, which were measured directly in the field using a portable electric balance and metre tape. The number of fruits per kg was recorded for fifteen trees (five trees per site) and the yield was recorded for three trees from the Cox's Bazar site. However, the approximate fruit yield was recorded for fifteen trees at three sites (five trees per site). For the nutritional analyses, fruits were collected from the same trees and refrigerated (at -4^{o}C) soon after collection. All fruits were then transferred to Bangor University in the UK by DHL courier service for further analysis. Fruits were subsequently cleaned and separated into peel, pulp and kernel and stored in a freezer at -20°C. The frozen pulp samples were freeze dried and stored in air-tight bags and kept in the freezer at -20°C before further analysis. These samples were used for proximate analysis (dry matter content, moisture content, ash content, crude fat, crude fibre, crude protein, carbohydrate and energy). Apart from that, total soluble sugar (using refractometer), pH (using pH meter), Vitamin A (using HPLC-UV), Vitamin C (using HPLC-UV), macro and micro minerals (using total X-Ray fluorescence analyser), total phenolics (using folin-ciocalteu reagent) and phenolic compound were identified (using HPLC-UV detector), and fatty acid methyl ester analysis (using gas chromatography) was also analysed. Details of the methods used are given as supplementary material.

4.2.3 Statistical analysis

Descriptive statistics were derived using Minitab 17. For nutritional analysis, means, standard error (SE) and one-way ANOVA were performed. For phenotypic variation, the extent of trait variation between sites was determined from average and standard error for each trait (fruit length, fruit width, fruit mass, peel mass, kernel mass and flesh mass). Correlations between measured fruit traits were calculated based on tree average. Nested ANOVA was conducted to find the percentage of variation of different fruit traits at three levels (between site, within site and within trees). Finally, a linear regression model ($Y = a + bX$) was developed between fruit weight, pulp weight and kernel weight.

4.3 Results

4.3.1 Morphological traits

M. sylvatica is a small mango fruit with a big kernel of more than 40% its body weight (Figure 4.2). The mean fruit weight varied among trees from 17.85g to 35.96g (Table 4.1), while the kernel weight and the pulp weight varied from 7.29g to 15.02g and 4.96g to 11.63g (Table 4.1), consequently. It was also observed that the biggest fruits were found from the Cox's Bazar site and the Sylhet site produced second largest fruits. 67% of fruit weights were above 25g, kernel weights were above 10g and pulp weights were above 5g, and 77% of fruit lengths and widths were above 3cm and 2cm (Supplementary Figure 4.1). The variation within sites was significantly higher (P=0.01) than between sites, indicating that a sample of ten fruits per tree and five trees per local site was sufficient to characterise the morphological variation (Table 4.2). Tree DBH and other fruit traits were weakly correlated, whereas fruit weight, pulp weight and kernel weight were strongly correlated with fruit length and fruit width, suggesting that the indirectly measurable traits can be measured through the directly measureable traits (Table 4.3). There were highly significant and strong relationships between fruit weight, pulp weight and kernel weight. Yield of pulp weight and kernel weight (g) could be predicted from fruit weight (g) by utilising the following model.

Pulp Weight = 0.3919 (Fruit Weight) - 2.1559, (R^2 = 0.973)

Kernel Weight = 0.3714 (Fruit Weight) + 1.0415, (R^2 = 0.973)

Table 4.1: Morphological characteristics of fruits and components of *M. sylvatica*

Variables	Local provenance			
	Site 1: (Cox's Bazar)	Site 2: (Chittagong)	Site3: (Sylhet)	All average
	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Fruit length (cm)	4.88±0.21	3.85±0.40	4.79±0.58	4.51±1.16
Fruit width (cm)	3.28±0.19	2.79±0.37	3.05±0.38	3.04±0.78
Fruit weight (g)	35.96±3.43	17.85±3.08	27.84±4.51	27.22±7.03
Peel weight (g)	8.87±0.98	5.23±0.92	6.97±1.19	7.02±1.81
Kernel weight (g)	15.02±1.14	7.29±1.16	11.14±1.39	11.15±2.88
Pulp weight (g)	11.63±1.59	4.96±1.16	8.95±1.98	8.51±2.20
Fruit : Pulp	3.17±0.17	4.07±0.69	3.95±0.96	3.73±0.96
Fruit : Kernel	2.38±0.08	2.45±0.15	2.42±0.18	2.42±0.62
Pulp : Kernel	0.77±0.07	0.67±0.10	0.74±0.13	0.73±0.19

N.B. SE (Standard Error)

Table 4.2: Variation of traits (in percentage) from nested analysis of variance (*P*=0.05)

Source of variation	Fruit length	Fruit width	Fruit weight	Peel weight	Kernel weight	Pulp weight
Among provenance	10.92 (*P*=0.20)	0.00 (*P*=0.56)	45.73 (*P*=0.01)	24.92 (*P*= 0.08)	57.18 (*P*=0.00)	33.77 (*P*= 0.03)
Within provenance						
Trees within provenance	64.20 (*P*=0.00)	65.84 (*P*=0.00)	45.35 (*P*=0.00)	57.81 (*P*=0.00)	31.29 (*P*=0.00)	48.62 (*P*=0.00)
Within trees (error)	24.88	34.16	8.92	17.27	11.53	17.61
Total	100	100	100	100	100	100

Table 4.3: Matrix of correlations between DBH, measured and derived variables on fruit and components of *M. sylvatica*

	Fruit Length	Fruit width	Fruit Weight	Peel Weight	Kernel Weight	Pulp Weight	DBH
Fruit Length	1,00						
Fruit width	0,91	1,00					
Fruit Weight	0,87	0,77	1,00				
Peel Weight	0,77	0,66	0,94	1,00			
Kernel Weight	0,81	0,72	0,97	0,90	1,00		
Pulp Weight	0,90	0,81	0,97	0,87	0,91	1,00	
DBH	-0,20	-0,01	-0,36	-0,42	-0,35	-0,32	1,00

4.3.2 Nutritional and medicinal properties

M. sylvatica fruits were, on average, juicy (85±0.57% moisture), low in carbohydrate (1.95%), rich in fibre (2%), Na (54.58 ± 9.97 mg/100g), K (172.76 ± 13.98 mg/100g), Vitamin C (41.51 ± 6.06 mg/100g) (Table 4.4, Table 4.5). The peel of this wild mango is thin (7.02 ± 1.81g) and contains high amounts of minerals (Na, K, Fe, Zn and Mn), which can be utilised as well (Figure

4.5). *M. sylvatica* fruit (kernel) is a rich source of carbohydrate (20.36%), fibre (9.93%), fat (11.57%), minerals (K and Fe) and also very rich in medicinal compounds. Wild mango kernel contains higher amounts of phenolic compounds (115.44 ± 1.53mg/100g) compared to pulp (23.77 ± 0.30mg/100g) indicating that the kernel can be utilised for medicine purposes (Supplementary Figure 4.2). Wild mango pulp is a source of mangiferin, quercetin and kaempferol (Table 4.6).

4.4 Discussion

Morphological traits have been used traditionally to obtain information on variation within species. Continuous variation in fruit traits has important implications for domestication, suggesting opportunities for cultivar development through identification of elite individuals (Leakey and Page, 2006). Moreover, the domestication of wild fruit species depends on the expansion of the market demand for non-timber forest products (Leakey, 1999). So, it is recommended that selection of elite trees should not be based only on morphological traits, but also nutritive traits should be given priority. Many recent studies have documented variation in fruit traits in fruit tree species (Abasse et al., 2011; Assogbadjo et al., 2011; Fandohan et al., 2011; Gouwakinnou et al., 2011). In Bangladesh, very little information is available on morphological and nutritional traits for many wild fruits species. This pioneer study quantifies variation in fruit traits of wild mango (*M. sylvatica*) and provides basic knowledge on the range of variation of several morphological and nutritional fruit traits within and between local sites of Bangladesh. The findings of this study highlight the opportunity to exploit natural populations of *M. sylvatica* for selection and identification of superior quality trees for production (pulp or kernel) and the development of suitable cultivars for domestication programmes (Leakey et al., 2004; Leakey and Page, 2006; Bationo et al., 2008; Allendorf and Luikart, 2007). It was observed that nutritional properties were rich in wild mango compare to domesticated mango in most cases. Such as *M. sylvatica* pulp is a very rich source of Vitamin C (41.51 mg/100g) compared to different variety of *M. indica* fruit pulp such as Amarpali (34.96 mg/100g), Fazlee (28.23 mg/100g), Himsagar (46.53 mg/100g) (Ara et al., 2014). Though *M. sylvatica* has small precent of pulp portion but they contain good amount of vitamin A (2.44 mg/100 g) compared to *M. indica* such as Amelia 5.442±0.80 mg/100g (Ara et al., 2014). *M. sylvatica* fruit pulp contain higher amount of Na (54.58 mg/100g). *M. indica* pulp also contain a wide range (7.99-91.15 mg/100g) of Na in pulp. *M. sylvatica* fruit pulp (172.26 mg/100g) is a good source of Potassium (K). *M. indica* pulp content 10.29-64.04 mg/100g and kernel content 0.22 mg/100g of Potassium (Ara et al., 2014). *M. sylvatica* pulp is a good source of Calcium (16.92 mg/100g). *M. indica* may contain a wide range of Calcium (6.45-30.56 mg/100g) depending on varieties (Ara et al., 2014). *M. sylvatica* fruit pulp content (0.21 mg/100g) Fe. Fe content in mango pulp is 0.30-8.43 mg/100g (Ara et al., 2014). Here it is important to point out that, sugar content on average in M. sylvatica fruit is usually also lower (12 Brix°) compared to *M. indica* (14 to 22 Brix°). *Mangifera indica* is around 5.28 -15% oil (Gunstone, 2011).

Continuous variation was observed for the morphological fruit traits of *M. sylvatica*, which is important for the selection of elite trees. Selection of trees with an average fruit weight of 27g would result in considerable improvement in the quality and uniformity of marketable products. Screening a larger number of trees would almost certainly allow even greater benefits to be achieved, with the possibility of raising average fruit weights to 30g. However, to identify elite trees, it is recommended to include at least two morphological traits. Therefore, it will be worth including average fruit length (4.50 cm), which is strongly correlated with pulp weight and kernel weight (Table 4.3). Although between-site variation was found to be relatively high, particularly for fruit weight, pulp weight and kernel weight, within-site variation was prominent. Similar findings have been reported by Fandohan et al. (2011), Gouwakinnou et al. (2011), Leakey et al. (2004), Waruhiu et al. (2004), Anegbeh et al. (2005) and Assogbadjo et al. (2011) for African plum (*Dacryodes edulis Lam*.) and *Sclerocarya birrea*. The within-site variation was high, indicating that tree samples were sufficient in number for measuring the variation of *M. sylvatica* for breeding purposes and the improvement through vegetative propagation does not narrow the genetic diversity in the studied species (De Smedt et al., 2011; Allendorf and Luikart, 2007). However, limiting the selection process based only on morphological traits could lead to the loss of some essential market prospects. Therefore, it is better to consider nutritional traits for the selection of elite trees. From the proximate analysis, we found that the tree-to-tree variations were not too large in moisture and fibre for fruit pulp. Moisture content is an important measurement in the processing, preservation and storage of food (Akpabio and Ikpe, 2013). *M. sylvatica* contains higher quantities of moisture (85%), which is an indication of the low shelf life of the fruit, indicating that fresh fruit may not be able to be stored for long time due to its susceptibility to microbial attack (Ogunbanwo et al., 2013; Onwuka, 2005). *M. sylvatica* fruit has a wide range of variation in sugar (7 to 17 Brix°) content (Supplementary Figure 4.2). Fruits from the Sylhet site contained higher amounts of sugar where the climate is (temperature and rainfall) cooler compared to the Chittagong and Cox's Bazar sites (ZaferSiddik et al., 2013). Moreover, fruit phenotypic traits also support these findings, shown in Table 4.1 and Table 4.4. Similar findings are mentioned by Maranz and Wiesman (2003) for *Vitellaria paradoxa* in Africa. A high correlation was observed between sugar and Vitamin A (Table 4.4). It was realised that fruits from the Sylhet site hold higher amounts of Vitamin A compared to the other two sites (Table 4.4). Therefore, it can be concluded that fruits from the Sylhet site are sweeter and a rich source of Vitamin A, which can be considered during cultivar development for pulp production. On the other hand, a reasonable array of variation in kernel fat (9.00 to 14.00 %) was observed for *M. sylvatica* fruits, where higher fat content can be utilised in the food, pharmaceutical or cosmetic industries (Leakey et al., 2008). Our study demonstrates that fruits from the Cox's Bazar site, which is the hottest zone in terms of temperature and rain fall (ZaferSiddik et al., 2013) contained bigger kernels (Table 4.1) and higher fat percentages (Table 4.4) compared to the other two local sites. On the other hand, kernels with lower saturated and unsaturated fat ratios (Figure 4.3a), higher oleic and linoleic acid (Figure 4.3b) and lower fat content can be suitable as edible kernels. Although there was no significant site variation in the fatty acid composition of *M. sylvatica* butter/fat, there are similarities with cocoa butter (Lipp and Adam, 1998). Therefore, there is the potential for *M. sylvatica* butter to be used in the chocolate and confectionery industries as a partial or total replacement for cocoa butter. Thus, the domestication activity should not only contemplate the continuous variation but also take into account the resemblances in the population, and a higher level of selection pressure can be applied based on the ideotype selected (Leakey and Page, 2006). As the *M. sylvatica* tree produces fruits that have the potential to be used for pulp and kernel, this may be considered a new crop plant. For the utilisation of this rich wild fruit, it is necessary to develop novel foods from these fruits. Therefore, there is an urgent need for the food industry to work with foresters and horticulturists to domesticate this species, so that the process of genetic selection can include the traits important for the food industry. In contrast, the use of these fruits can lead to the initiation of the domestication processes by local farmers.

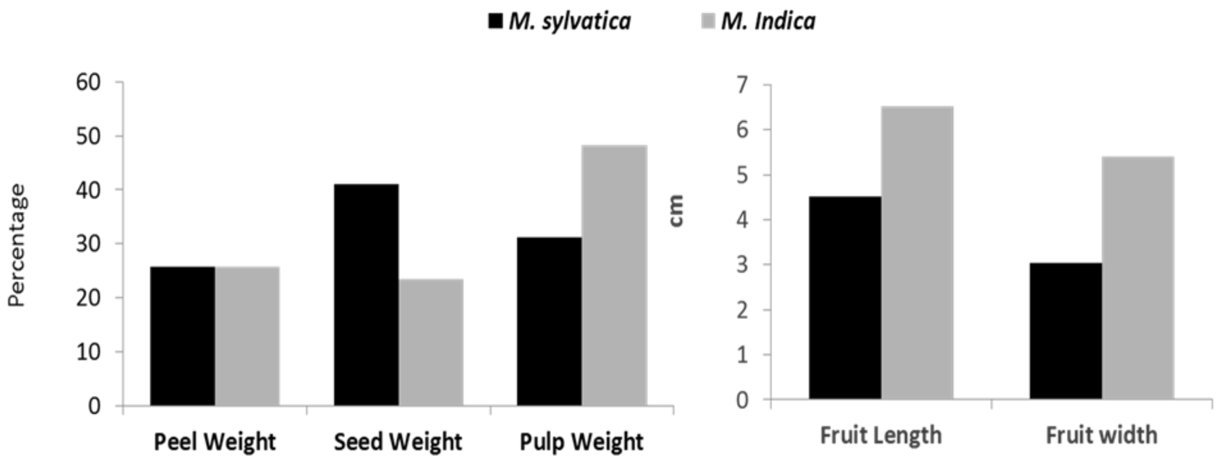


Figure 4.2: ***M. sylvatica*** **and** ***M. indica*** **fruit proportion weight**

Table 4.4: Proximate composition of *M. sylvatica* fruit pulp and kernel

Parameter	Nutritional profile of wild mango (*M. sylvatica*) pulp and kernel based on dry weight										
	Fibre (%)	Moisture (%)	Carbohydrate (%)	Ash (%)	Fat (%)	Protein (%)	Calorific value (KJ/100g)	Vitamin C (mg/100g)	Vitamin A (mg/100g)	Sugar (Brix°)	pH
Fruit pulp											
Sylhet	2.08 ±0.07[a]	85.20 ±0.62[a]	2.16±0.82[a]	2.17 ±0.24[a]	3.07 ±0.27[a]	5.33 ±0.19[a]	57.59 ±4.05[a]	35.81 ±12.45[a]	4.61 ±1.87[a]	14.44 ±0.72[a]	2.65 ±0.13[a]
Cox's Bazar	2.34 ±0.15[a]	85.46 ±1.13[a]	1.74±1.00[a]	1.58 ±0.28[a]	3.40 ±0.25[a]	5.05 ±0.29[a]	59.47 ±2.90[a]	52.88 ±10.75[a]	1.34 ±1.00[a]	13.04 ±1.27[a]	2.66 ±0.09[a]
Chittagong	2.14 ±0.06[a]	85.29 ±1.21[a]	2.00±0.83[a]	2.09 ±0.30[a]	3.43 ±0.22[a]	5.48 ±0.60[a]	59.09 ±4.32[a]	52.86 ±5.50[a]	2.63 ±1.01[a]	11.30 ±1.94[a]	2.65 ±0.13[a]
Average	2.19 ±0.06	85.32 ±0.57	1.95±0.48	1.93 ±0.17	3.32 ±0.14	5.29 ±0.23	58.80 ±4.08	41.51 ±6.06	2.44 ±0.65	12.93 ±0.83	2.65 ±0.06
Fruit kernel											
Sylhet	9.99 ±0.95[a]	44.91 ±4.63[a]	28.41±5.54[a]	0.06 ±0.03[a]	11.42 ±0.45[a]	5.21 ±0.19[a]	237.20 ±21.22[a]	NE	NE	NE	NE
Cox's Bazar	9.36 ±0.48[a]	57.30 ±5.70[a]	17.65±6.79[a]	0.16 ±0.06[a]	11.76 ±0.26[a]	5.48 ±0.35[a]	196.11 ±21.62[a]	NE	NE	NE	NE
Chittagong	10.44 ±0.56[a]	54.85 ±6.29[a]	16.64±5.16[a]	0.08 ±0.04[a]	11.51 ±0.75[a]	4.78 ±0.37[a]	191.53 ±21.35[a]	NE	NE	NE	NE
Average	9.93 ±0.37	52.89 ±3.37	20.36±3.47	0.10 ±0.03	11.57 ±0.29	5.15 ±0.20	206.22 ±17.26	NE	NE	NE	NE

Table 4.5: Macro and micro mineral composition of *M. sylvatica* fruit pulp, kernel and peel

Site	**Mineral composition of *M. sylvatica* fruit** based on dry weight					
	Na	K	Ca	Mn	Zn	Fe
Mango pulp (mg/100g)						
Cox's Bazar	34,15±11,90[a]	175,21±22,07[a]	16,83±2,78[ab]	1,50±0,47[a]	0,19±0,04[a]	0,21±0,05[a]
Chittagong	59,57±10,63[a]	191,98±31,18[a]	22,85±3,71[a]	1,91±0,67[a]	0,19±0,03[a]	0,23±0,01[a]
Sylhet	73,90±27,85[a]	145,68±11,35[a]	9,61±1,45[b]	1,00±0,35[a]	0,11±0,00[a]	0,18±0,03[a]
Average	54,58±9,97	172,76±13,98	16,92±2,15	1,50±0,30	0,17±0,02	0,21±0,02
Mango kernel (mg/100g)						
Cox's Bazar	9,62±2,10[a]	84,57±7,50[a]	8,20±1,69[a]	0,77±0,17[a]	0,17±0,02[a]	0,89±0,16[a]
Chittagong	13,47±4,92[a]	75,14±6,02[ab]	9,91±1,77[a]	0,68±0,23[a]	0,16±0,01[a]	0,63±0,18[a]
Sylhet	4,07±0,99[a]	54,79±0,39[b]	6,37±1,02[a]	0,32±0,08[a]	0,14±0,02[a]	0,31±0,02[a]
Average	9,41±2,07	72,69±4,61	8,29±0,94	0,61±0,11	0,16±0,01	0,63±0,10
Mango peel (mg/100g)						
Cox's Bazar	30,99±7,96[a]	128,76±10,05[a]	17,82±1,80[a]	1,37±0,31[a]	0,38±0,14[a]	0,52±0,06[b]
Chittagong	28,09±6,45[a]	164,51±8,32[a]	23,84±2,74[a]	1,57±0,43[a]	0,36±0,14[a]	0,44±0,05[b]
Sylhet (n=4)	43,57±13,03[a]	176,81±21,62[a]	28,27±8,31[a]	1,65±0,56[a]	0,20±0,02[a]	0,95±0,07[a]
Average	33,55±5,09	155,25±9,02	22,95±2,67	1,52±0,23	0,32±0,07	0,61±0,07

N.B. Macronutrients: Na K, Ca; Micronutrients: Zn, Mn, Fe; SE = Standard Error

Table 4.6: Medicinal compounds detected (by HPLC-UV) in *M. sylvatica* and their medicinal use

Medicinal compound in *M. sylvatica* pulp	Anti-cancer	Anti-diabetic	Anti-inflammatory	Prevention of liver steatosis	Anti-allergic	Anti-viral	Anti-microbial	Anti-ulcer	Heart diseases	Anti-coagulant	Anti-obese	Anti-mutagenic	Anti-osteoporotic	Anti-estrogenic	Neuroprotective	Anti-anxiety	Analgesic	Anti-angiogenic	Immunomodulatory	Reference
Mangiferin	√	√	√	√	√	√	√			√	√						√		√	Benard and Chi, 2015; Mujawdiya and Kapur, 2015; Matkowski et al., 2013; Vyas et al., 2012; Masibo and He, 2008
Quercetin	√		√		√	√	√		√		√	√			√					Hossen et al., 2015; Nabavi et al., 2015; Maalik et al., 2014; Asif and Khodadadi, 2013; Yoshida et al., 1990; Hollman et al., 1996
Kaempferol	√	√	√		√		√	√			√		√	√	√	√	√			Zang et al., 2015; Calderón-Montaño et al., 2011; Ackland et al., 2005
p-coumaricacid	√	√	√					√												Pei et al., 2016; Krishna et al., 2014; Srivastava et al., 2007
Gallic acid	√		√			√	√			√	√	√						√		Choubey et al., 2015; Pandey et al., 2014; Vazirian et al., 2011; Madsen and Bertelsen, 1995
Ellagic acid	√		√			√					√	√								Kang, 2015; Park et al., 2014; Favarin et al., 2013; Usta et al., 2013; Mandal et al., 1988

