Genetic diversity of Bangladeshi jackfruit (*Artocarpus heterophyllus*, Moraceae)

Colby Witherup

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The student Colby Witherup defended his/her thesis, titled Genetic diversity of Bangladeshi jackfruit (Artocarpus heterophyllus, Moraceae)

on the date of June 4, 2012. The committee members have rendered the following decision:

- ☐ Pass with no revisions
- ☒ Pass with minor revisions
- ☐ Pass with major revisions
- ☐ Fail to pass

Committee members:

Printed name	Signature	Department/Affiliation
Nyree Zerega (Major adviser)		PBC/NU/CBG
Jeremie Fant		PBC/NU/CBG
Stuart Wagenius		PBC/NU/CBG
Allison Miller		

In the case of revisions, specify below, on back, or on additional attached pages what they are and who on the committee (major adviser, or everyone) will review the revisions before the thesis is officially accepted as complete. After this approval has been met, the major adviser needs to sign again below and this form, along with a pdf file of the final approved thesis must be turned into the PBC program assistant. Students need to check the deadlines for June and December graduations with TGS to make sure these forms are completed on time.

Minor re-writes and analyses required as discussed

Signature of major adviser confirming final approval — Date: 5/14/2013

This form, along with a pdf file of the final approved thesis, must be submitted to the PBC program assistant, Susan Black, in Hogan 2-144. The program director will then sign off on it and approval will be communicated to TGS.

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Background and objectives

Jackfruit History and Origins

The Moraceae family includes several species producing edible fruits, including mulberry, fig, breadnut, and osage-orange. The *Artocarpus* genus contains over 50 species of latex-producing trees and shrubs, with at least 25% of these species producing edible fruits (Zerega et al. 2010). The two most widely cultivated and economically important species in the genus are *Artocarpus altilis* (breadfruit) and *Artocarpus heterophyllus* (jackfruit). Breadfruit is considered a staple food of many countries in its native Oceania and in the Caribbean, where it was introduced in the 18th century (Ragone 1997). Jackfruit is a major crop in Southeast Asia and is growing in popularity in Australia, Europe, the Middle East, and the United States, fueled by demand from Asian immigrants and migrant workers (Rahman and Talukder 2001). Thailand, China, and Malaysia are the dominant exporters of fresh jackfruit and jackfruit products (Haq 2006). Exact numbers are not known for the economic value of the world's jackfruit crop, however, it is estimated that nine million metric tons of jackfruit are produced annually in Asia (Haq 2006).

Jackfruit has been cultivated for millennia and was referred to as early as 300 B.C. by Theophrastus from his travels in India (Hort 1916). Despite a long history of cultivaiton, almost nothing is known about the relationships between geographic populations or the history of jackfruit domestication. Today it can be found in cultivation at low elevations throughout the Indian subcontinent, Bangladesh, Myanmar, Sri Lanka, Southern China, Nepal, Laos, Vietnam, Cambodia, Malaysia, and has also been introduced in the Philippines, Indonesia, and throughout Africa, Australia, and the Neotropics. It is now so widely cultivated that it is unclear in which region it is indigenous and which region holds its greatest diversity. The rainforests of the Western Ghats in India (where it is a commercially minor fruit and is seldom found in regular plantations) are generally cited as both the area of origin and center of diversity of jackfruit (Jagadeesh et al. 2007). This citation, however, is based in part on Wight (1843) who observed jackfruit trees growing in primary forest away from human habitation; no update has since been provided on the presence of wild trees in the Western Ghats. Barrau (1976) suggests that the point of origin could be Malaysia, due to the great diversity of cultivars there, but no wild trees have been observed there. Jackfruit has been reported in forests away from human habitation on the Andaman Islands (Bashar and Hossain 1993) and trees with "wild" characteristics have been reported in Bangladesh (Khan et. al 2010), though the status of these trees has not been confirmed. It is possible that jackfruit has a broader center of origin than previously proposed, or that the true point of origin will never be known (Jarrett 1959).

Vavilov proposed that the center of origin for a crop would be the same as the center of diversity; the center of origin is located where the highest diversity of crop wild relatives can be found (Shantz 1927). Jackfruit's closest wild relative, the sister species *A. integer*, is believed to be indigenous to Indonesia (Zerega et al. 2010). There are several possible explanations for jackfruit's origins, though neither fossil data nor molecular data has yet

been presented to support any claim. It is possible that jackfruit was domesticated from a wild species that has since gone extinct. This domestication could have occurred in either the Indo-Burma region or Siam-Malaya-Java region, both of which were proposed as centers of diversity by Vavilov (Shantz 1927), followed by introduction into other parts of Asia. Alternatively, jackfruit was domesticated directly from another related species, such as *A. integer*, but more molecular analysis would need to be done to support that relationship. Hybridization is a third possible route of domestication, and it has been identified elsewhere in the genus with *A. altilis* (Zerega et al. 2010). However, our microsatellite analysis shows consistent diploid markers for jackfruit, making hybridization, which often results in polyploidy, a less likely possibility.

Jackfruit Uses

Although the jackfruit tree is used in many ways, it is primarily produced for the fruit, which is difficult to process because of the latex. Before opening the fruit, it is recommended that hands and knives first be coated in vegetable oil for easier clean up. The fruit flesh may be eaten unripe, like a vegetable when cooked or dried, and is often used in curries. Ripe flesh is eaten raw; it has a strong odor when opened, described as a combination of pineapple and banana. Although the large, heavy and perishable fruit is not well suited for the fresh fruit export trade, it is canned and processed into products that are exported to Australia, Europe, and, more recently, the United States. These products include wine, ice cream, chips, and jellies (Asquieri et al. 2005, Odoemelam et al. 2005, Jagadeesh et al. 2009). The seeds can be eaten boiled, roasted or dried and salted as table nuts, or they can be ground to make flour (ICUC 2003). The leaves are also used as fodder for livestock (Das et al. 2007), and the latex is used as glue. Whole trees are harvested for timber for furniture, construction, and musical instruments because the wood is termite-proof and fairly resistant to fungal and bacterial decay (Morton 1987). Traditionally, jackfruit was used medicinally throughout Southeast Asia to relieve several different ailments, including using the pulp and seeds as a cooling tonic, roots for relieving diarrhea, wood as a sedative and skin lightener, and leaves for vermifuge and to