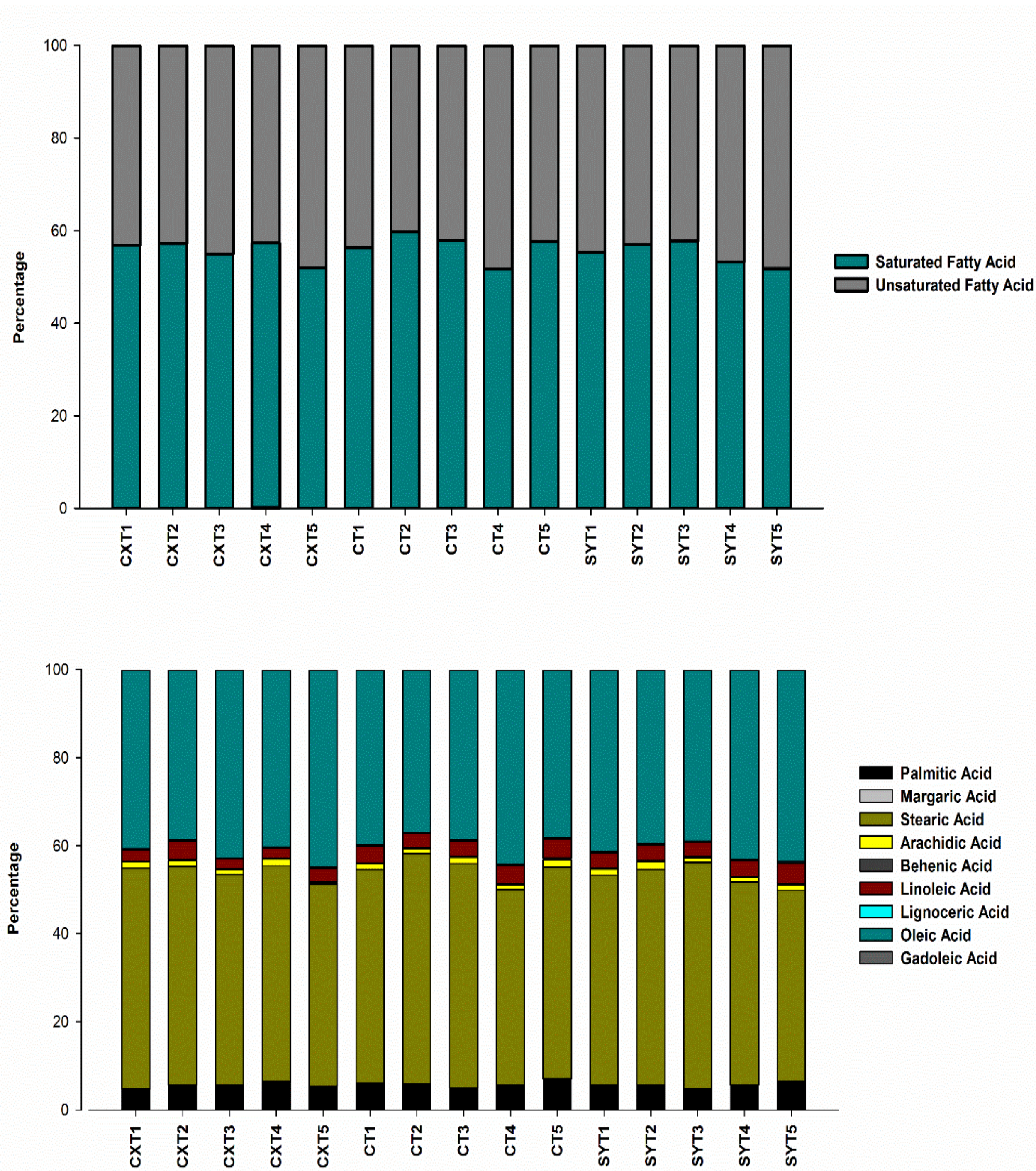


Figure 4.3: (a) saturated and unsaturated lipid content; (b) fatty acid profile of *M. sylvatica* kernels

In the 21st century, the world has made much progress in reducing hunger, poverty and malnutrition. However, these problems, particularly in the developing world, are an unresolved issue. The number of undernourished people in developing countries was 780 million in 2014–16 (FAO, 2015). According to the WHO, one in three people in developing countries suffers from nutrition (vitamin and mineral) deficiency. Every second pregnant woman and 40% of preschool children are estimated to be anaemic; and 250 million preschool children are Vitamin A deficient in developing countries (WHO, 2016). Wild fruits can play a crucial role in mitigating the effects of nutrient deficiencies and consumption of a variety of wild fruits can potentially contribute to health improvement (Judprasong et al., 2013). Wild fruit is an important source of food, medicine and income for forest dwellers, and tribal and marginalised rural people (Judprasong et al., 2013). In the rural countryside of many developing nations, wild fruits are consumed as people cannot afford commercial, domesticated fruits (apple, grapes, pomegranate and orange). Some wild fruits have been identified as having better nutritional value than cultivated fruits (Mahapatra et al., 2012). They are nutritionally rich and traditionally used as supplements to the staple diet. Several wild foods are used only in times of scarcity and famine (FAO, 1992). Many of these famine foods have a higher protein, energy, vitamin (A and C) and mineral content than domesticated varieties. Still, there are many wild fruits available in the forest that are underexploited and information on their nutritional values and medicinal properties, storage ability and economic potential are unknown, except for their consumption value and taste. As a result, in recent years, a growing interest has emerged to evaluate the edibility, nutritional features and therapeutic potential of various wild fruit species (Nazarudeen, 2010; Aberoum and Deokule, 2009; Musinguzi et al., 2007; Nkafamiya et al., 2007; Glew et al., 2005) and other authors suggest an inventory of wild food resources may promote conservation of wild fruit species. *M. sylvatica* Roxb. is one such wild fruit species for which there has been no information to date on its morphological and nutritional value. The present study provides noteworthy information on the morphological traits and nutritional profile of the *M. sylvatica* species, which can open up opportunities for cultivar development that can assist in the commercialisation and conservation of this underutilised wild fruit species.Information on these aspect will benefit commercial users such as timber, pickle and butter etc; potential investors in butter, nutraceuticals, flavourings etc, minister responsible for foreign direct investment in Bangladesh. For conservation of *M. sylvatica* morphological and nutritional traits should be utilised. It is clear from this study that fruit pulp and kernels even from a small population of individual trees of *M. sylvatica* vary considerably in morphological and nutritional traits, although the site variation was not great. This variability provides excellent opportunities for the domestication of the species through the development of multiple-trait cultivars from elite trees, using simple horticultural techniques (Leakey et al., 2008). So, if we can conserve the *M. sylvatica* species, this can help to combat nutritional deficiency at local levels and village-level domestication can potentially enhance the livelihoods of local people, while maintaining genetic diversity at the national level (Judprasong et al., 2013; Leakey and Page, 2006, Leakey et al., 2003). The current study can promote the development of a strategy for domestication of *M. sylvatica* to empower smallholders and enhance their livelihoods and income. This strategy aims to fulfil the demand of producers and consumers and offers opportunities for wider-scale marketing of *M. sylvatica* fruits and kernels.

4.5 Conclusion

M. sylvatica is a wild species that could be used as an alternative source of nutrition to satisfy hidden hunger. Some interesting and valuable morphological and nutritional information has come to light that can be used as a tool for nutrition education and to encourage people to consume *M. sylvatica* fruit. The current study may lead to the beginning of a domestication programme of this wild underutilised fruit species. The results also highlight a continuous variation in fruit traits, which are important for consumption and market-orientated selection. The kernel of this wild fruit species is a good source of mango butter that can be utilised, although further research on this sector is also needed. Additionally, contribution from the food industry is also required to identify potentially novel food products. Moreover, research on the agroforestry suitability of this species would also be necessary for adequate and appropriate conservation of this wild species. This paper calls for greater collaboration between foresters, horticulturists and the food industry to promote the conservation and commercialisation of the *M. sylvatica* fruits in Bangladesh.

Supplementary material: Method for proximate, vitamin, mineral and fatty acid analysis

Moisture and ash content

Samples of 0.2g mango pulp and 1g of kernel was oven dried at 105°C for three hours and dry matter and moisture content were determined. Samples were subsequently put in a muffle furnace at 600^0C for six hours and total ash content was recorded (Jaafar et al. 2009).

Crude fat

About 0.5g of mango pulp sample was weighed and 150ml of petroleum ether added to the flask and refluxed for four hours. The flasks were removed and dried with a rotary evaporator (Buchi Rotavapor R-114). Weight was recorded as crude fat content. For kernels, 4g of frozen mango kernel sample was weighed and then finely chopped, a solution of 2:1 = Chloroform: Methanol, ten times the weight of the mango kernel was added to the chopped mango kernels in a conical flask and homogenised using Ultra-Turrax (14A Ultra Turrax T25). The homogenised mixture was filtered three times and evaporated using a rotary evaporator (Buchi Rotavapor R-114) at 40^oC. Finally, the weight was recorded as crude fat content.

Crude fibre

About 0.5g of mango pulp and kernel sample was sealed in fibre filter bags (ANKOM filter bags) which were put into 250ml conical flasks. Later, 200ml of 1.25% sulphuric acid solution was added into the conical flask and heated for 30 minutes and washed until traces of acid were detected using pH paper. After that, the acid extracted in the filter bags were transferred into 250ml conical flasks and again 200ml of 1.25% NaOH solution was added. The sample was heated for 30 minutes and washed with water until trace of base was detected by pH paper. The filter bags were oven dried for two hours at 105°C (Sanyo convection oven MOV-212F). After that, the samples were transferred into pre-weighed crucibles and placed into a muffle furnace (Carbolite CWF 1200) at 600°C for six hours and the weight of crucible was recorded (Nwanekezi et al. 2010).

Crude protein

Mango pulp and kernel powdered samples were oven dried (Sanyo convection oven MOV-212F) for two hours at 105°C and a 0.1g sample was taken for nitrogen analysis. Samples were wrapped in tin foil and analysed using a Leco CHN 2000 analyser. The results were converted to protein content using a 6.25 conversion factor (FAO, 1992).

Carbohydrate and energy value (calories)

Carbohydrate was determined by the following formula (Nwanekezi et al., 2010). Carbohydrate content (%) = 100 – (ash + dietary fiber + fat content + protein contents) %. Energy value was calculated using the formula Energy value (KJ/100 g) = [(% available carbohydrates x 17) + (% protein x 17) + (% fat x 37)].

Determination of total soluble solids, pH

Fresh mango pulp was used for this analysis. Total soluble solids (TSS) were determined using a hand refractometer in terms of °Brix. pH was measured using a pH meter. A total of 150 fruits were used from 15 trees (10 fruits from each tree and five trees from each provenance (sample area) to measure TSS and pH.

Vitamin analysis of *M. sylvatica* fruit pulp

Mango pulp samples were freeze dried and then powdered using ball mill. A 0.5g sample was first extracted with 16ml of 10mM ammonium acetate/methanol 50:50 (v/v) containing 0.1% BHT. After 15 minutes of shaking to achieve good sample dispersion in the extraction liquid, samples were placed in an ultrasound bath for 15 minutes. Bath temperature was controlled to

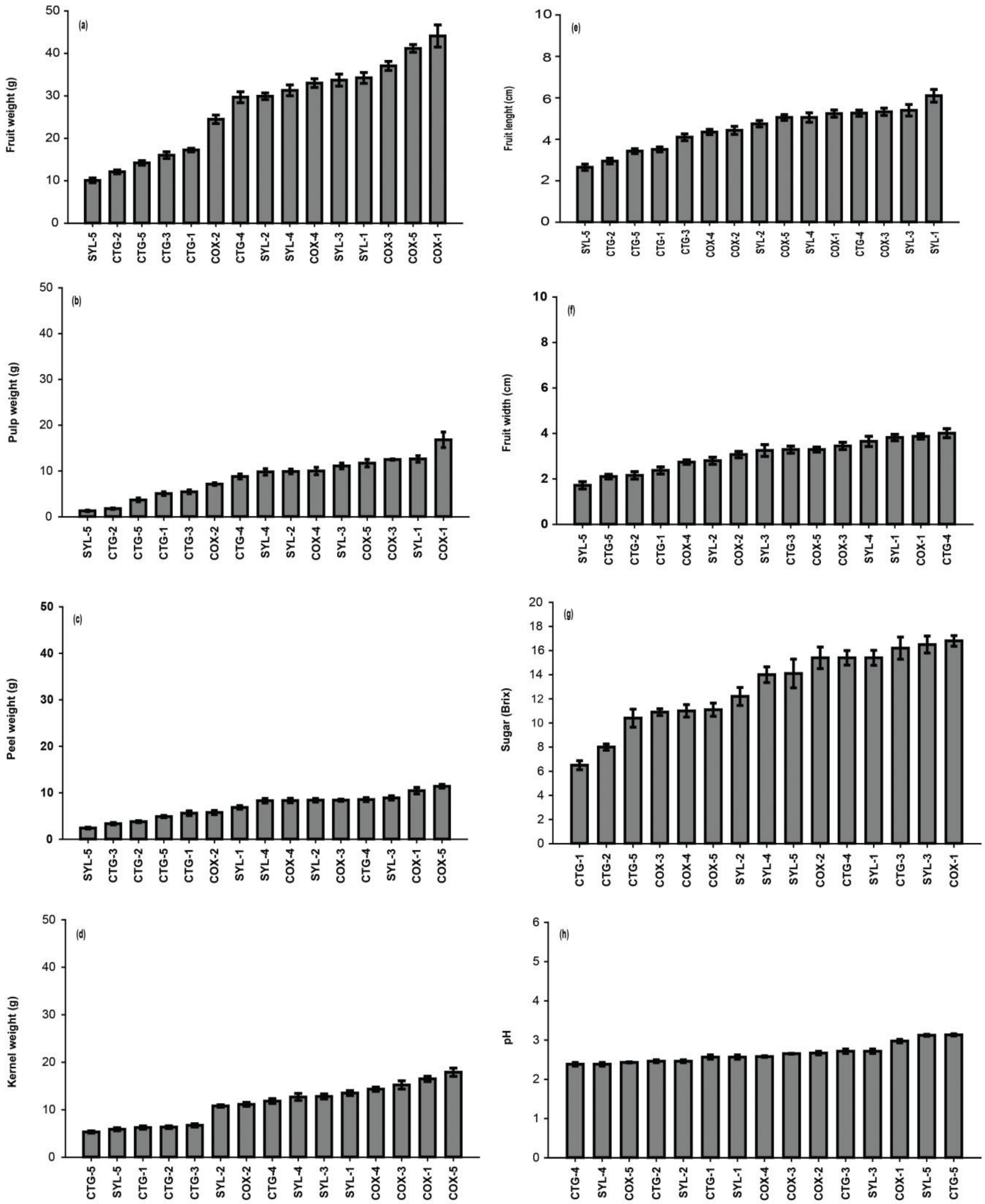
25^{o}C. The samples were centrifuged at 14,000rpm for 15 minutes and the supernatant was collected. Three millilitres of the supernatant was concentrated into a N_2 stream and 1ml of 10mM ammonium acetate was added to the dried sample. This final sample was injected into a HPLC–UV system to determine the Vitamin C content. The solid residue from the first extraction was re-extracted with 12ml ethyl acetate containing 0.1% BHT, kept in the ultrasonic bath for 15 minutes. The samples were centrifuged (14,000 rpm for 15 minutes) and 3ml supernatant dried under a N_2 stream. Finally, the residue dissolved in 1ml of ethyl acetate and injected in a HPLC–UV system to monitor the Vitamin A content (Santos et al. 2012).

Mineral analysis of *M. sylvatica* fruit

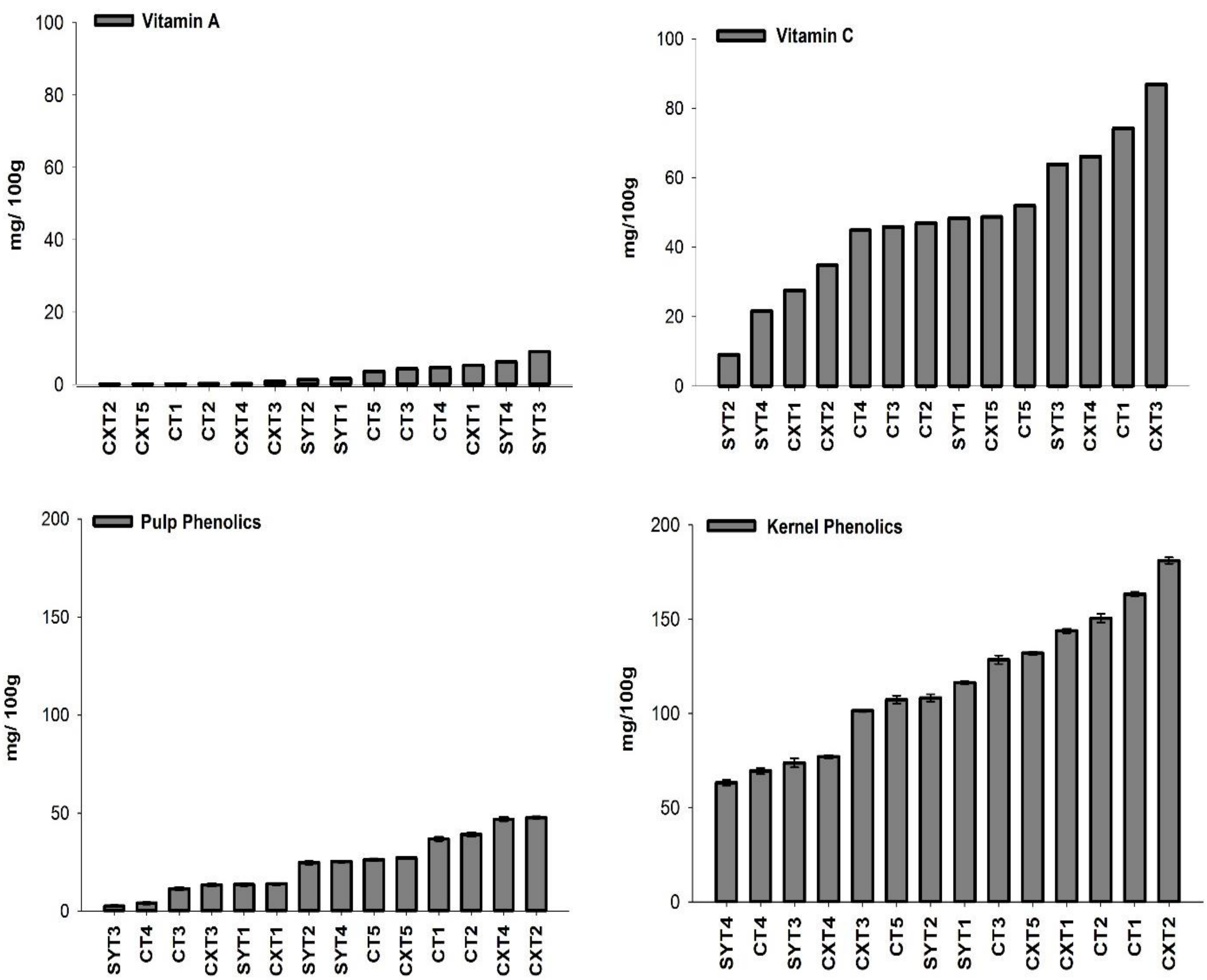
For Sodium (Na) analysis, 0.2g of dried mango pulp and kernel sample was ashed in a muffle furnace (Carbolite CWF 1200) at 600°C for six hours. After that, cooled ash samples were dissolved in 1ml of 20% HCl solution and 9ml of deionised water was added. Where necessary, the solution was filtered through acid-washed filter paper and finally analysis was done by flame photometer (Sherwood 410). For Potassium (K), Calcium (Ca), Phosphorus (P), Sulphur (S), Chlorine (Cl), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu) and Nickel (Ni), total X-Ray fluorescence analysis was done. Here, oven dried samples were ground as fine powder using a ball mill and stored in airtight bags. A 0.02g sample was transferred to Eppendorf tubes and 1ml of Tritinx solution and 10µl of Ga (1000 mg/l Ga internal standard) were added to the suspension. Samples were mixed well using a vortex and immediately after vortexing 10µl of sample was pipetted into the center of a siliconised disc before the powder settled out of suspension. Samples were dried on a hot plate and assembled in the sample cassette. The first position of the cassette was reserved for Ga standard disc. All the samples were then run in the total X-Ray fluorescence machine.

Determination of fatty acid

The fatty acid composition of kernels was analysed by g GC-MS. Kernels were separated from flesh and peel and the fresh kernel was used for fatty acid analysis. Frozen, chopped kernel was added to a mixture of 2:1 = Chloroform: Methanol and homogenised using an Ultra-Turrax. The homogenous mixture was filtered three times and evaporated using a rotary evaporator (Buchi Rotavapor R-114) at 40^{o}C. The butter produced was dissolved in methanol and chloroform (1:1) using a rotary evaporator and transferred into fresh vials. Afterward, 0.1 ml of solution mixture was placed in a 5ml vial for drying while stirring. Then, 1ml of heptane and 0.05ml of 1N Methanolic NaOH was added and stirred at 50^{o}C for two minutes. After two minutes, when the two layers were separated, the supernatant was collected and transferred to GC-vials for GC-MS analysis.



Supplementary Figure 4.1: Tree to tree variation of different morphological fruit traits. From top left (a) fruit weight (b) pulp weight (c) peel weight (d) Kernal weight (e) fruit length (f) fruit width (g) sugar (h) pH



Supplementary Figure 4.2: Tree to tree variation of different medicinal properties. From top left (clockwise): Vitamin A, Vitamin C, Kernal phenolics, pulp phenolics.

Chapter 5. *M. sylvatica* (wild mango): a new cocoa butter alternative[3]

Abstract

Cocoa butter is the pure butter extracted from cocoa beans and is a major ingredient in the chocolate industry. Global production of cocoa is in decline due to crop failure, diseases and ageing plantations, leading to price fluctuations and the necessity for the industry to find high-quality cocoa butter alternatives. This study explored the potential of a wild mango (*M. sylvatica*), an underutilised fruit in Southeast Asia, as a new cocoa butter alternative (CBA). Analyses showed that wild mango butter has a light-coloured fat with a similar fatty acid profile (palmitic, stearic and oleic acid) and triglyceride profile (POP, SOS and POS) to cocoa butter. Thermal and physical properties are also similar to cocoa butter. Additionally, wild mango butter comprises 65% SOS (1, 3-distearoyl-2-oleoyl-glycerol), which indicates its potential to become a cocoa butter improver (an enhancement of CBA). It is concluded that these attractive properties of wild mango could be prompted by a coalition of policy makers, foresters, food industries and horticulturists to promote more widespread cultivation of this wild fruit species to realise the market opportunity.

[3] Akhter, S., McDonald, M. A., Marriott, R. 2016. *Mangifera sylvatica* (Wild Mango): A new cocoa butter alternative. *Sci. Rep*, 6, 32050; doi: 10.1038/srep32050.

5.1. Introduction

Cocoa butter (CB) is a light-yellow fat obtained from the beans of the cocoa plant (*Theobroma cacao*L.). It is one of the unique natural fats highly demanded by the food, pharmaceutical and cosmetic industries (Issara et al., 2014; Jahurul et al., 2014a). Cocoa butter is the major ingredient of the chocolate industry (Menezes et al., 2016). Palmitic acid (C 16:0), stearic acid (C 18:0), oleic acid (C 18:1) and linoleic acid (C 18:2) account for more than 98% of the total fatty acids (Shekarchizadeh et al., 2009) in cocoa butter. This is the only commercially available natural fat that is rich in saturated and monounsaturated fatty acids, 13.65–15.5% of 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), 33.7%–40.5% of 1-palmitoyl-3-stearoyl-2-oleoyl-glycerol (POS) and 23.8%–31.2% of 1,3-distearoyl-2-oleoyl-glycerol (SOS) (Bootello et al., 2012; Shekarchizadeh et al., 2012). This relatively simple triglyceridesin cocoa butter confers desirable melting profiles prized by the confectionery industry, being solid at 20°C and melting between 27°C and 35°C, which is appreciated by consumers as well as desirable in confectionery applications (Jahurul et al., 2014b). Moreover, the price of cocoa butter is one of the highest among all tropical fats and oils (Bootello et al., 2012; Jahurul et al., 2014b; Zaidul et al., 2006). According to the ICCO (2014), the price of cocoa butter more than doubled between 2005 and 2015 (ICCO, 2014), from \$1433/tonne to \$3360/tonne (Supplementary Figure 5.1). Cocoa is cultivated on a land area of over 70,000km^2 worldwide (WCF, 2014), while Africa (68%), Asia (17%) and America (15%) contribute the major proportion of global production of CB (WCF, 2014). According to the ICCO (2014), annual global cocoa production was reported to be more than 4 million tonnes per season. However, global demand for cocoa is growing annually by 2% to 3% due to low productivity, price fluctuations and uncertainty in supply (Supplementary Figure 5.1), which has forced the confectionery industry to seek CBAs (Jahurul et al., 2014a; Bootello et al., 2012) from other natural sources (Council of the European Union, 2000). Cocoa butter equivalents (CBEs) are commercially available fats containing a similar mixture of triacylglycerol to cocoa butter that can be mixed with cocoa butter up to 5% (Gunstone, 2011). Very few tropical fats are considered to be CBAs but include those sourced from illipe butter, kokum butter, shea butter and mango *Mangifera indica* L. butter (Jahurul et al., 2013). The mango kernel contains about 7%–15% fat that is rich in palmitic, stearic and oleic acids (Council of the European Union, 2000; Jahurul et al., 2013). Cocoa butter from the domesticated mango species, *M.indica*, is a natural fat containing high saturated and monounsaturated fatty acids containing symmetrical triglycerides such as POS (10% to 16%), SOS (25% to 59%) and POP (1% to 8.9%) (Jahurul et al., 2013). These are relatively simple triglyceride combinations, which are desirable for confectionery applications, especially in chocolate processing. *M. indica* kernels have, therefore, been heavily researched for their potential as a cocoa butter alternative (Jahurul et al., 2014c).

Wild mango (*Mangifera sylvatica* Roxb.) belongs to the Anacardiaceae family. It is found in Bangladesh, India, China, Cambodia, Myanmar, Nepal and Thailand (Figure 5.1) (Baul et al., 2016). It is one of the closest genetic species to *M. indica* in the world (Nishiyama et al., 2006) but it is underutilised and unmarketed in its native home of Bangladesh, as well as in other tropical countries due to a lack of information and awareness of its potential value as a source of food, nutrition or medicine. The species is already threatened in Bangladesh (Khan et al., 2001) due to habitat loss and deforestation, but is not afforded any conservation protection due to its lack of documented value. The seed germination rate and early growth of seedlings indicates that this species could be easily domesticated and incorporated into small-scale forestry programmes (Baul et al., 2016). There is growing evidence of beneficial medicinal properties, such as a recent study showing that *M. sylvatica* leaves possess thrombolytic properties that could lyse blood clots (Zaman et al., 2015a). The leaves can also be used as antidiarrhoeal drugs (Zaman et al., 2015b). Until now, no research has been conducted on its market potential, which will ultimately promote domestication and commercialisation of the species. Therefore, the present study constitutes an assessment of the potential of *M. sylvatica* as a CBA for the food, pharmaceutical and cosmetic industries. In this study, the fatty acid and triglyceride composition, and the physiochemical and thermal properties of *M. sylvatica* were determined and compared to those of the domesticated mango and cocoa butter to assess the potential for *M. sylvatica* as a new source of cocoa butter.

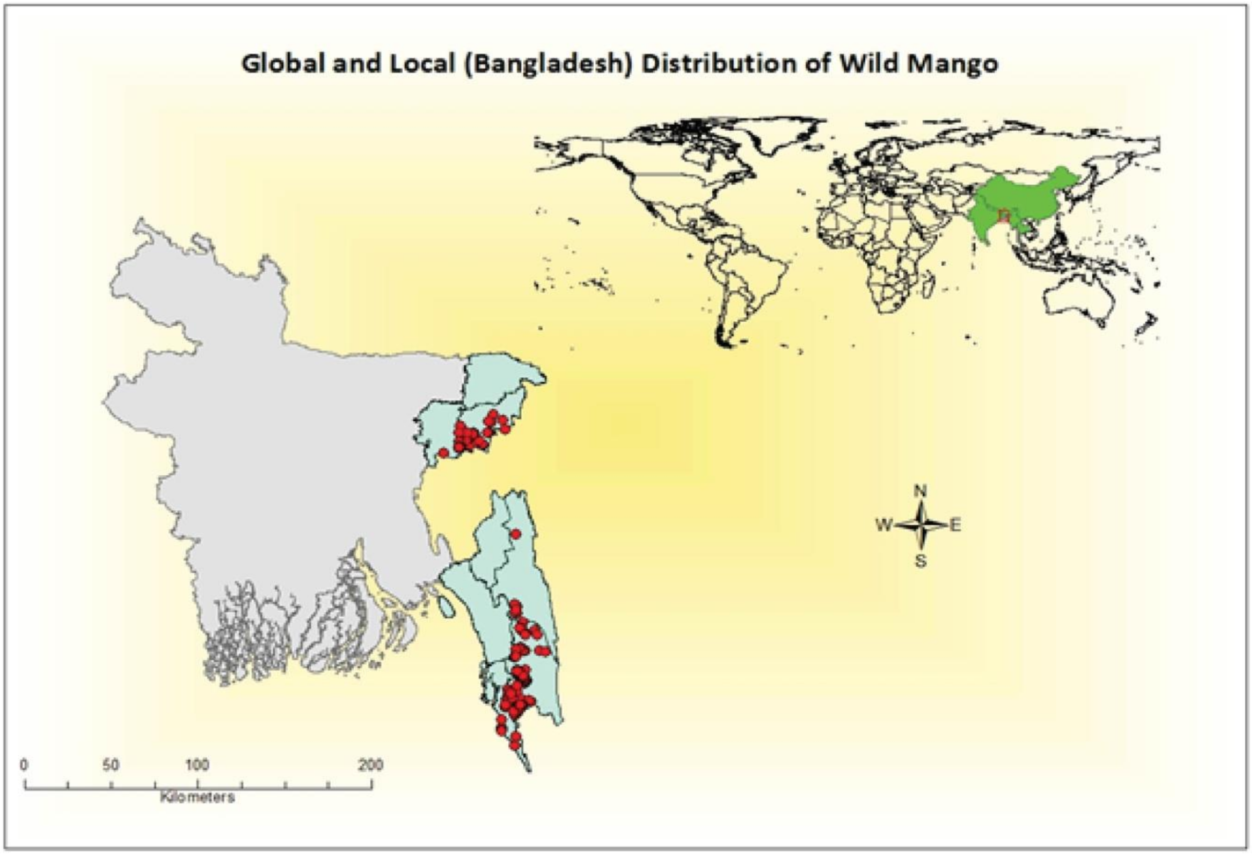


Figure 5.1: Global and local distribution of wild mango (*M. sylvatica*). The spatial positions of the site locations were plotted in the global geo-political boundary available from Esri (http://www.arcgis.com/) and species presence locations were plotted

5.2 Material and Methods

5.2.1 Sample collection, preparation, extraction and analysis

Mature fruits of *M. sylvatica* were collected from Cox's Bazar, Bangladesh (Figure 5.1), during April to June, 2014. After collection, fresh fruits were retted, de-pulped and sun dried. The nuts were then separated from the kernels using a hand betel nut cutter (Figure 5.2). Phosphine fumigation was carried out before the nuts were sent to Bangor University, UK, for further processing. Mango butter was extracted using the SC-CO_2 (Supercritical Carbon Dioxide Fluid Extraction) method. We obtained two cocoa butter samples from the Callebaut chocolate company (UK) and purchased 99% pure *Mangifera indica* butter (domesticated mango butter

(DMB) from the Soapery. Finally, we analysed the physical (saponification value, iodine value, moisture content, specific gravity, refractive index, acid value and glycerol percent) and chemical (fatty acid profile and triglyceride composition) and thermal (melting profile) parameters of *M. sylvatica* butter (wild mango butter (WMB) and compared the results of WMB with the other three butter samples.

Figure 5.2: Collection, processing and preparation of wild mango butter from *M. sylvatica*: (a) *M. sylvatica* tree; (b) fruit of *M. sylvatica* with the big kernel in the left and the fruit of *M. indica* with big pulp in the right; (c) seed of *M. Sylvatica*

5.2.2 Wild mango butter extraction by SC-CO_2

The wild mango kernels were extracted using the supercritical fluid extraction method (Jahurul et al., 2014a). A total 18.85kg of dry ground mango kernel samples were loaded into the extraction vessel. The continuous methods of SC-CO_2 extraction were carried out at pressures of 50MPa, temperatures of 40°C and at a constant CO_2 flow rate of 30kg/hour. When pressurisation was initiated, the CO_2 from the cylinder passed through the chiller at 0°C and was pumped into the extraction vessel by a high-pressure pump. The fat was extracted from the fat-rich CO_2 by separators at one end of the instrument. Two separators were used through the entire process, with the first separator being at a fixed pressure and temperature of 80MPa and 40°C, respectively. The second separator was maintained at room temperature and 55MPa and desiccated the samples. CO_2 was recirculated throughout the run time. Yield was calculated on a dry weight basis at the end of the process as g fat/kg mango kernel.

5.2.3 Fatty acid and triglycerides profiling

The fatty acid composition of the mango (*M. indica* and *M. sylvatica*) and cocoa butter (deodorised and non-deodorised) samples was done by GC (PerkinElmer Clarus 680)-MS (PerkinElmer Clarus 600 C). Five to 7mg of frozen butter dissolved in 1ml of heptane and 0.05ml of 1N Methanolic NaOH were shaken at room temperature for two minutes. After two minutes, when the two layers were separated, the lower layer was discarded and the supernatant was used for GC-MS analysis. The analysis was done in triplicate. On the other hand, a direct infusion mass spectrometry method (API 150 EX MS System) was used for the determination of triglycerides. The nebuliser gas was N_2. Scanning was done for masses 100 to 1000 in an ESI positive mode with a flow rate of 90µl/min. Samples were run once for triglyceride profiling (Jahurul et al., 2014a; 2014b) and the percentage of triglycerides was calculated based on the peak intensity.

5.2.4 Physio-chemical and thermal properties

Determination of the saponification value, glycerolpercentage, acid value, iodine value, moisture content, refractive index and specific gravity were carried out according to methods described by Jahurul et al. (2014a; 2014b; 2014c) and US Pharmacopeia (2016). Quantitative analyses were performed in triplicate and the results were expressed as average ± standard deviation. One-way ANOVA was conducted to find any significant difference among the four types of butter. Differential scanning calorimetry (DSC) was used to monitor the melting profiles of the samples. A modified method of Afoakwa (2010) was used for this analysis. The method followed was a heating-cooling-heating cycle. The first cycle (-20^{o}C to 60^{o}C) and second cycle (60^{o}c to -20^{o}C) were done to erase thermal memory and also to get rid of any unwanted materials. The final cycle (-20^{o}C to 60^{o}C) was recorded to get the melting profile of the samples. The heating rate was 10^{o}C/ min and the cooling rate was 2^{o}C/min.

5.3 Results

5.3.1 Fatty acid profile and triglyceride compositions

Wild mango butter (WMB) is a light-yellow fat that is not greasy to touch and has a characteristic nutty flavour. WMB is a rich source of saturated fatty acids (Figure 5.3a). WMB consists of three major fatty acids, namely, palmitic acid (C 16:0), stearic acid (C 18:0) and oleic acid (C 18:1) (Figure 5.3b). The saturated fatty acid content of *M. sylvatica* (56%) approximates to that of DMB (57%) but is lower compared to CB (65%). Stearic acid, oleic acid and palmitic acid account for 95% of the total fatty acid in WMB from *M. sylvatica,* followed by CB (96%º97%) and DMB (94%). Apart from that, *M. sylvatica* butter contains small amounts of arachidic acid and linoleic acid (also known as Omega-3 fatty acid), which is similar to CB and DMB (Figure 5.3b). Triglycerides are complex mixtures of a variety of fatty acids, which are the major constituents in fats and oils. The major triglycerides found in WMB are 1,3-distearoyl-2-oleoyl-glycerol (SOS), 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS) and 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), which are also the main features of cocoa butter (Table 5.1). POP, POS and SOS account for 79% for WMB and 82%–

85% for CB (Figure 5.4h). This similarity in fatty acid and triglyceride profiles indicates considerable potential for WMB to be used as a source of cocoa butter alternative.

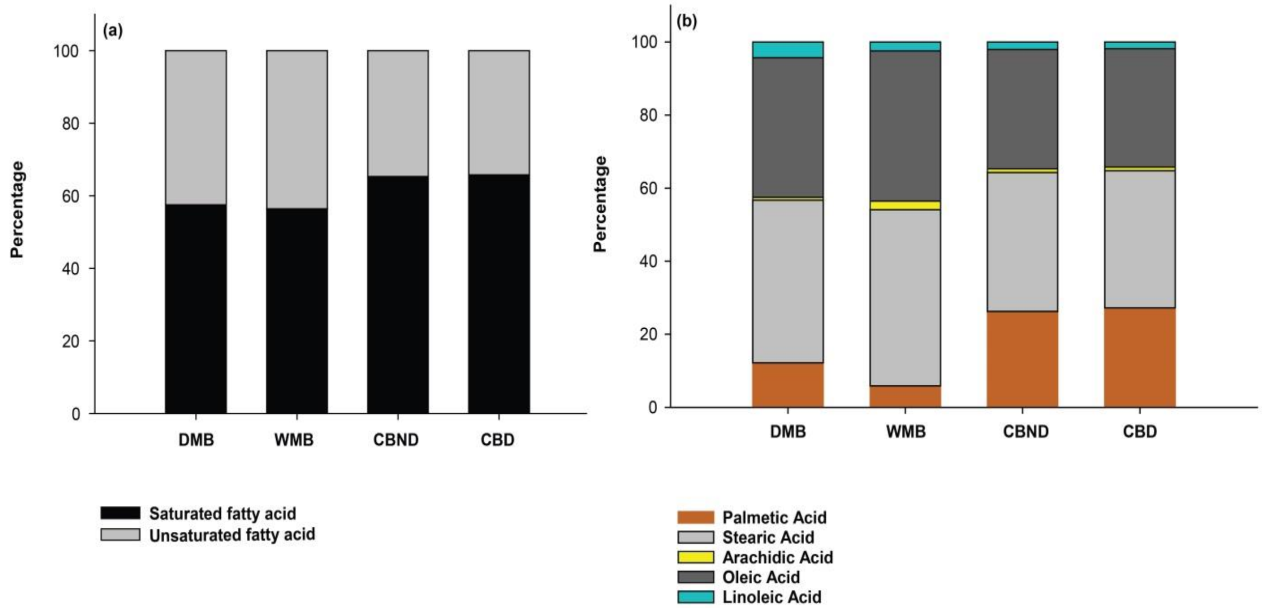


Figure 5.3: (a) total saturated and unsaturated fatty acid content; (b) fatty acid profiles of WMB, DMB and CB (CBD and CBND)

Table 5.1: Triglyceride profiles of *M. sylvatica* butter, *M. indica* butter and cocoa butter

Triglycerides	Triglycerides	WMB	DMB	CBD	CBND
1,3-dipalmitoyl-2-oleoylglycerol	POP	√	√	√	√
1-palmitoyl-2-oleoyl-3-stearoyl-glycerol	POS	√	√	√	√
1,3-distearoyl-2-oleoyl-glycerol	SOS	√	√	√	√
Trioleoyl-glycerol	OOO	√		√	
1-arachidoyl-2-oleoyl-3 linoleyolglycerol	AOLo	√			√
1,2-palmitoyl-3-linoleoylglycerol	PPLo			√	√
1-stearoyl-2,3-dioleoyl-glycerol	SOO		√		
1-stearoyl-2-oleoyl-3-arachidoyl-glycerol	SOA		√		

5.3.2 Physical and thermal properties of WMB

The saponification value, glycerol percentage, iodine value, free fatty acid percentage, moisture content, specific gravity and refractive index were determined for the wild mango butters as important parameters of butter quality. In WMB, the saponification value is slightly lower than CB (Figure 5.4a), which means WMB consists of long-chain carbon molecules but is close to DMB. WMB contains bigger carbon chain molecules so it has fewer glycerol molecules as indicated by the glycerol percentage (Figure 5.4b). The iodine value of WMB is slightly higher than CB (Figure 5.4d) but close to DMB. An elevated iodine value and acid value indicates high susceptibility of fat to oxidative rancidity due to the high degree of unsaturation. Moisture content and free fatty acid content in WMB are high compared to other butter samples (Figure 5.4c and Figure 5.4g), which also indicates the possibility of WMB oxidation. These results suggest that proper and controlled processing can produce high-quality butter with decreased degradation. The refractive index and specific gravity of WMB butter were very similar to CB and DMB (Figure 5.4f and Figure 5.4g). This indicates that the double bond present in WMB is similar to CB and the weight of WMB is very similar to CB. WMB has a melting point close to CB, though DMB has a higher melting point (Figure. 5.5). WMB is characterised by one leading peak around 16.18^{o}C with a 'shoulder' around -4.71^{o}C. *M. sylvatica* is similar to CB (Table 5.2) where the main melting peak appears around 20^{o}C. A complete melting of WMB was observed around 26^{o}C, and 27^{o}C for CB. The results from *M. sylvatica* are very different to *M. indica*, where the main melting peak was observed around 16.88^{o}C, but with multiple shoulders and with a very high melting point observed around 53^{o}C (Figure 5.5).

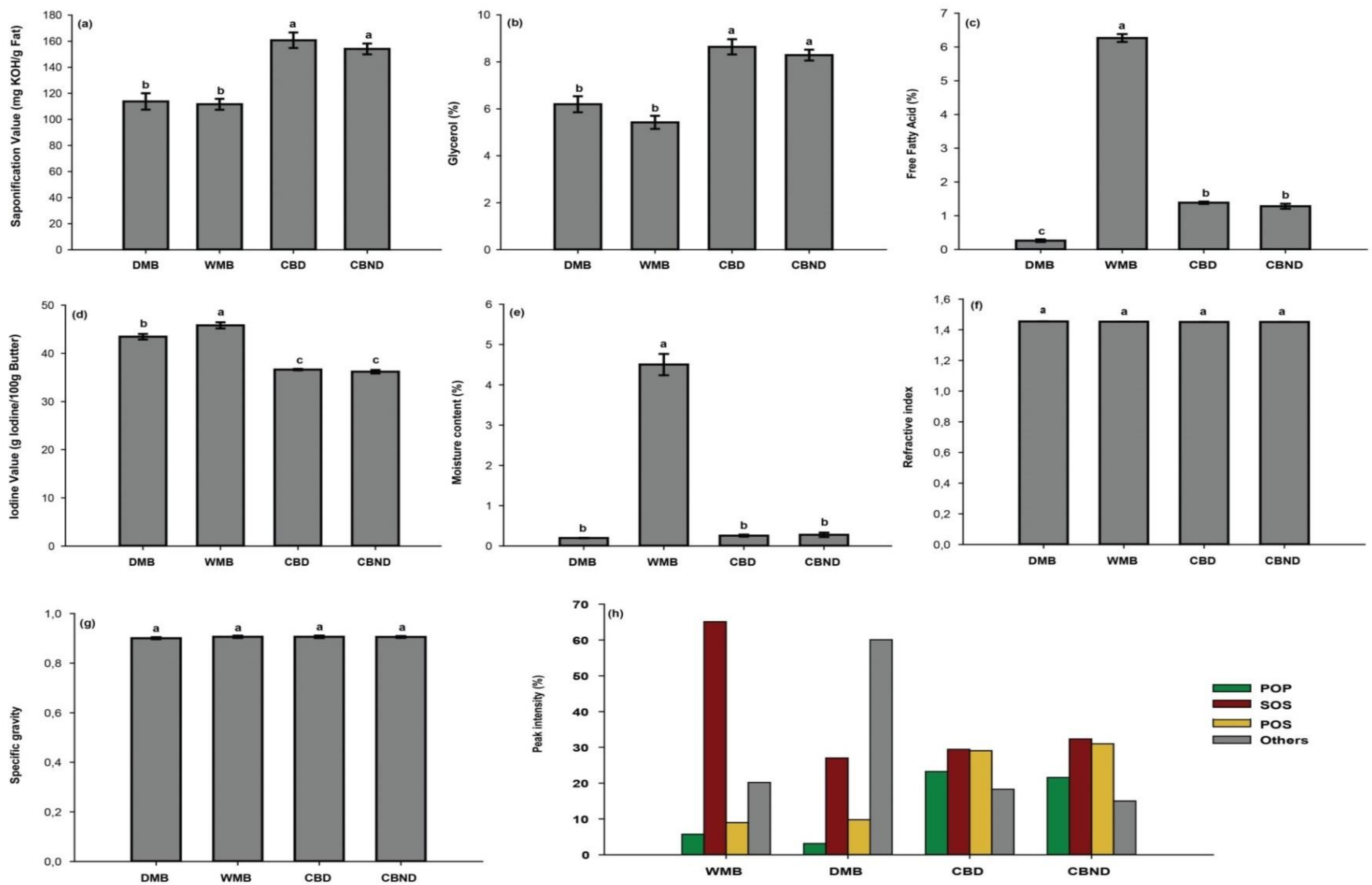


Figure 5.4: Physical properties of butter samples: (a) saponification value; (b) glycerol; (c) free fatty acid; d) iodine value; (e) moisture content; (f) refractive index; (g) specific gravity; (h) major triglyceride percentage. Error bars indicates standard deviation

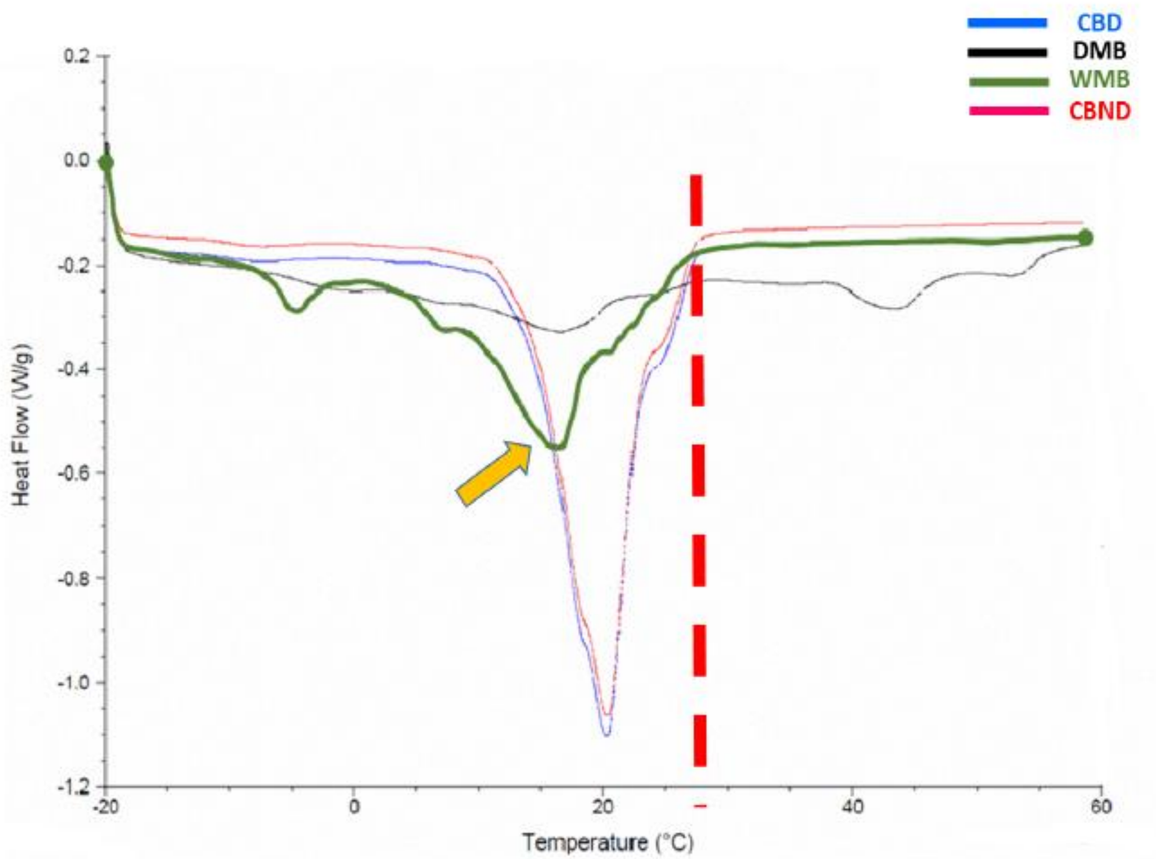


Figure 5.5: Melting profile of different butter using DSC

Table 5.2: Melting characteristics of different butter samples

Sample	T_{onset}**(°C)**	T_{offset}**(°C)**	T_{peak}**(°C)**
Mangifera sylvatica butter	-7.20	25.88	16.18
Mangifera indica butter	-5.36	58.00	16.66
Cocoa butter deodorised	14.64	26.99	20.27
Cocoa butter non-deodorised	14.58	26.99	20.34

5.4 Discussion and conclusion

The majority of studies report palmitic acid, stearic acid, oleic acid and linoleic acid to be the major fatty acid components of CB (20). Minor components of lauric acid (C 12:0), myristic acid (C 14:0), linolenic acid (C 18:3) and arachidic acid (C 20:0) have also been reported (Lipp and Anklam, 1998). The main difference between CB and WMB observed in this study was in the palmitic acid content, 27% and 6%, respectively (Supplementary Table 5.2). Other studies on the triglyceride content of DMB have reported highly variable results; POP (6%–16%), SOS (2%–59%) and POS (1%–74%) and POO, SOO, SOA, OOO (Jahurul et al., 2013; Jahurul et a., 2014a) compared to CB, which has more consistent concentrations of POS (37%–47%), SOS (26%–33%) and POP (16%–23%) (Lipp and Anklam, 1998; Jahurul et al., 2013). TGAs in WMB are similar to CB, where POP, POS and SOS are the major TGAs but with a higher percentage of SOS (65%). WMB contains a slightly lower SFA content compared to CB but the fatty acid profile is comparable (Figure 5.3a and Figure 5.3b). Therefore, it is evident that the fatty acid and triglyceride composition of WMB are close to that of CB derived from *Theobroma cacao,* indicating good prospects for WMB to be used as a cocoa butter alternative.

Key parameters in conferring high fat quality, distinctive flavour and aroma in butters are the saponification and acid values. WMB has a lower saponification value than CB (Jahurul et al., 2014a; Chaiseri and Dimick, 1989), which means that the fatty acids in WMB are significantly longer carbon chain compounds (Figure 5.4a). Such long chain fatty acids (saturated and unsaturated) are prone to oxidation and breakdown, which provides characteristic flavours and aromas. High acid values indicate breakdown of triglycerides into free fatty acids (FFA) relating to inadequate processing and storage conditions. Cocoa butter is reported to have acid values in the range of 0.42% to 3.11% (Chaiseri and Dimick, 1989). The acid value of fat extracted from DMB varies from 1.22% to 7.48% (Jahurul et al., 2014b; Sonwai et al., 2014). Our analyses showed that WMB has a significantly higher acid value compared to CB, which suggests there might be processing or storage problems. With respect to iodine values, the higher the value the more reactive, less stable, and softer the fat and, hence, more susceptible to oxidation and rancidification (Jahurul et al., 2014b). In general, the iodine value for CB was found to be 34–38g I_2/100g (Zaidul et al., 2006; McPherson et al., 2007; Norton et al., 2009) and for DMB 40–75g

I_2/100g (Jahurul et al., 2014b, Sonwai et al., 2014). The iodine value of WMB fat is higher than that of CB. This study, again, suggests that adequate storage will be essential if it is to be used more widely. The moisture content of WMB is higher than CB, which may render WMB more susceptible to microbial attack and oxidation. However, the moisture content is easily managed during the extraction process. On the positive side, there is a growing body of evidence that butters with higher moisture content produce lower-fat chocolate, which may help to prevent obesity, heart diseases, diabetes, stroke and arthritis (McPherson et al., 2007). Indeed, there is ongoing research to produce low-fat chocolate by adding water to the CB (Norton et al., 2009). Manipulation of the extraction process to best manage moisture levels will eliminate the need to add water to the final product. The refractive index and specific gravity of WMB were very similar to CB. It has been suggested that the physicochemical characteristics of WMB can be manipulated through controlled processing, chemical or physical refining and natural blending processes to adjust the properties of WMB to CB (Jahurul et al., 2014c; Jahurul et al., 2015). The melting point is important to determine the storage temperature. The melting temperature of CB is slightly higher than that of WMB, which could be due to of the higher saturated fatty acid content (Figure 5.3a), as previously noted (McPherson et al., 2007). Similar results have been reported for CB by many researchers (Jahurul et al., 2014a; 2014c; Maheshwari and Reddy, 2005; Yamoneka et al., 2015; Solis-Fuentes and Duran-de-Bazua, 2004). So, there were some significant similarities in the physical and thermal properties of WMB compared to CB, which, again, shows the potential of WMB to be used as a cocoa butter alternative.

Chocolate commands an enviable position among food products due to its premium cost, taste and unique physicochemical properties (Lipp and Anklam, 1998). The consumption of chocolate products has significantly increased worldwide (Afoakwa, 2010) whilst 30% of the world's cocoa crops have been destroyed by pests and disease and are deteriorating due to climate change and ageing plantations. Demand is increasing and supply is inadequate as cocoa is cultivated in only a few tropical countries, making its availability unstable, expensive and subject to price fluctuations (Lipp and Anklam, 1998; Supplementary Figure 5.1). Moreover, poor-quality harvests and some technological problems such as fat blooms and high tempering times during chocolate production make it necessary for the food industries to look for alternatives to CB and intensive efforts are ongoing to find suitable cocoa butter alternatives (Afoakwa, 2010). Cocoa butter alternatives are divided into three subgroups (Supplementary Figure 5.2). Cocoa butter replacers (CBRs) are non-lauric fats with a fatty acid profile similar to cocoa butter, but a completely different triglyceride composition (e.g. PEE, SEE). Cocoa butter substitutes (CBSs) are lauric plant fats (containing lauric acid), chemically totally different to cocoa butter (e.g. major TGAs LLL, LLM, LMM), with some physical similarities, suitable only to substitute cocoa butter to 100% and often incompatible with CB (Cooper et al., 1990). Cocoa butter equivalents (CBEs) are non-lauric plant fats, which are relatively similar in their physical and chemical properties (e.g. major TGAs are POP, POS and SOS) to cocoa butter and can be proportionately mixed without affecting the properties of the cocoa butter. CBEs can be either cocoa butter extenders (CBEXs), which are a subgroup of CBEs, not mixable in every ratio with cocoa butter or cocoa butter improvers (CBIs), which have a higher solid triglyceride (SOS) content, used for improving soft cocoa butters (Lipp and Anklam, 1998; Naik and Kumar, 2014). The chocolate and confectionery industries give priority to fats that are rich in palmitic acid or stearic acid and are based on symmetric (POP-rich and SOS-rich) fats. Some research has shown that SOS-rich fat confers a higher solid fat content that inhibits fat blooms and decreases the tempering time (Maheshwari and Reddy, 2005). Therefore, SOS-rich fat could be used as a suitable raw material for the production of temperature-resistant hard butters in tropical countries (Jahurul et al., 2014a) and could also be used to improve the quality of soft cocoa butter (Jahurul et al., 2014a). Generally, lauric acid and hydrogenated fats are used to replace CB; these increase the levels of cholesterol, whereas CBEs contain high oleic and stearic acids, which do not alter the levels of cholesterol in blood. Thus, CBEs represent a healthier and promising alternative to CB. The CBEs used up to now are tropical SOS-rich fat butters from species such as shea (*Vitellaria paradoxa*), kokum (*Garcinia indica*), illipe (*Shorea stenoptera*), mango (*Mangifera indica*) and sal (*Shorea robusta*) butter and usually blended with palm (*Elaeis guineensis*) kernel oil stearin rich in POP (Naik and Kumar, 2014). Palm kernel oil consists of higher amounts of lauric acid, and relatively lower stearic and oleic acid than cocoa butter. The production of CBA from palm oil needs intensive processing (Zaidul et al., 2006). Palm kernel oil is used in preparing CBA as it is a very rich source of POP (51%). Sal butter is green in colour, which limits its use in chocolate and confectionery products (Jahurul et al., 2013). It is feasible to lighten butters but it is a very energy-intensive and costly procedure, so industries prefer light-coloured butters (Council of the European Union, 2000). Kokum butter is grey coloured and mainly used as a CBE by blending with Mahua (*Madhuca longifolia*) and Phulwara (*Madhuca butyracea*) butter (Reddy and Prabhakar, 1994). However, the extraction is only practiced at the cottage scale and has no industrial application as yet (Reddy and Prabhakar, 1994; Vidhate and Singhal, 2013). Shea butter is known to have the highest unsaponifiable fat content (up to 10%) of any natural fat and the highest iodine value of 52–56g Iodine/100g fat and is used as a cocoa butter substitute (Megnanou and Niamke, 2015) in the European chocolate and confectionery industry (Olajide et al., 2000). Illipe and mango butters can be used directly as CBE, and mango butter *(Mangifera indica*) is comparatively as good quality as CBEs, although the melting point (34 ^{o}C–43^{o}C) is quite high (Lipp and Anklam, 1998; Naik and Kumar, 2014). There are, therefore, not enough reliable sources of CBE available from natural fat sources (Lipp and Anklam, 1998). Our study suggests that WMB is a potential high-quality new CBE or improver as the fatty acid and triglyceride composition are very similar to CB, as are the physical parameters.

However, going beyond an industrial utility, wild fruit is an important source of food, medicine and income for forest dwellers, tribal and marginalised rural people (Judprasong et al., 2013). There are many wild fruits available in the forests that are underexploited. Moreover, information on their nutritional value and economic potential are unknown. Adding value to underutilised products through processing for products that have market value could generate a way to conserve those species and help to generate alternative income sources and reduce household poverty (Shackleton et al., 2011). Additionally, collection and processing of these products can reduce household vulnerability to shocks and seasonal variations in other income sources (Ellis, 2000). For example, shea kernels from *Vitellaria paradoxa* are widely exported for use in the international cosmetic and chocolate industries. The annual value of total exports of shea kernels from Africa was estimated at USD30 million in 2004 (Lovett, 2004) and they represent one of Burkina Faso's main export commodities (Pouliot, 2012). Moreover, income from shea kernels has been shown to contribute as much as 12% of total household income for poor households and 7% of total household income for better-off households (Pouliot, 2012). In Bangladesh, there are 47 edible wild forest fruits available (Das, 1987), an important one of which is the wild mango species (*M. sylvatica).* Wild mango is a multipurpose tree species used for purposes including edible fruits, pickles, fodder, fuelwood, vegetables, plywood, tea chests and match boxes (Das, 1987; Das and Mohiuddin, 2012). A close genetic relationship between *M. indica* and *M. sylvatica* has been reported (Das et al., 2014; Mukherjee, 1957), which indicates that *M. sylvatica* may have the potential to fulfil nutritional and livelihood needs. It is underutilised in Bangladesh as well as in other tropical countries due to a lack of awareness of its potential as a source of food and no established market demand (Malik et al., 2010). However, this research confirms that this underutilised wild mango has the potential to be used as a unique cocoa butter alternative.

Bangladesh is one of the most densely populated countries in the world, with 2.14 million hectares of forest area (BBS, 2016). Millions of the poor and forest dwellers earn their livelihoods from the forest (CBD, 2007). Therefore, there is a socio-economic imperative to allow access for these forest-dependent people to the natural resource. However, finding alternative income-generating activities can secure income, improve livelihoods and conserve forest resources sustainably (Negi, 1996). There is enormous potential for the development of a wild-mango-kernel-based enterprise in Bangladesh as well as in other tropical countries for the production of wild mango butter. This will not only provide raw materials for the chocolate and confectionery industries but also offer opportunities to empower forest-dependent people and small-scale farmers. There is, therefore, an urgent need to promote the domestication and commercial plantation of wild mango species to satisfy global and local demand for cocoa butter alternatives. A recent study shows that it can be domesticated and introduced in small-scale forestry programmes (Baul et al., 2016). However, larger-scale plantings will require field trials and an improved knowledge of the species silviculture. The current study may lead to the beginning of a domestication and commercialisation of this wild underutilised fruit species. However, more research on chocolate production using this butter and the silviculture of this species is necessary to fully capture the value of this wild mango species. Additionally, collaboration between foresters, horticulturists, the food industry and policy makers is required to promote the domestication and commercialisation of the *M. sylvatica* fruits of Bangladesh and other tropical countries. Commercial butter production value of this species will benefit food technologists, chocolate industry and government actors to initiate conservation effort.

Supplementary Table 5.1: Fatty acid content of different butters

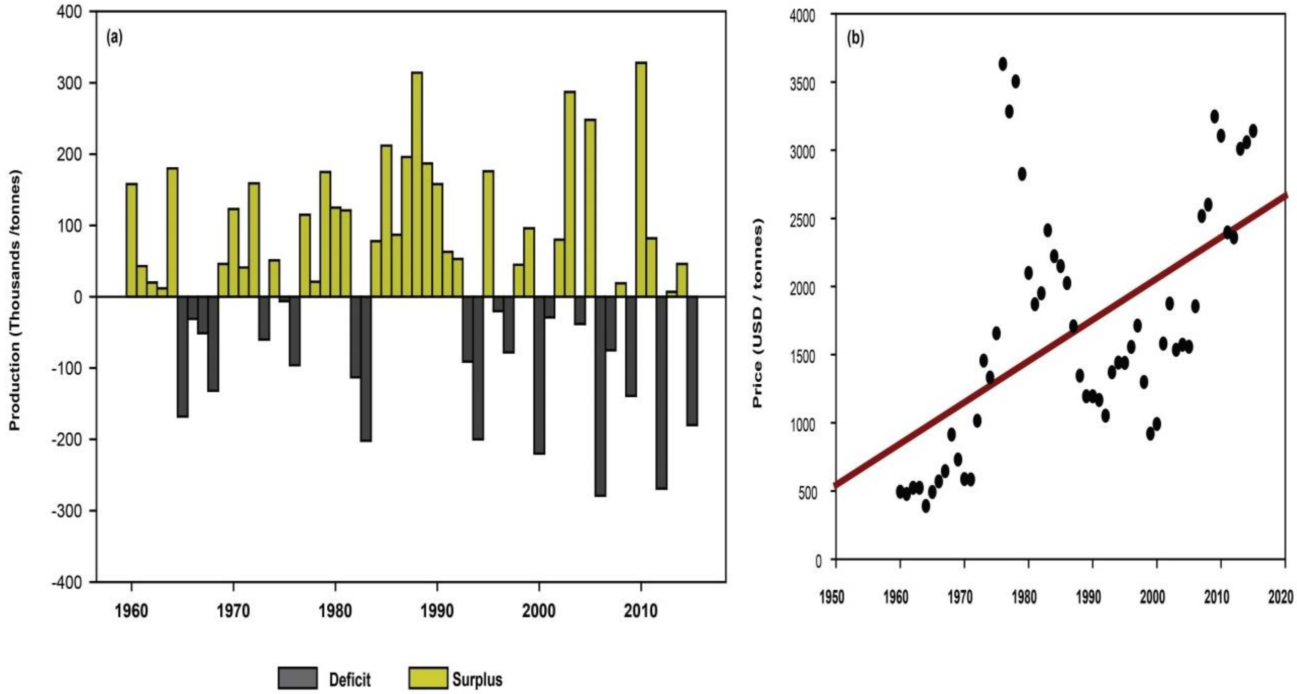
Species	Butter	Saturated fatty acid (%)	Unsaturated fatty acid (%)
M. sylvatica	Wild mango butter (WMB)	56,44[a]	43,56[a]
Mangifera indica	Domesticated mango butter (DMB)	57,51[a]	42,49[a]
Theobroma cacao	Cocoa butter deodorized (CBD)	65,78[b]	34,22[b]
Theobroma cacao	Cocoa butter non-deodorised (CBND)	65,30[b]	34,70[b]

Different letters represent significant differences (P< 0.05)

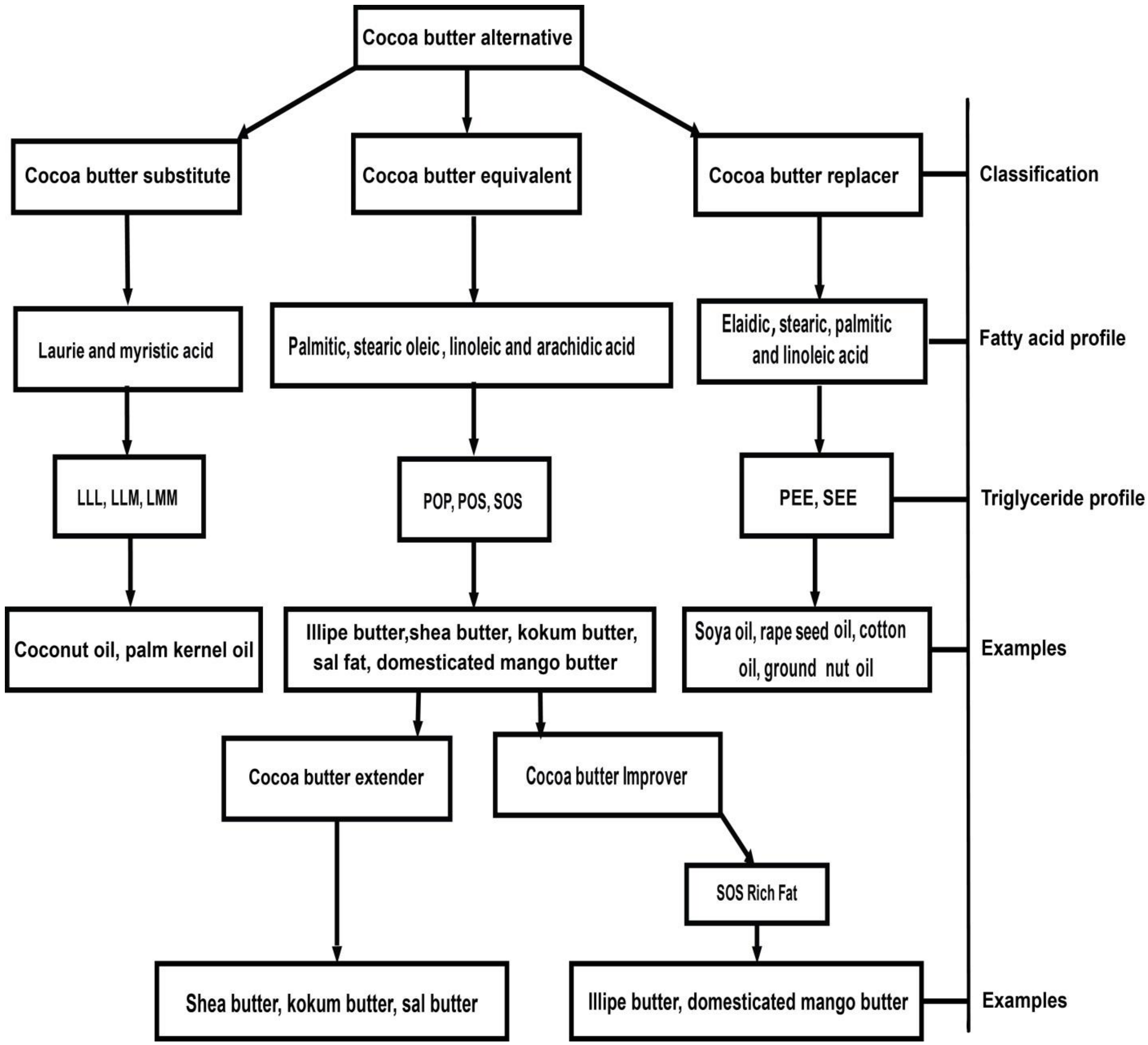
Supplementary Table 5.2: Fatty acid profile of different butters

Butter	Saturated fatty acid (%)			Unsaturated fatty acid (%)	
	Palmitic acid (16:0)	**Stearic acid (18:0)**	**Arachidic acid (20:0)**	**Oleic acid (18:1)**	**Linoleic acid (18:2)**
DMB	12,18[b]	44,53[b]	0,80[c]	38,18[b]	4,31[a]
WMB	5,86[c]	48,23[a]	2,34[a]	41,12[a]	2,45[b]
CBD	27,24[a]	37,53[c]	1,01[b]	32,42[c]	1,80[b]
CBND	26,25[a]	38,02[c]	1,03[b]	32,66[c]	2,04[b]

Different letters represent significant differences (P< 0.05)



Supplementary Figure 5.1: (a) surplus and deficit of cocoa butter production from 1960 to 2015; and (b) cocoa butter price from 1960 to 2015 (ICCO 2016a; ICCO 2016b)



Supplementary Figure 5.2: Cocoa butter alternative classification (adapted and modified from Lipp and Anklam, 1998)

Chapter 6. Agroforestry potential of a wild mango (*M. sylvatica* Roxb.)[4]

Abstract

In Bangladesh, very few native species have been incorporated into agroforestry systems, and commonly observed species are generally exotic pan-tropical species. This neglects the wealth of native genetic resources available, and means that wild, native species are under-valued, which can result in a lack of conservation interest in threatened species The present study seeks to address this by considering the potential for including a locally threatened native species, *Mangifera sylvatica* Roxb. (wild mango) into the agroforestry systems of Bangladesh. We investigated farmers' preferences, and tree architecture and root competitiveness in comparison with other agroforestry species to assess the potential and to identify any barriers to use. According to farmers, *M. sylvatica* is a multipurpose tree species, with the main uses being for timber and provision of wild fruit. In addition, unripe fruits are sold to the pickle industry for seasonal income. The average observed height of *M. sylvatica* is approximately 14m with a straight bole. The crown architecture is similar to other popular agroforestry species (*Mangifera indica, Artocarpus heterophyllus* and *Acacia auriculiformis*). The root competitive index of *M. sylvatica* is also similar to *M. indica* and *A. auriculiformis* but less than *A. heterophyllus.* This indicates that *M. sylvatica* has the potential for introduction to agroforestry practices in a similar manner to *M. indica, A. auriculiformis and A. heterophyllus*. Due to over-exploitation, illegal logging and habitat destruction, *M. sylvatica* is currently threatened in Bangladesh. The major barriers to use include: fruit collection being time consuming and laborious; the ripe fruit quality being not as good as the common mango (*Mangifera indica*); fruits contain small amounts of flesh that often harbour insects; storage problems; irregular fruiting; and limited consumption. Promotion of the species' positive attributes will be necessary to develop its potential as a new native agroforestry tree species, which can provide a valuable conservation initiative that ultimately protects the genetic diversity and quality of this native tree species.

[4]Akhter, S., McDonald, M. A, Jashimuddin, M. 2016. Agroforestry potential of a wild mango (*Mangifera sylvatica* Roxb.). This will be submitted to Journal from Elsevier/Sciencedirect in 2016.

6.1 Introduction

Agroforestry is the integration of agricultural and forestry systems simultaneously on the same unit of land (Lundgren and Raintree, 1983). Agroforestry is increasingly seen as a future land-use strategy due to the potential for income generation, biodiversity conservation and ecosystem regulation, particularly in less-developed countries. One of the main challenges today, particularly in the tropics, is to meet the ever-growing demand for agricultural products while at the same time conserving biodiversity, providing and regulating critical ecosystem services, and maintaining rural livelihoods (Bhagwat et al., 2008). Agricultural expansion has been widely recognised as a major driver of forest and biodiversity loss (Sala et al., 2000). Since deforestation continues in many parts of the tropics, the driver is to find suitable approaches that can reduce deforestation and provide income to rural livelihoods simultaneously with conservation of biodiversity (Bhagwat et al., 2008). Multifunctional agroforestry systems also have the intrinsic potential to provide food, fuel, fodder, green manure, plant-derived medicines and timber resources (Kumar et al., 2012). With the growing realisation that agroforestry is a practical, low-cost alternative for food production as well as environmental protection, the forestry departments of many countries are integrating agroforestry programmes with conventional slivicultural practices (Swaminathan, 1987).

Bangladesh is exceptionally rich in biodiversity (Appanah and Ratnam, 1992). The country hosts a wide range of agroforestry systems that have been managed by local or indigenous communities for decades. However, as is typical to many other tropical countries, the country is now facing the challenge of intensified management of its traditional agroforestry management systems by market forces, rapid economic development, the need for more food and other products necessary for sustaining the livelihoods of a growing population. To make traditional agroforestry systems more productive and sustainable, the choice of species and development of new varieties are crucial. In Bangladesh, the major timber tree species used in agroforestry are four exotic species, namely, mahogany (*Swietenia macrophylla*), akasmoni (*Acacia auriculiformis*), mengium (*Acacia mengium)* and minjiri (*Senna siamea*) (Ahmed et al., 2013). These trees are favoured for their rapid growth rates, timber value and potential income generation. There are five major fruit tree species used in agroforestry, which are all exotic, namely, mango (*Mangifera indica*), jackfruit (*Artocarpus heterophyllus*), papaya (*Carica papaya*), litchi (*Litchi chinensis*) and guava (*Psidium guajava*) (Ahmed et al., 2013). This scenario indicates that the agroforestry systems of Bangladesh are heavily dependent on exotic species and farmers have less species choice to improve the diversity of their agroforestry systems. The invasion of habitats by non-native species has become a global concern with serious consequences for ecological, economic and social systems (Holzmueller and Jose, 2011; Kohli et al., 2008). It has been estimated that up to 80% of endangered species worldwide (with the possible exception of the USA) are threatened due to pressures from non-native species (Pimentel, 2005). According to the Encyclopedia of Flora and Fauna of Bangladesh (2007–2009; Volumes 5–12), 36 pteridophyte species (all vulnerable; 18.46% of 195 recorded species), one gymnosperm species (endangered; 14.29% of seven species), and 449 angiosperm species (30 critically endangered, 126 endangered, 293 vulnerable; 12.43% of 3,611 recorded species) are threatened in the country (Haseeb, 2011). According to Hossain and Pasha (2001), more than 300 exotic species have been introduced to date. Herbaceous and lianas are the dominant groups, followed by trees and shrubs (Hossain and Pasha, 2001; Uddin et al., 2013). All of these exotics are already naturalised (Hossain and Pasha, 2001). Several of these exotic plant species have significant detrimental effects on Bangladeshi forest ecosystems such as slow degradation of leaves; altered nutrient cycling; inedible fruits creating food shortages for wildlife; pollen grain allelopathy; and suppression of native species by their luxurious growth (Uddin, 2011). The controversy surrounding non-native species and their effects on native flora, fauna and the environment has refocused attention on native species in many parts of the world (Jose, 2011). Therefore, the present study considers the potential of including a new native species in agroforestry systems.

Mangifera sylvatica Roxb. is a wild fruit tree species native to Bangladesh, India, Myanmar, Nepal, Thailand, China and Cambodia, belonging to the Anacardiaceae family. There are 40 species of *Mangifera* in this family, 26 of which produce edible fruits, but only a few have been domesticated (*Mangifera indica*, *Mangifera caesia*, *Mangifera foetida*, and *Mangifera odorata*) and only one commercially cultivated (*Mangifera indica*) in the world (Ariffin et al., 2015;

Mukherjee and Litz, 2009). *M. sylvatica* also produces edible fruits and is genetically one of the closest to *M. indica* (Mukherjee, 1950, 1957; Nishiyama et al., 2006), and also looks very similar (Das and Alam, 2001; Das, 1987). It grows in the hill forests of Bangladesh but is unfortunately considered threatened in the country (BFRI, 2013; Khan et al., 2001), despite being valued for its timber, fruit and medicinal properties (Baul et al., 2016). The kernel of *M. sylvatica* can be used as a cocoa butter alternative (Akhter et al., 2016). A recent study by Baul et al. (2016) reported that the seed germination rate and the early growth of seedlings are fast and that the species can be easily domesticated and incorporated into small-scale plantation programmes. With the increasing demand for fruit, timber and fuelwood, wild mango could be a potential native tree species to fill a market gap and the needs of farmers. Insufficient knowledge of its present status, uses and compatibility with other agroforestry tree species indicates that research is needed for the domestication and introduction of this species in agroforestry practices. The overall objective of this study is to identify possible barriers to use, and the potential for domestication of this species as a first step in the improvement process.

6.2 Methodology

6.2.1 Study area

The study was conducted at three sites:

1. Ukhia Natural Forest Range in Cox's Bazar South Forest Division. The total area of Ukhia forest range is 8611ha. Geographically, the area lies between 21°268´ North latitude to 92°569´ East longitude. The average maximum temperature is 34.2°C and the minimum temperature is 15.6°C. This area has low hills of less than 100m elevation from the mean sea level (Akhter et al., 2009). Approximately 10% of the landscape of the area is occupied with well-drained flood plains. The climate is mainly monsoon. The total annual average rainfall is 3,595mm. The average relative humidity is over 90% during July to August, whereas in December it decreases to approximately 74% (BBS/UNDP 2005).

2. Padua Forest Range in Chittagong South Forest Division (Figure 6.1). For the present study, Tankawati reserve forest under Chittagong South Forest Division was included, which is 66km

away from Chittagong city. It lies between 21°57′ to 22° 9′ North latitude and 92°7′ to 92°02′ East longitude. The total area of Tankawati Forest Reserve is 1124ha (Motaleb and Hossain, 2007). The study area has a moist tropical climate with high rainfall from June to September (Alamgir and Al-amin, 2008). The relative humidity remains high (70% to 85%) with only minor variation throughout the year. Temperature also remains high, with only small seasonal differences. The mean minimum and maximum temperatures are 21.97°C and 30.51°C, respectively (Alamgir and Al-amin, 2008). The elevation of the study area ranges between 14m and 87m above mean sea level.

3. Rema-Kalenga Wildlife Sanctuary (RKWS) is located approximately 130km East-Northeast of Dhaka and 80km South-Southeast of Sylhet in Chunarughat Thana, a sub-district of Habiganj District of Sylhet Division. Geographically, the area lies between 24°06' to 24°14' North latitude and 91°34' to 91°41' East longitude (Uddin et al., 2002). The area falls under the Sylhet Hills zones. In 1982, the government designated 1,795ha of the reserve forest as the Rema-Kalenga Wildlife Sanctuary. The sanctuary encompasses several hills of different elevations and the low-lying valleys. The highest peak of the hills is approximately 67m from sea level (Rizvi, 1970). The average temperature in winter is 9.6°C (January) and in summer 34.8°C (April). The area covered under the WS is one of the wettest in the country and so the rainfall is quite high with an approximate annual average of 4,000mm, with maximum rainfall falling during June to September from South-West monsoon. The humidity is high in the wildlife sanctuary throughout the year, with monthly average humidity varying from 74% in March to 89% in July.

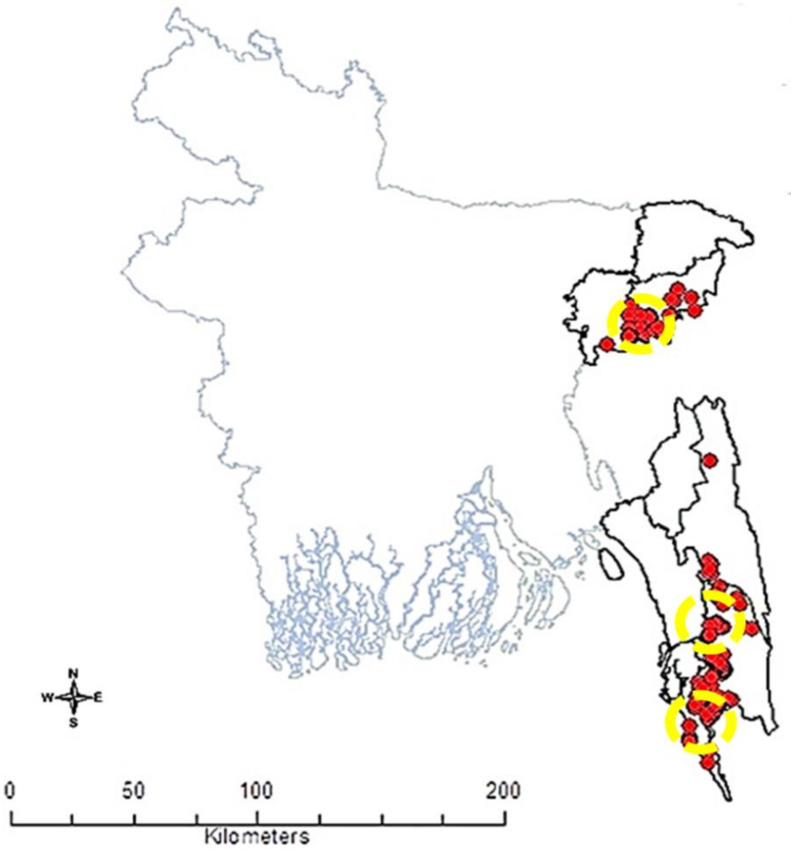


Figure 6.1: Distribution of *M. sylvatica* in Bangladesh (red dots) and study locations (yellow circles)

6.2.2 Barriers of conservation of wild mango

To identify the barriers to the conservation of wild mango and for the development of the conservation recommendations, we used the DPSIR framework (Song and Frostell, 2012; Kelble et al., 2013; Pîrvu and Petrovici, 2013). Drivers (D) are human demand and lifestyle such as changes in social, economic or cultural aspects that may generate environmental effects. Pressures (P) refer to the effect of human activities affecting the species under investigation. State change (S) refers to the changes in physical and biological conditions resulting from pressures. Impact (I) is defined as the impact caused by the physical and biological changes on human well-being. Responses (R) are interventions to minimise the negative effects of an impact. For the application of the DPSIR, we combined knowledge from different local experts through participatory approaches. For the participatory exercise, five to seven local experts were consulted. They were selected based on: a) their familiarity with the area and the species, and b) an adequate knowledge of the forest area. Selected experts included: local forest department officials, NGO officials, nursery staff and local people living near the forest areas. Two focus group discussions (FGDs) were arranged. During the discussions, both the local experts and the authors participated in the discussion. Before starting the participatory discussion, a briefing was given about the DPSIR framework in relation to the wild mango conservation. In order for a consensus to be reached among the participants, they were asked to evidence and justify their observations. A consensus was reached among the participants when supported by the highest number of participants.

6.2.3 Potential for conservation of wild mango

A reconnaissance survey and transect walk was carried out in the study areas to determine the specific localities where the species was abundant. In addition, a total of 30 key informant interviews were conducted through semi-structured questionnaires. Key informants were selected with consideration of their knowledge about the species. Various aspects were surveyed including uses (food, fodder, wood, medicine); attractive features (colour, taste, aroma, size); frequency of fruit consumption (once, twice, three times, more than three times during the fruiting season); fruit quantity consumed (1–5kg, 5–10kg, 10–20kg and >20kg) seasonally; seasonal income (Taka, Bangladeshi currency) of the fruit sellers (< 500, 500–1,000, 1,000–1,500, >1,500); and fruit yield. A sensory analysis was conducted as a proxy for fruit quality. Fruit samples were evaluated for other quality attributes in terms of colour, odour/smell, taste, texture and overall acceptability. A typical taste panel consisted of 15 panellists. A modified five-point hedonic scale was used to evaluate the samples, where: 1 = dislike a lot, 2 = dislike, 3 = neither like nor dislike, 4 = like and 5 = like a lot (Russell and John, 1987).

6.2.4 Suitability of *M. sylvatica* as an agroforestry tree species in Bangladesh

To determine the suitability of *M. sylvatica* as an agroforestry species, farmers' perceptions, transect walks and ecological surveys were conducted at the study sites. From the species observed, three alternative prevalent agroforestry tree species (based on abundance and economic importance in agroforestry) were considered as a comparison for agroforestry suitability. For each species (except *M. sylvatica)*, fifteen individual trees were selected and the parameters recorded included diameter at breast height (DBH), tree height, crown architecture, light transmission and light quality under the canopy. In the case of *M. sylvatica*, 21 trees were selected. Trees were sampled randomly. Additionally, farmers were asked about their knowledge of the fruiting season, fruiting duration, storage possibility and harvesting patterns.

6.2.4.1 Crown architecture

The assessment involved measurement of tree DBH, total height, crown length (Cl), crown diameter (Cd), crown ratio (Cr) and crown shape ratio (Csr) for each species. Crown length (Cl) was calculated as Cl = total tree height - height to lowest live branch; Cr = total tree height - lowest live branch height divided by total tree height (i.e. Cr = Cl/total height), and Csr = Cl/Cd (Foli et al. 1999). Tree DBH was measured using a diameter tape and height measured with a relascope. The diameter of the crown in two directions was measured with a metre tape, projecting the crown onto the ground, and the average of the two measurements was calculated for each tree.

6.2.4.2 Root structure

Though the standard method of the root shallowness study needs field calibration, for the preliminary study reported here, root structure analysis was based on seedlings from the nursery because of difficulties in finding target seedlings of known age from the forest floor or plantation. A comparison was made with similar-aged seedlings of wild mango (*M. sylvatica*), domesticated mango (*M. indica*), jackfruit (*A. heterophyllus)* and akashmoni (*A. auriculiformis*) raised in the same nursery of Chittagong University, Bangladesh. Five seedlings per species were collected and measured in 2014. Seedlings were one year old. The basal part of the roots at the stem base of each selected tree was carefully cleaned and washed slowly with water before analysis. Stem diameter D_{stem} was measured as the root collar diameter and used to estimate the stem basal area. Root diameter was measured with a digital calliper for both the vertical and horizontal root and the basal area for the horizontal and vertical root was calculated. These measurements were subsequently used to calculate an index for root shallowness/root competitiveness, as the ratio of the basal area of all horizontally orientated roots and the stem (Das and Chaturvedi, 2008).

6.2.4.3 Light quality and quantity

Light transmission was measured as photosynthetically active radiation (PAR) through the canopy and the quality of light (Red: Far-Red) under the canopy was measured. PAR was measured using a handheld ceptometer, (AccuPAR PAR-80 ceptometer) while the Red: Far Red (R:FR) radiation was measured with a handheld SKR 110/100 660/730 measuring system (Skye Instruments Ltd.). Three trees of the same species were used for this analysis. Data was collected over six days/tree from 7.00am to 17.00pm. On each occasion, PAR and R/FR readings were taken in the open and recorded as above-canopy PAR and R/FR, respectively. Readings beneath the canopy of each species were then taken and recorded as below-canopy PAR and R/FR. On each recording occasion for each species, eight to twelve above-canopy and twelve to eighteen below-canopy readings were taken at different locations outside and beneath the canopy of each tree species and the average was computed.

6.2.5 Data analyses

Data collected through interview is represented as frequency of response. Crown and root architecture, PAR and Red: Far-Red data are presented as mean values ± SE together with significance testing using one-way ANOVA (Minitab 17).

6.3 Results

6.3.1 Barriers to conservation of *M. sylvatica*

Respondents gave their opinions regarding conservation barriers (Figure 6.2). The main factors responsible for decreasing *M. sylvatica* in the hilly areas of Bangladesh are over-population, increased demand for wood, lack of awareness about the species potential, lack of interest about the species and lack of policy implementation on conservation of native species. In addition, the implementation of environmental management and conservation activities has largely failed due to poverty, corruption and political issues. A number of crucial response elements are lacking, including clear and consistent forest policy, motivation and education of local people, marketing facilities and financial incentives. Major pressures creating barriers to conservation of wild mango in Bangladesh are over-exploitation, illegal logging, clear felling, slash-and-burn agriculture, fuelwood collection and land clearance for farming or other development activities. The impacts are shortage of seasonal fruit, shortage of raw material for the plywood industry, shortage of sawn timber, shortage of fuelwood/charcoal and shortage of fodder. Plywood industries have sourced alternative raw material from *M. sylvatica* and as a result these species can also become threatened due to over-extraction (e.g. *Swintonia floribunda* Griff.) (Hossain et al., 2012; Akhtaruzzaman, 2012; Chowdhury, 1996). However, for the conservation of *M. sylvatica*, a few initiatives have been taken by government and non-governmental organisations, though among them the major conservation activities were seed collection, raising seedlings in nurseries and a few plantation programmes initiated recently.

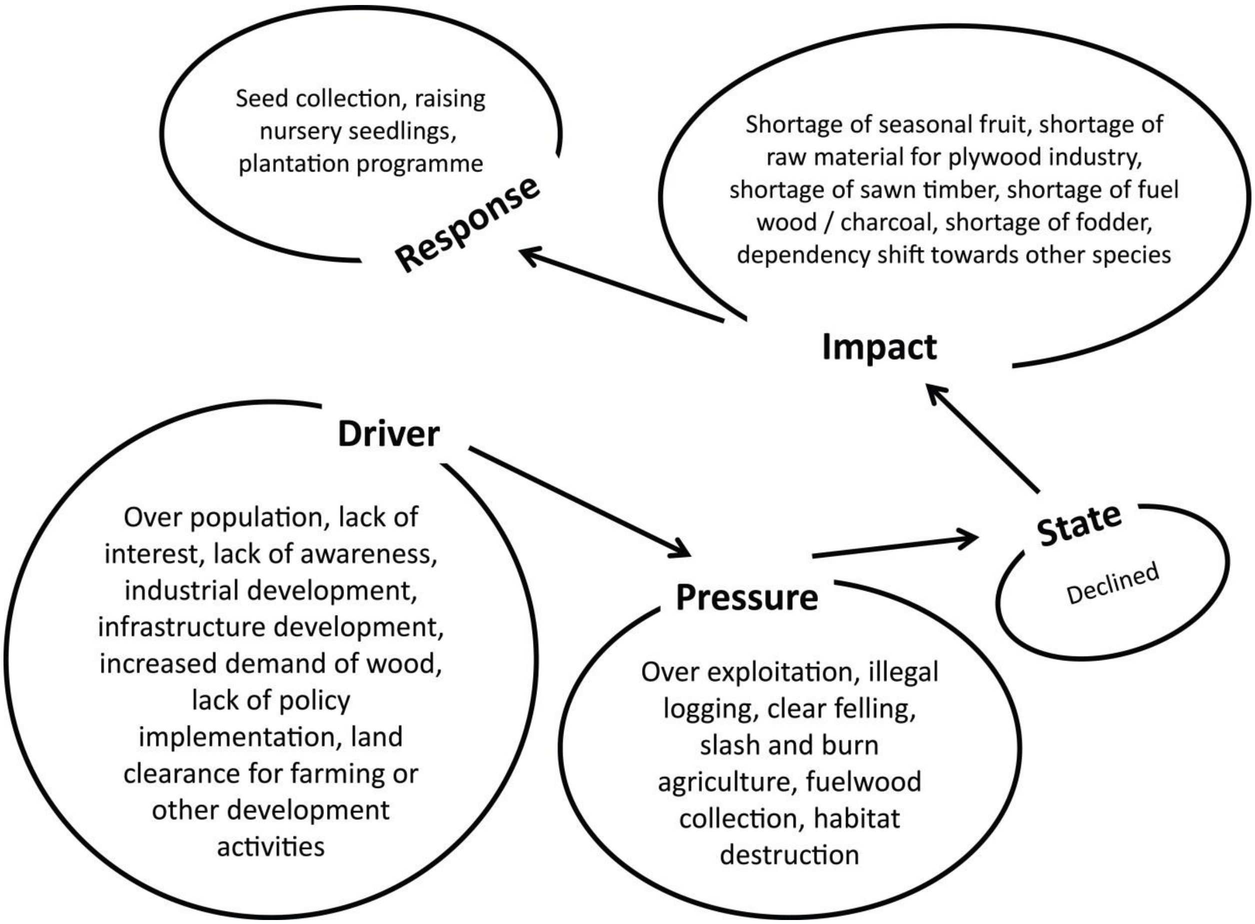


Figure 6.2: Barriers to conservation of *M. sylvatica*

The respondents indicated several concerns related to marketing and domestication (Figure 6.3). Collection of wild mango is laborious and time consuming as it is quite scarce. Moreover, the fruit is not very well known but they sell it together with *M. indica* to pickle companies. The fruit has a pleasant aroma when it is raw but the ripe fruit contains only a small portion of pulp that often has insects inside and is not appreciated by customers. Only small consumer groups (e.g. tourists) buy the ripe fruits as the taste, colour and smell of the fruit is very attractive. Prolonged storage of fruits is difficult and, according to the respondents, it can be stored for only up to two weeks, even with a cooling system. Most of the respondents did not show much enthusiasm for planting the tree in home gardens as trees can grow tall in size and the respondents were anxious that the trees might break during a storm. Moreover, *M. sylvatica* gives fruit from the age of 7 or 8 years, while the *M. indica* tree gives fruits after only three years from planting. Lack of space, low seedling survival rate and lack of planting materials were also given as reasons that discourage farmers from planting *M. sylvatica* in their home gardens (Figure 6.3).

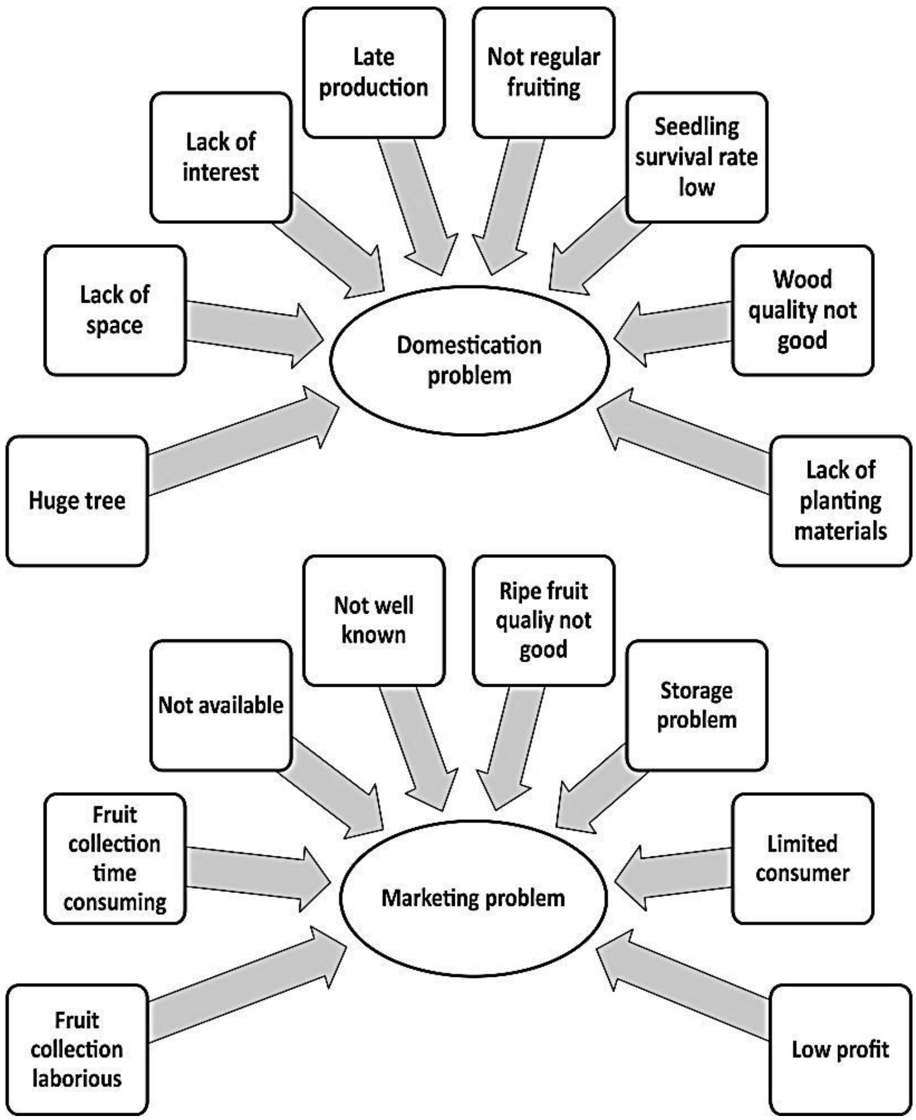


Figure 6.3: Domestication and marketing barriers of *M. Sylvatica*

6.3.2 Potential of *M. sylvatica*

The respondents believed that people consume *M. sylvatica* either for its taste (86%) or just as a source of fruit (62%). Other attractive features of this wild mango are colour (79%), aroma (62%) and sweetness (51%). However, the respondents also mentioned that wild mango is not consumed on a regular basis as it is considered as only food for wild animals (75%). Moreover, the fruit is not readily available (72%) and the pulp often contains insects (65%). The farmers mentioned that *M. sylvatica* is a multipurpose tree species (Table 6.1) used as timber (93%), fuelwood (80%), plywood (50%), fresh fruit and vegetables, fodder, indigenous medicine and pesticide (Figure 6.4). The timber is used for furniture, house ceilings, rickshaw bodies, bus and truck bodies, wooden utensils, musical instruments and toys. The branches and dried leaves are used as fuelwood. In addition, it is also used in the plywood industry for making matchboxes and tea chests. The respondents mentioned that the fruits are either consumed fresh or used to prepare pickle and chutney. Moreover, the fruits are also cooked with fish and lentils as a vegetable. *M. sylvatica* is seen as a preferred food for wild animals, especially monkeys, hoolock gibbons, birds and squirrels. It is also used as a traditional medicine (Figure 6.4). The medicinal properties are not well known, but a few respondents mentioned that the kernel is used for diarrhoea control (40%), dandruff (16%) and as a body fat reducer (10%). A few respondents also mentioned that the bark is used as a pesticide in the paddy fields (10%) since the bark has a smell that deters insects. More than 50% of the respondents consume and sell *M. sylvatica*. The fruiting season is from April to May with a fruiting period of about two to three weeks (Figure 6.5). Trees do not produce fruits every year but when there is fruiting the yield can be more than 30kg. More than 55% of the respondents consume between 1kg and 10kg of fruit per season and collection is predominantly done once or twice per season. The price is not fixed but more than 60% of respondents mentioned that the price is higher than 50 taka per kg and the seasonal income of the farmer from the sale of the fruit can be around 500-1,000 taka (Figure 6.5).

Table 6.1: Farmers' perceptions of the uses of *M. sylvatica* and other agroforestry tree species

Uses	*M. indica*	*M. sylvatica*	*A. heterophyllus*	*A. auriculiformis*
Fruits/Nuts	√	√	√	×
Vegetables	√	√	√	×
Condiments	√	×	×	×
Timber	√	√	√	√
Fuelwood	√	√	√	√
Fodder	√	√	√	×
Plywood	×	√	×	×
Furniture	√	√	√	√
Cosmetics	×	×	×	×
Medicine	√	√	√	×
Organic manure	√	√	√	×
Building materials	√	√	√	√

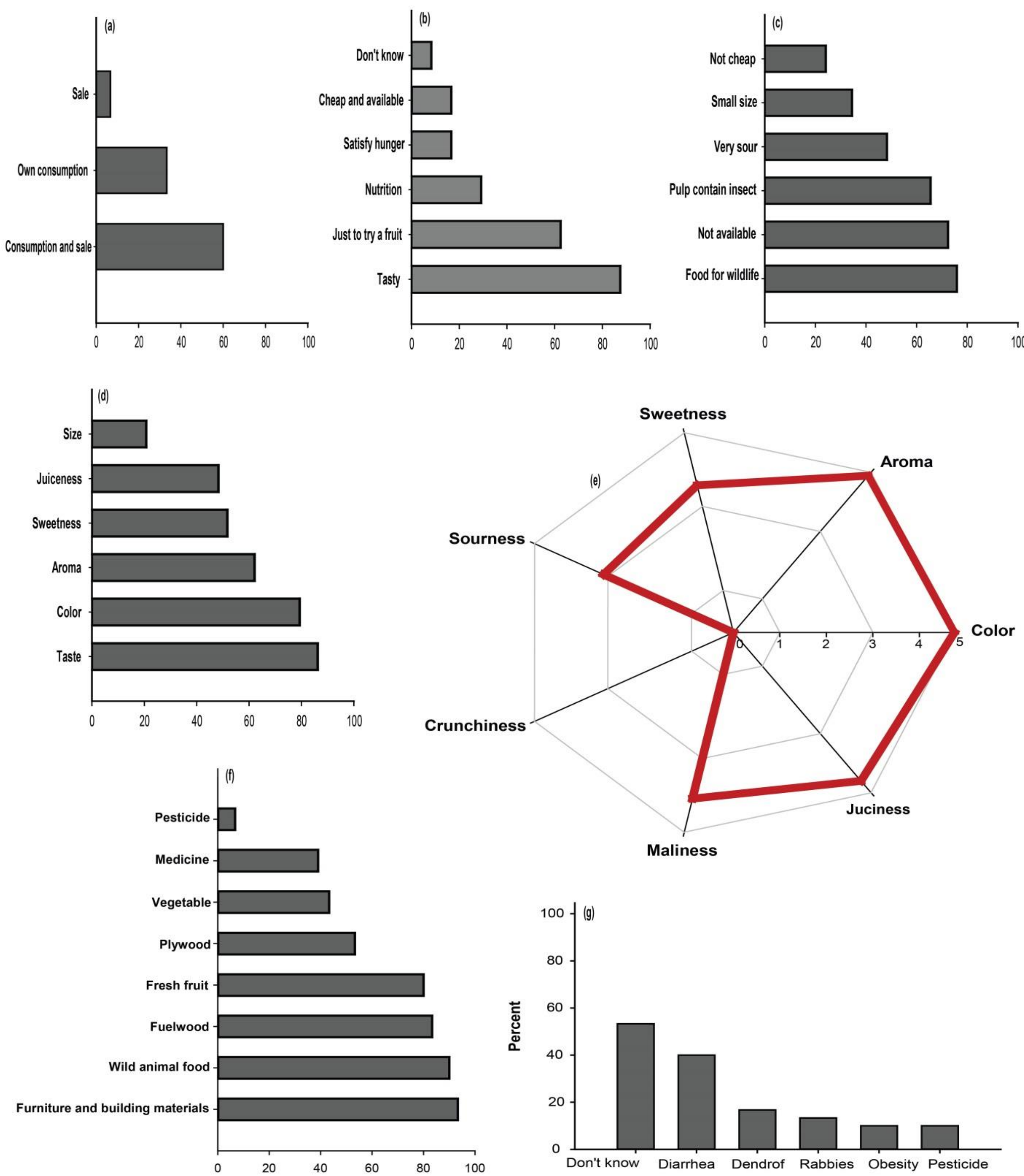


Figure 6.4: Farmers' perceptions of and preferences for *M. sylvatica* fruit: (a) purpose of consumption; (b) why consumed; (c) why not consumed; (d) attractive features; (e) sensory evaluation; (f) use; (g) traditional medicine

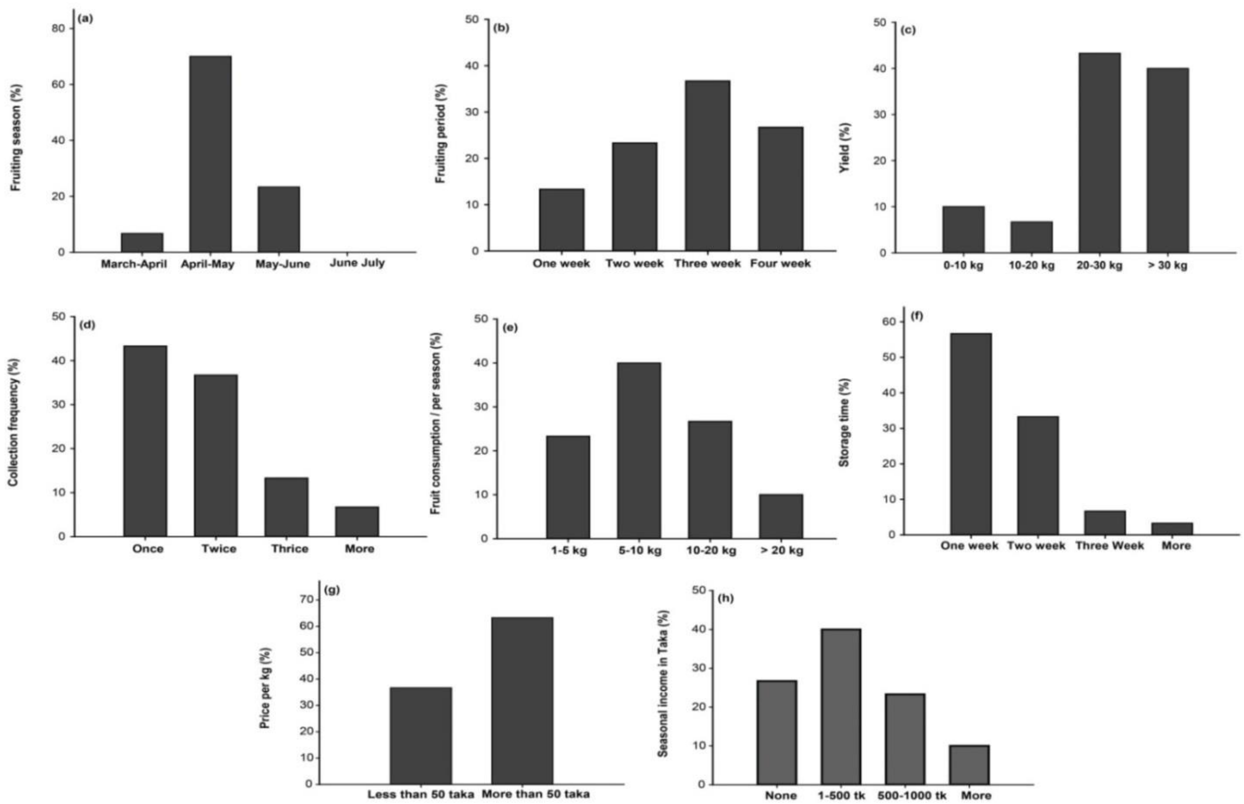


Figure 6.5: Farmers' preferences for and perceptions of *M. sylvatica* fruit: (a) fruiting season; (b) fruiting period; (c) yield; (d) collection frequency; (e) fruit consumption per season; (f) storage time; (g) fruit price per kg; (h) seasonal income

6.3.3 Crown architecture, light transmission and root structure

Mean tree heights, bole height, DBH, crown length, crown ratio, crown shape ratio and fruiting patterns of *M. sylvatica, M. indica, A. heterophyllus and A. auriculiformis* are shown in Figure (6.6). *M. sylvatica* is a comparatively tall tree with an average tree height of 14.14±3.87m, similar to *A. auriculiformis* (12.22±1.69m) and somewhat different from *M. indica* (9.71±3.14m) and *A. heterophyllus* (10.84±2.02m). *M. sylvatica*'s DBH, crown length and crown shape ratio are similar to *M. indica* and *A. heterophyllus*. The bole height of *M. sylvatica* (8.62±2.93m) is higher than *M. Indica* (2.79m), *A. heterophyllus* (5.59m) and *A. auriculiformis* (1.56m). *M. sylvatica* crown width is similar to all the other observed species. The crown ratio of *M. sylvatica* is similar to *A. heterophyllus* but not to *M. indica* or *A. auriculiformis*. PAR and R/FR recorded for all the observed species was highest below the canopy between 11.00am and 15.00pm (Figure 6.7). Highest PAR was recorded at 752.89±27.18µmol $m^{-2}s^{-1}$ between 13.00pm and 15.00pm for *M. sylvatica* during sunny days. At the other end, the lowest PAR was recorded at 453.94±101.96µmol $m^{2}s^{-1}$ between 15.00pm and 17.00pm for *M. sylvatica* during rainy days (Table 6.2). The highest R/FR was recorded for all species from 7.00am to 9.00am and the lowest and R/FR was recorded from 13.00pm to 15.00pm (Figure 6.7). There is no significant difference in light quality and quantity between *M. sylvatica*, *M. indica*, *A. heterophyllus* and *A. auriculiformis* (Table 6.2). The seedling height of *M. sylvatica* is similar to *M. indica* but different from *A. heterophyllus and A. auriculiformis*. Seedling diameter is significantly similar to *M. indica* and *A. heterophyllus* and different from *A. auriculiformis*. The number of side roots of *M. sylvatica* is similar to the other observed species (Figure 6.8). *M. sylvatica* has the second highest number of horizontally orientated roots among all four species studied, with a mean horizontal root basal area of 11.82±3.43cm^2, while *A. heterophyllus* has the least number of horizontal roots, with a mean basal area of 4.79±1.00cm^2 and a greater proportion of its roots (mean basal area of 2.91±0.29cm^2) orientated in the vertical plane. The root architecture of *M. sylvatica* is similar to *M. indica*, *A. heterophyllus* and *A. auriculiformis*. Rooting depth orientation of *M. sylvatica* is similar to that of *M. indica* and *A. auriculiformis* with more horizontally orientated roots than vertically orientated roots. The values obtained for index of tree root shallowness (CI) ranged from 1.21±0.21 to 5.70±0.67, with the highest value for *M. indica* and the lowest value for *A. heterophyllus* (Table 6.3). *M. indica* and *A. auriculiformis* trees have more shallow lateral roots with relatively higher CI values, while *A. heterophyllus* has fewer shallow lateral roots and more vertically orientated roots, with relatively smaller CIs. The CI obtained for *M. sylvatica* was 3.00±0.69. Based on CI, this preliminary study suggests that *M. sylvatica* can be planted together with *A. heterophyllus* in the agroforestry system. However, no significant difference was found in the root shallowness of *M. sylvatica, M. indica* and *A. auriculiformis* (Figure 6.8 and Table 6.3).

Table 6.2: Light quality and quantity below canopy and above canopy for different species

Species	Photosynthetically active radiation (µmol $m^{-2}s^{-1}$)		
	Average	Highest	Lowest
M. sylvatica	596.56±57.51[a]	752.89 ±27.18	453.94±101.96
M. indica	550.56±232.4[a]	794.67 ±75.72	398.45±204.14
A. heterophyllus	607.97±115.51[a]	771.94 ±21.85	405.76 ±83.77
A. auriculiformis	560.86 ±97.03[a]	747.79 ±25.06	355.89 ±41.41
Species	**Red/Far-red light ratio**		
	Average	**Highest**	**Lowest**
M. sylvatica	1.11±0.00[a]	1.13±0.00	1.09±0.02
M. indica	1.11±0.01[a]	1.13±0.02	1.09±0.01
A. heterophyllus	1.11±0.01[a]	1.13±0.01	1.08±0.02
A. auriculiformis	1.11±0.01[a]	1.14±0.05	1.10±0.01

Table 6.3: Root structure of different species

Species	Stem basal area (cm^2)	Vertical root (cm^2)	Horizontal root (cm2)	Root shallowness
M. sylvatica	3.80±0.48	3.420.46	11.82±3.43	3.00±0.69
M. indica	2.70±0.36	2.27±0.33	15.15±2.50	5.70±0.67
A. heterophyllus	3.95±0.35	2.91±0.29	4.79±1.00	1.21±0.21
A. auriculiformis	1.99±0.07	1.47±0.06	10.65±0.89	5.40±0.57

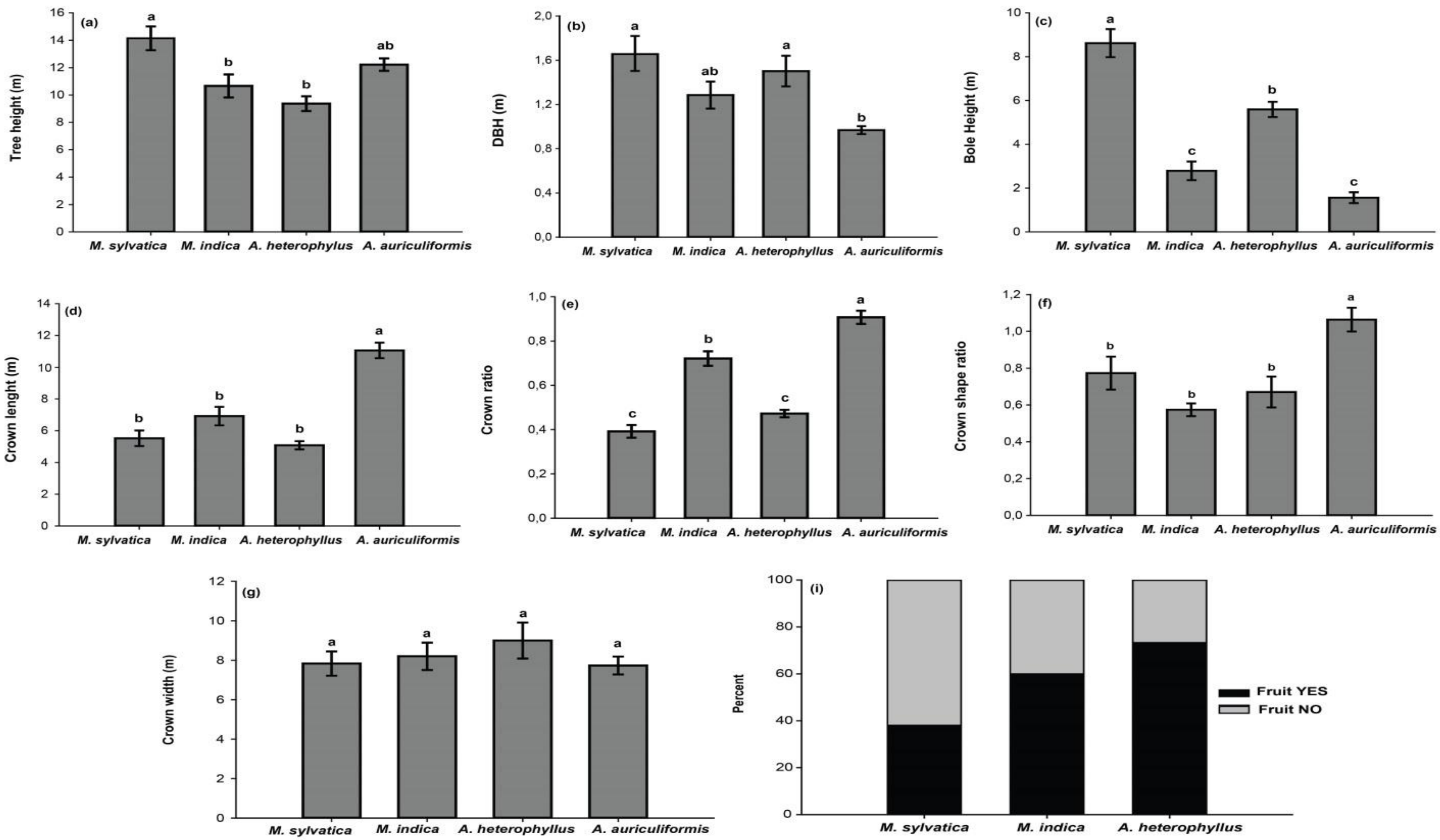


Figure 6.6: Crown architecture of *M. sylvatica* and comparison with other popular agroforestry species: (a) tree height; (b) DBH; (c) bole height; (d) crown length; (e) crown ratio; (f) crown shape ratio; (g) crown width; (h) fruit availability

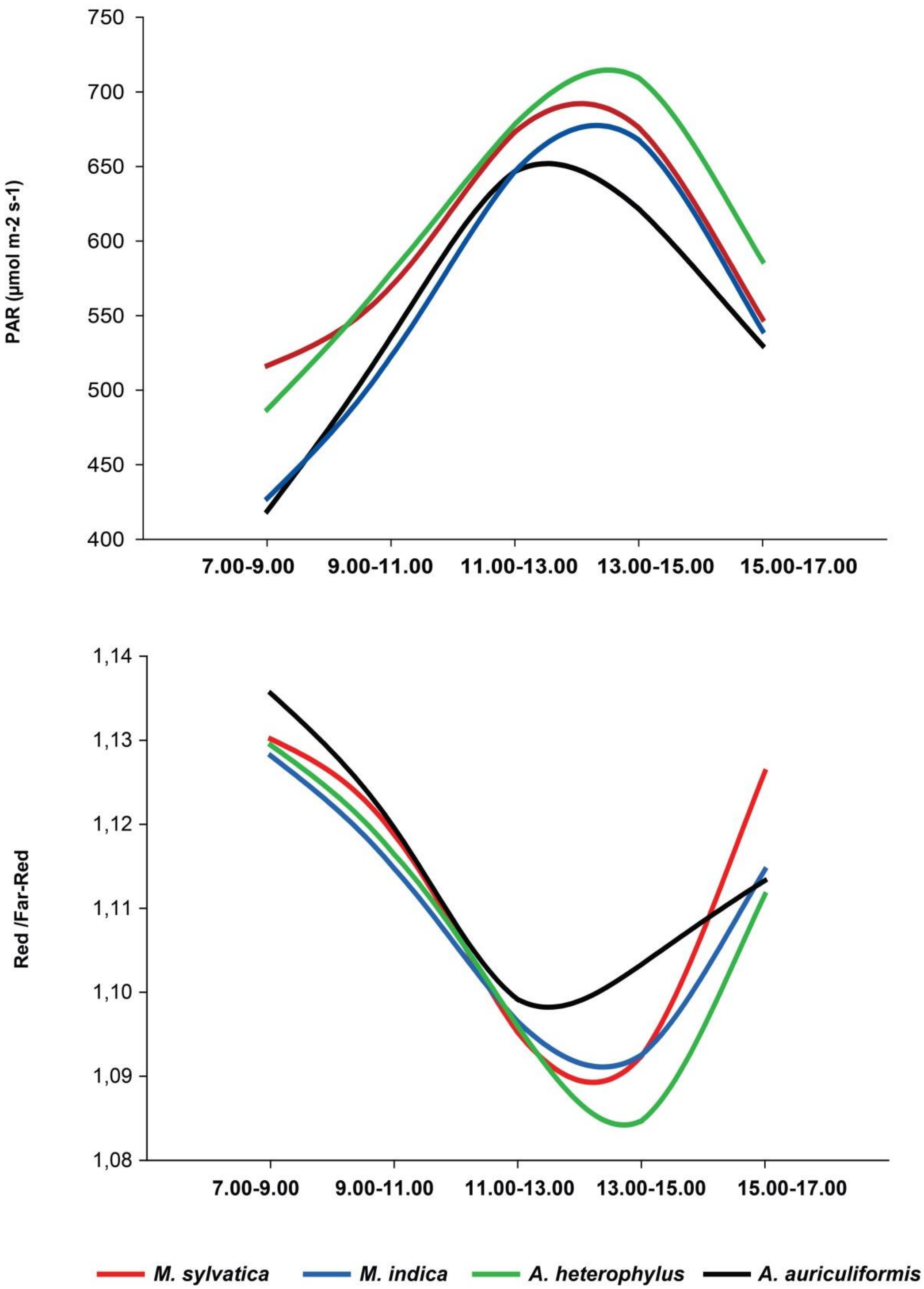


Figure 6.7: Light quantity (a) and quality (b) under *M. sylvatica* canopies in comparison with other popular agroforestry species

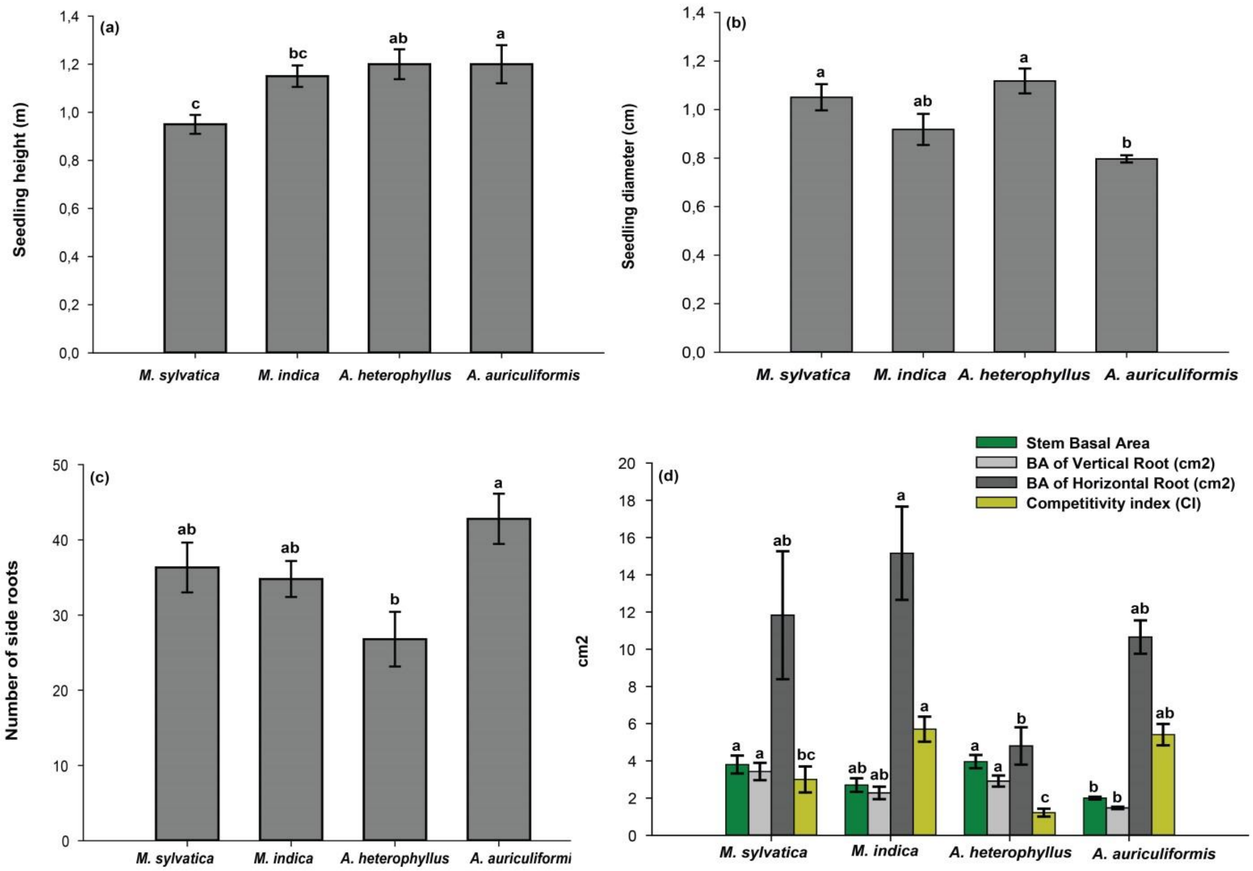


Figure 6.8: Root architecture of *M. sylvatica* nursery seedling and comparison with nursery seedlings of other popular species: (a) seedling height; (b) seeding diameter; (c) total number of side roots; (d) root competitiveness

6.4 Discussion

In 21st-century forest degradation, biodiversity loss and rural poverty are among the major challenges, especially in less-developed countries where rural people closely depend on forests (Mohammed et al., 2016). Thus, forest conservation for improving the livelihoods of forest-dependent people is receiving more attention from the world community (Amoah and Wiafe, 2012; Beaumont et al., 2011, Mahdi et al., 2009). However, despite increased conservation efforts by millions of people, organisations and world political powers, the pressures on biodiversity continue to increase with approximately one species lost every 20 minutes (Joes, 2012). Several factors causing biodiversity loss have been identified by researchers (Uddin, 2011). Introduction of exotic of species is one of the major causes (Uddin, 2011). Many exotic species have replaced native species because of their superior growth rates and timber production. These exotic species sometimes develop into invasive species even in highly valued conservation areas and can have detrimental effects on the native species (Biswas et al., 2012). However, according to the CBD strategic plan, multifunctional landscapes, including agroforestry, will continue to play a major role in conserving and enhancing biodiversity from farms to landscape level in tropical and temperate regions of the world (Jose, 2012). Agroforestry is considered one of the effective strategies for forest management and biodiversity conservation as well as poverty reduction (Rahman et al., 2012a; Garrity, 2004). Agroforestry not only helps to increase the production and diversity of food and fodder but also creates opportunities for employment amongst the rural poor (Rahman et al., 2012a). Agroforestry is practiced by more than 1.2 billion people worldwide (Garrity 2004). Agroforestry improves soil fertility, conserves biodiversity and ensures a continuous supply of food and fuelwood (Snelder and Lasco, 2008). Agroforestry contributes to farmers' income and well-being (Thorlakson and Neufeldt, 2012; Verchot et al., 2007; Garrity, 2006). Combining fruit tree species in agroforestry systems, based on the seasonal calendar of fruit harvest times, could ensure a year-round supply of key nutrients (Jamnadass et al., 2011). The cultivation and management of trees by smallholders in agroforestry systems provide a variety of food products, some of which enter local, national and international markets, while others meet subsistence needs (Powell et al., 2013). The product and services obtained from agroforestry provide substantial resilience for local farmers (Shackleton et al., 2007; Shackleton, Delang and Angelsen, 2011) and for nutritionally balanced global food system multifunctional landscapes. It is also crucial to ensure market chains developed for nutritionally important tree and forest products so that such products can reach distant consumers while remaining affordable for everyone (Powell et al., 2013). Worldwide domestication of many wild fruits has taken place and these species have been integrated into agroforestry systems. Examples of such species include *Adansonia digitata, Canarium indicum, Irvingia gabonensis*, *Sclerocarya birrea* and *Vitellaria paradoxa* (Hunter and Fanzo, 2013; Vinceti et al., 2013; Leakey and Newton, 1993).

Bangladesh is a densely populated country with a total land area of 12,736,000 hectares (FAO, 2010). Natural forests occupy 9.5%, plantation forests 1.8%, shifting cultivation 2.6% and home garden agroforestry 21.7%. In Bangladesh, natural forest is disappearing at an alarming rate and threatening the populations of many native species (Appanah and Ratnam, 1992). It is estimated that there are 13 million home-garden agroforestry systems in the country, which generate about 70% of the fruit, 70% of the timber, 90% of the fuelwood and 40% of the vegetables in the country (Miah and Hussain, 2010). However, most of this production comes from only a few exotic species (Shams et al., 2015). This study demonstrates the potential to integrate a new native species, *M. sylvatica*, in agroforestry systems. We compared *M. sylvatica* (wild mango) with *M. indica*, *A. heterophyllus* and *A. auriculiformis*, which are three of the most common agroforestry species already domesticated in Bangladesh. Our study found that wild mango is a multipurpose tree providing wood, fruit, fuelwood, medicine and fodder. These traits are generally considered as suitable criteria in an agroforestry species and also preferred by farmers (Aladi and John, 2014; Mekoya et al., 2008). However, the population of the species has suffered decline due to human over-population, over-exploitation, excessive demand for wood and lack of awareness about the species' declination, potential and conservation needs. Under-storey microclimatic conditions are influenced by the crown architecture of the upper-storey species in the agroforestry system (Rao et al., 1998). A good shade tree should be tall, with a light crown to ensure good air circulation for the under-storey crop, which minimises the incidence of disease (Anglaaere, 2005). The crown architecture of wild mango (*M. sylvatica)* is interestingly similar to *M. indica*, *A. heterophyllus* and *A. auriculiformis*. The tree height, crown ratio and crown shape ratio are very similar, indicating its potential to be integrated into agroforestry systems. The light quality (R/FR ratio) and quantity (PAR) under the canopy are very important factors for the development and production of under-storey crops (Khattak et al., 2011; Tsegay et al., 2005). Plants use 400nm to 700nm of light for photosynthesis (Tegelberg et al., 2004). According to our observations, the amount of light present below *M. sylvatica*'s canopy (596.56µmol $m^{-2}s^{-1}$) is very similar to other species. This indicates that the crown of this species in not over-shading. The R/FR ratio influences the physiological flowering, fruiting, leaf phenology and shoot growth of plants and crops (Khattak et al., 2011; Tsegay et al., 2005; Tegelberg et al., 2004). The light quality under the

M. sylvatica tree is similar, thus indicating that the growth and production of under-storey crops might be optimal if this native species is integrated into the agroforestry systems in Bangladesh. The root structure of the nursery seedlings of *M. sylvatica* indicates its potential to be used in agroforestry systems based on *M. indica*, *A. heterophyllus* and *A. auriculiformis.* Mango-based agroforestry (*M. indica*) is widely practiced in the Padma floodplain (Rahman et al., 2012b); jackfruit (*A. heterophyllus*) and pineapple (*Acacia. comosus*) together are commonly grown in tribal areas, including Madhupur and Maulvibazar (Hasan et al., 2008); jackfruit (*A. heterophyllus*) and Burmese grape (*Baccaurea ramiflora*) have recently been introduced in the Narsingdi district (Shams et al., 2015); akashmoni (*A. auriculiformis*) and mengium (*A. mangium*) are two of the major agroforestry species in the cropland agroforests of the Khulna, Jessore and Satkhira districts (Hasanuzzaman et al., 2014; Chakraborty et al., 2015). Though the experiment was based on nursery seedlings, our study suggests that co-planting of *M. sylvativa* with *A. heterophyllus* might also be possible due to their low root competitiveness (Schroth, 1995). Mango (*M. indica*), jackfruit (*A. heterophyllus*), mengium (*A. mangium*) and a few other species are planted together in agroforestry systems of Char Kukri-Mukri Island of the Bhola district (Islam et al., 2015) and the Char Gobadia area of Mymensingh (Zico et al., 2011). Mango (*M. indica*), jackfruit (*A. heterophyllus*) and akashmoni (*A. auriculiformis*) are planted in the Jhenaidah district of Bangladesh (Sharmin and Rabbi, 2016). Planting mango (*M. indica*) and jackfruit (*A. heterophyllus*) together is a very popular trend in home-garden agroforestry systems of Bangladesh (Zaman et al., 2010; Bardhan et al., 2012). So, the *M. sylvatica* species can provide food and enhance the livelihoods of local people. Additionally, there are many agroforestry options available where this native species could possibly be integrated into agroforestry in Bangladesh. Among all the species used in Bangladesh as agroforestry species *M. indica* contributes 22.6% (Hasan et al., 2008) and as it shares similarities with *M sylvatica,* indicating a potentiality to introduce this as an agroforestry species in the country.

6.5 Recommendations and conclusion

M. sylvatica is an underutilised native species, which could be used as an alternative source of food and income. The many attractive features of this species indicate its potential for integration into agroforestry systems and also to encourage people to consume more *M. sylvatica* fruit. The findings from the root compatibility study were based on nursery seedlings. Root competition can change with the age of the seedling and also can be very different in the field. So, it is recommended that field trials are conducted to confirm the actual root compatibility. Light quality studies also needs to be conducted in plantation and natural conditions rather than single standing tree investigations. The tree is tall compared to other popular agroforestry species but can be modified through different tree improvement techniques (pruning, gaffing, budding and breeding). *M. sylvatica* has been successfully used in the nursery as root stock for *M. indica*. No experiment has been done to date to investigate the compatibility of *M. sylvatica* as scion and *M. indica* as root stock. Therefore, our study recommends further research in this sector. Developing new varieties through breeding would be good for early and regular production. Training on pest and disease management would be a potential solution for controlling the insects in the pulp. To improve the shelf life of *M. sylvatica* fruit, post-harvest techniques (e.g. aloe vera coating, chitosan coating from shrimp shell, lipid coating from beeswax and resin, polythene packaging, etc.) can be used. The development of a market niche is needed to popularise the species. The information generated in this pioneer study may help to promote the conservation of *M. sylvatica* fruit through agroforestry practices in Bangladesh. Finally, collaboration between farmers, forest department, industry partner (e.g plywood industry), horticulturists, the food industry and policy makers is required to reduce the risk of extinction of this native, locally threatened species.

Chapter 7: Discussion

7.1. Overall discussion

The global population is estimated to reach 9 billion people by 2050 (Vinceti et al., 2013). With increasing climate change and biodiversity loss, the global food system may not be able to support this huge population. Therefore, there is an urgent need for collective action towards this problem to ensure global food security (Hunter and Fanzo, 2013). In recent years, there has been increasing concern to reduce malnutrition around the world (Swaminathan, 2012). Policymakers historically gave priority to energy-rich staple foods originating from cereals, starchy tubers and root vegetables, though these are often not sufficient to combat micronutrient deficiency (Swaminathan, 2012; Stephenson et al., 2010). Micronutrient deficiencies are often called 'hidden hunger' and can result in severe and lifelong health issues (e.g. increased mortality; physical and mental impairment). It was estimated in 2015 that over 795 million people in the world are undernourished (FAO, 2015) and over 30% of the world's population are anaemic, many due to iron deficiency (WHO, 2016). Consumption of fruits and vegetable is a sustainable way to improve nutrient quality and diet (Slavin and Lloyd, 2012; Stephenson et al., 2010; Bharucha and Pretty, 2010). Many traditional agricultural activities give less attention to the cultivation of fruit and vegetables (Powell et al., 2013). These nutrient-dense, non-staple foods are often harvested from wild and usually consumed by rural populations around the world (Penafiel et al., 2011; Bharucha and Pretty, 2010). Globally, an estimated 1 billion people use wild edible plants to supplement protein, vitamins and minerals and improve the palatability of staple foods. Typical wild forest foods are wild fruits and nuts, wild leaves, mushrooms, wild meat, wild roots and tubers. They are rich in proteins, fats, carbohydrates, vitamins, minerals and phytochemicals such as phenolics (Karjalainen et al., 2010). There is a strong negative relationship between income and malnutrition (FAO, 2012). Forests contribute to the livelihoods of more than 1.6 billion people (FAO, 2010). Forest incomes can be diverse such as income from selling these wild fruits, nuts, vegetables, medicines, firewood and timber (Powell et al., 2013). Forests in some cases provide the only cash income that makes it possible to purchase food for the local households (Powell et al., 2013). The majority of rural households in developing countries rely on wild forest produce to meet some part of their food, nutritional, health and livelihood needs (Arnold, 2011). The access to wild food has diminished due to deforestation as the forest has become distant from settlements and the natural forests have been converted to plantation forests, which contain less diversity, thereby affecting diet diversity and nutrition. Wild biodiversity such as wild fruits and wild vegetables can contribute to the dietary diversity of the local people (Powell et al., 2013; Powell, 2012; Frison et al., 2011). Wild fruits and vegetables contribute to food supply during lean seasons and also can be sold for income to enhance food security (Vinceti et al., 2013). Wild fruits play an important role in supplying many essential micronutrients for human nutrition (Powell et al., 2013; Sunderland, 2011; Arnold et al., 2011), which are commonly lacking in the diets of developing countries (Vinceti et al., 2013). For example, Vitamin A deficiency causes blindness in up to 500,000 children every year (Kennedy et al., 2003). Wild forest fruits provide a 'safety-net' and provide emergency food during the hunger periods (Powell et al., 2010; Colfer, 2008).

Bangladesh is a densely populated country (156.5 million total population), with land and resources in limited supply. Rates of malnutrition in Bangladesh remain among the highest in the world, with an estimated 6 million children chronically undernourished (DHS, 2011a). Bangladesh's malnutrition burden is significant, with 41% stunting, 16% wasting (DHS, 2011b), 22% with low birthweight and 2% overweight (UNICEF, 2014). Deficiencies in Vitamin A, iron, and iodine are acute problems in Bangladesh. 69% of Bangladeshi households experience some food insecurity (FSNSP, 2011). Agriculture in Bangladesh is dominated by small and marginal farms. A large proportion of cropped land is given to rice farming. 70% of the energy required comes from rice. 40% of households in Bangladesh spend 60–70% of their income on consumption of food (BBS, 2010). When rice prices were high in 2011, 69% of Bangladeshi households experienced some food insecurity (FSNSP, 2011). The intake of fruits and vegetables increased in 2010 (211g), which is still half of the FAO/WHO recommendations (400g per day). Average fruit consumption is 44.8g which is only 1% of the total energy required (FAO, 2014). Bangladesh relies heavily on imports for fruit, vegetables, pulses and oilseeds. In 2012, Bangladesh imported nearly Euro 2 million of fruit. However, there are varieties of wild underutilised fruits grown in forest areas without much care. There is little awareness of the nutritional value of those species among the people. These wild fruits can be a good source of micronutrients and can be used as an alternative source to combat hidden hunger, such as Vitamin A and Vitamin C deficiencies (Deshmukh and Waghmode, 2011; Bioversity International, 2004). These fruits are well adapted to the local climate and contribute to the poverty reduction and household food security of rural and tribal people, and play a significant role in herbal medicine (Powell et al., 2013; Rahim et al., 2009). Little attention has been given so far to increase the use of wild fruits to overcome the malnutrition in Bangladesh (Rahim et al., 2008; Shajib et al., 2013). These underutilised fruits can play an important role in the local and national nutrition and economy of Bangladesh.

The contribution of wild fruits is often overlooked (Powell et al., 2013). Wild foods are especially important for the poorest communities, rural populations and women, particularly during critical food shortages. Commercialisation of wild forest products has been promoted by researchers, conservationist, development organisations and governments to achieve rural livelihood improvement in an environmentally sound way (Belcher et al., 2005). These products contribute significantly to household food security, but only if local management systems and national policies ensure their sustainable extraction and use. Wild fruits are not only an important food for poor people in developing countries in order to cope with seasonal shocks (Ræbild et al., 2011; Arnold, 2008) but also are considered as a significant store house of genetic resources. In order to use wild forest fruits to supplement diets in balanced ways, more knowledge is needed on the nutritional values of different types of wild forest fruits (Leakey, 2005; Leakey et al., 2003). However, due to the lack of effective regulation, over-exploitation, climate change and habitat degradation, these wild edible resources often face rapid degradation (Karjalainen et al., 2010). The impact of losing these resources may not be immediate but could reduce the global capacity for plant breeding to produce new crop varieties needed for agricultural systems to adapt to global climate change (Frison et al., 2011). They also make a significant contribution to ecosystems (Ford-Lloyd et al., 2011).

Biodiversity conservation and food security are two sides of the same coin and the majority of today's modern crop varieties are derived from their wild relatives (Sunderland, 2011). The value of crop wild relatives has historically been unappreciated (Jansky, 2013). It is estimated that 20% of all plant species will be in danger of extinction in the near future (Brummitt and Bachman, 2010). The World Health Organisation estimates that in many developing countries up to 80% of the population relies on biodiversity for primary healthcare and around 1 billion people rely on wild-harvested products for nutrition and income (Pimentel et al., 1997). Policies governing forest management, food security and poverty alleviation are often not well coordinated. As a result, people are not aware of the benefits of the forest foods available to them and, because these resources are not valued or protected and their availability is shrinking (Bioversity International, 2006). The wild relatives have already proven to be important sources of genes for improving agricultural production. For example, wild relatives of the potato have provided resistance to late blight, the Colorado potato beetle, and wild relatives of the cultivated peanut have been used to breed resistance to root-knot nematode (Bioversity International, 2006). However, the significance of the CWR resource is now becoming more apparent to policymakers. Conservation and sustainable use has become a priority on the international conservation agenda (Maxted et al., 2012). Native species, those that were more closely related to widely cultivated crops with a high economic value, and those considered under threat were prioritised (Fielder et al., 2016). As native tree species are particularly adapted to local climates and often more resilient in the face of local climatic extremes (Powell et al., 2013), there has been growing interest in the conservation of wild relatives of fruit trees. In most tropical regions, the rich diversity of native fruit species is an important and valuable resource for enhancing nutritional security, reducing poverty and protecting the environment. Many species are currently underutilised. Many are also threatened and vulnerable due to habitat loss. Wild mangos are among the significant fraction that are on the verge of disappearance (ICEM, 2014) and which could be a new source of novel genetic traits (Bioversity International, 2006). The current study focused on one of the crop wild relatives of *Mangifera sylvatica*. The genus Mangifera belongs to the family of Anacardiaceae. There are 41 species found in this family. Twenty-six species produce edible fruits, but among them only a few have been domesticated (*Mangifera indica*, *Mangifera caesia*, *Mangifera foetida* and *Mangifera odorata*) and only one commercially cultivated (*Mangifera indica*) in the world (Ariffin et al., 2015; Mukherjee and Litz, 2009). *M. sylvatica* is valued for its timber, fruit and medicine (Baul et al., 2016). Unfortunately, *M. sylvatica* is currently threatened in Bangladesh (BFRI, 2013; Khan et al., 2001). Therefore, the current study investigated the conservation prospects of *M. sylvatica* by identifying and quantifying its current and likely future distribution; characterising its morphological traits; profiling its nutritional and medicinal properties; and identifying barriers to its conservation and potential to be integrated in agroforestry systems.

This pioneer study confirms that future climate change may cause a major decline in the potential distribution of this species in Bangladesh. Restoration of depleted populations may be an option in south eastern parts of the country. These findings might be a useful guide for government and non-governmental organisations responsible for the conservation and management of forest resources. Morphological variation in fruit traits is important for the identification of elite individuals (Leakey and Page, 2006) for cultivar development to start domestication process. The current study characterised morphological, nutritional and medicinal values and variation within and between local sites in the fruit traits of the wild mango (*M. sylvatica*) in Bangladesh. Our study suggests that fruits from Sylhet are sweeter and a rich source of Vitamin A, which can be considered during cultivar development for pulp production, and fruits from the Cox's Bazar site are big with bigger kernels and higher fat content, which can be utilised in the food, pharmaceutical or cosmetic industries (Leakey et al., 2008). *M. sylvatica* kernel fat has similarities with cocoa butter, indicating its potential to be used in the chocolate and confectionery industries as a partial or total replacer of cocoa butter. The current study provides information about the possibility of integration of *M. sylvatica* into agroforestry systems. Our study found that wild mango is a multipurpose tree providing wood, fruit, fuelwood, medicine and fodder, which are considered to be suitable criteria for an agroforestry species (Aladi and John, 2014; Mekoya et al., 2008). However, the population of the species has suffered a decline due to human over-population, over-exploitation, excessive demand for wood and lack of awareness about the species' declination, potentials and conservation need. Crown architecture, light properties under the canopy and root competitiveness with nursery seedlings of the wild mango (*M. sylvatica*) are interestingly similar to other popular species such as *M. indica*, *A. heterophyllus* and *A. auriculiformis*. So, *M. sylvatica* can provide food and enhance the livelihoods of local people. Additionally, there are many agroforestry options available where this native species could possibly be integrated into agroforestry in Bangladesh.

7.2. Reflections on findings in relation to identified actors/beneficiaries

It is concluded that the attractive features of wild mango could be promoted by a strong collaboration of different stakeholders such as land use planners, climate change policy makers, government or non-government organizations, commercial breeder, potential investors (chocolate, butter, nutraceuticals, flavourings), food industries, food technologists, minister responsible for foreign direct investment in Bangladesh and Bangladesh forest department to promote more widespread cultivation of this wild fruit species. Apart from this, before taking initiative for integrating this species in agroforestry system, the species should go through field trial.

Chapter 8. Conclusion

8.1. General conclusions

There are many underutilized wild fruits available in the forest. Many of them are threatened and conservation of those wild fruit species is directly related to livelihood and food security, health and nutritional security. However, information on their nutritional, medicinal and economic value and conservation value is unknown. *M. sylvatica* is an underutilised species that is genetically very close to the commercially cultivated common mango. This pioneer study provides a noteworthy information distribution, including the morphological, nutritional, medicinal and economic potential of *M. sylvatica* species. Fruits of the wild mango are nutritionally and medicinally rich and are considered a seasonal income source for the local people. This valuable nutritional and medicinal information can be used to encourage people to consume more *M. sylvatica* fruit. There is enormous potential for the development of a wild-mango-kernel-based enterprise in Bangladesh as well as in other tropical countries for the production of wild mango butter, which has similar features to cocoa butter. This will not only provide raw materials for the chocolate and confectionery industries but also offer opportunities to empower forest-dependent people and small-scale farmers. However, this species is currently threatened in Bangladesh due to over-exploitation for the wood and also by habitat destruction. The species distribution showed potential areas for reintroduction of this species. Additionally, the preliminary study showed that the species has enormous potential to be integrated as a new crop in the agroforestry system. The information generated in this study opens the path for domestication and commercialisation of this wild fruit species. The information generated in this pioneer study may help to promote the conservation of *M. sylvatica* fruits through agroforestry practices. The present study calls for greater collaboration between land use planners, climate change policy makers, government and non-government organizations, commercial breeder, potential investors (chocolate, butter, nutraceuticals, flavourings), food industries, food technologists, minister responsible for foreign direct investment in Bangladesh and Bangladesh forestry department to reduce the risk of extinction of this multipurpose underutilised fruit species. Finally, the method and approach used in this research can be applied for the conservation and utilisation of other underutilised wild forest fruit species for health and livelihood.

8.2 Conclusions for identified stakeholders

Objective conclusions are given below for different stakeholders:

Objective 1: To determine the current and future distribution to identify areas of high conservation value under climate change for *M. sylvatica.* The identified stakeholders are:

- Commercial users, timber, pickle and butter etc
- Wildlife, ecosystem services officers in government or NGOs
- Land use planners concerned with climate change
- Commercial breeder
- Climate change policy makers in Bangladesh

All the stakeholders mentioned above can jointly start conservation initiative in its suitable habitat such as establishment of PAs, participatory forestry and plantation, buffer zone with non-timber forest products

Objective 2: To characterise the fruit quality of *M. sylvatica* and to determine variations in fruit quality with site. Key stakeholders are:

- Commercial users, timber, pickle and butter etc
- Potential investors in butter, nutraceuticals, flavourings etc
- Minister responsible for foreign direct investment in Bangladesh

Information generated in this chapter will help to identify what traits a desirable new variety/ cultivar would have as an aid to future breeding efforts. If the potential investors come forward to promote plantation and conservation of this species, other stakeholders such as forest departments and local people may become interested as well.

Objective 3: To assess the potential of *M. sylvatica* butter as a cocoa butter alternative (CBA). The relevant stakeholders are:

- Food technologists
- Chocolate industry
- Government actors

The species has great potential to become an important raw material for chocolate industry as well as cosmetic industry. The relevant stakeholder's can take the necessary initiative for commercial production of butter.

Objective 4: To identify factors causing barriers for conservation of *M. sylvatica* and potential to be integrated as an agroforestry species. Specific stakeholders are:

- Farmers
- Industry partners eg. Plywood industry, Government actors: Forestry department

Information generated from this reseach shows that the species can be introduced as agroforestry species. However, to confirm the suitability, field trails are urgently needed. The relevant stakeholders should take the urgent step for field trials.

8.3 General reflections on methods

Although the SDM we developed were able to capture the observed distribution well, the need remains to consider other biophysical factors, such as soil characteristics, land cover and geology, to obtain more reliable and relevant predictions of where the species will be able to grow in the coming decades. In addition to the Maxent model, other SDM can be used. As *M. sylvatica* is a threatened species, we were able to use only fifteen trees for the characterisation of morphological variation from three study locations. Though the findings of the morphological variation were statistically significant, it may be useful to use a bigger sample size to determine morphological variation for a better understanding of the available range. *M. sylvatica* is nutritionally rich and contains different types of vitamin. For the present study, we estimated only Vitamin A and Vitamin C. However, to explore the full potential of this wild mango, other vitamin (Vitamin B1, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B6, Vitamin B12 and Vitamin E) analysis can be done, as well as micronutrients. Though the current study profiles the mango pulp phenolic compounds, there are many more compounds available that need to be explored. The wild mango kernel is a very rich source of phenolic compounds as well, which needs further research to maximise its potential. To investigate the nutritional and medicinal compounds, it is not necessary to study tree-by-tree, but an overall average would be a good starting point to begin to understand nutritional and medicinal properties. This will reduce both time and money required. Our study shows that wild mango kernel butter is a good cocoa butter alternative. However, research on chocolate production using the butter from this species is necessary to fully capture the value of this wild mango species. *M. sylvatica* has potential for integrating into agroforestry systems. This pioneer study suggests that *M. sylvatica* seedlings would benefit from further field trials to determine their root compatibility. The findings from the root compatibility in the present study were mainly based on nursery seedlings raised in plastic bags. Root competition can change with the age of the seedling and also can be very different in the field. Therefore, a field trial is recommended to check the actual root compatibility. To investigate light quality, the present study considers only three trees and measured for 6 days which is not sufficient. Also all those trees were single standing trees. The influence of light in the forest or in plantation conditions will be different from the measurement of singe standing trees. Therefore, is is necessary to investigate the light quality under canopy conditions. Also light quality could be investigated for different seasons.

8.4 Recommendations for identified stakeholders

The price of cocoa butter more than doubled between 2005 and 2015 from \$1433/tonne to \$3360/tonne (Supplementary Figure 5.1). On the other side, global demand for cocoa is growing annually by 2% to 3% limited by low productivity, price fluctuations and uncertainty in supply (Supplementary Figure 5.1), which has forced the confectionery industry to look for alternative sources. Given the evidence derived from this research, the market share and potential income for Bangladesh could be up to approximately 2000 USD/hectar. Therefore, the present study calls for greater collaboration among land use planners, climate change policy makers, government and non-government organizations, commercial breeder, potential investors (chocolate, butter, nutraceuticals, flavourings), food industries, food technologists, minister responsible for foreign direct investment in Bangladesh and Bangladesh forestry department to reduce the risk of extinction of this multipurpose underutilised fruit species. All these stakeholders can build a task force to take urgent step for new commercial plantation.

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Appendix 1: Questionnaire for Household Survey/Key informant interview

1. What are the local names of wild mango?

   a.   b.   c.   d.

2. Do you consume *M. sylvatica*?

   a. Yes   b. No

3. Purpose of collection:

   a. Own consumption   b. For sale   c. Others

4. If for own consumption purpose than why you consume it?

   a. Satisfy hunger
   b. Nutritious

c. Tasty
d. Cheap and available
e. Without any reason
f. Don't know

5. What attractive feature like most for consuming *M. sylvatica*?

Color	Size	Sweetness	Cheap
Taste	Smell	Sourness	Others

Note: 0 = Not Comments, 1 = Not Satisfactory, 2 = Moderately Satisfactory, 3 = Good, 4= Very Good

6. If no why you don't consume?

a. Not available
b. Not Cheap
c. Not Tasty
d. For wildlife only
e. Sourness
f. Size small
g. Others

7. What are the uses of *M. sylvatica*? Which one is the most important use?

Fruit	Timber	Plywood	Fuel wood	Fodder	Medicinal use	Others

8. What food product can be obtained from *M. sylvatica*?

a. Chatny b. Pickle c. Vegetables d. others

9. *M. sylvatica* as source of food for wildlife….

a. b. c. d. e.

10. What medicinal uses does it have?

11. What kind of products produced from this timber?

a. b. c. d. e.

12. Time of collection.

April	May	June	July

13. Period of collection (Fruiting time)

10-15 days	15-30 days	30-45 days	More than 45 days

14. Frequency of use (during the period of collection):

a. Once a week b. Twice a week c. Thrice a week

15. Quantity in each time: …………………………………………

16. Storability of the Fruit (Without Treatment)

0-10 Days	11-20 Days	21- 30 Days	>30 Days

17. Quantity of M. *sylvatica* sale by each family /season:

Less than 20 kg	20-40 kg	40- 60 kg	More than 60 kg

18. Market price of per unit amount of *M. sylvatica*: ………………………..

19. What are the major constraints in marketing of *M. sylvatica*?

a. Less supply
b. Less Demand
c. Inferior quality fruit
d. Less price
e. Not well known
f. Only certain group of people (Ex. Tourist)
g. Others

Reason of declining (FGD)

According to DPSIR framework

1. What are the Drivers (Human Demand and Life Style) that responsible for declining of *M. sylvatica*?

Drivers	response
Over population	
Lack of awareness	
Lack of interest	
Industrial and residential development	
Increased demand of wood (for plywood industry)	
Lack of policy	
Others	

2. Pressure (Human Activities affecting the species)

Pressure	response
Over exploitation	
Illegal logging	
Less or no planting activities	
Slash and burn agriculture	
Land clearance for farming or other development activities	
Others	

3. State (Physical condition of the species)

(a)…..Availability of wild mango at present compare to 10 years before?

a. Decreased b. Increased c. no change d. no idea

Decreased	A Lot	Moderate	A Little	No Idea
Increased	A Lot	Moderate	A Little	No Idea

4. Impact (Effect due to changed vegetation)

Shortage of….

Seasonal fruit		Wood used for utensils and tools	
Plywood		Fuel wood / Charcoal	
Swan timber		Fodder	
Building materials		Shade	

5. Response (Policy and management issues)

Financial or Technical support available in the locality, providers (micro credit, NGOs support etc) and agreements for wild fruit species conservation

Types of support	Provider	Agreement

Domestication and Marketing Problem (FGD)

1. Major threats/constraints to domestication according to the local peoples

Threats/constraints	Opinion
Lack of planting material (seed, seedling)	
Less Seedling survival rate	
Lack of knowledge about the species use	
Pest and Diseases	
Lack of space	
Less Fruit production	
More time for to get the 1st yield	
Less demand in the market as a fruit tree	
Fruit Quality not good	
Regeneration	
Others	

2. What can be done to overcome threat/constraints?

Providing free planting material	
Need training on pest and diseases control	
Information about the potential use species	
Awareness program	
Facilitating small scale industry	
Need information about vegetative propagation	
Need to improve the fruit quality	

Use of Species (FGD)

1) Function / Use of tree species used in agroforestry (FOOD).

Species	Fruits / Nuts / Seeds / Pods	Vegetable	Spices / Condiments	Oil	Others

M. indica					
M. sylvatica					
A. heterophyllus					
A. auriculiformis					

2) Function / Use of tree species used in agroforestry (WOOD).

Species	Fuel wood	Swan timber	Building materials	Wood used for utensils and tools	Plywood	Others
M. indica						
M. sylvatica						
A. heterophyllus						
A. auriculiformis						

3) Miscellaneous Aspect of tree species used in agroforestry.

Miscellaneous use	*M. sylvatica*	*M. indica*	*A. heterophyllus*	*A. auriculiformis*
Resin-Gum-latex				
Cosmetic use				
Medicinal use				
Others				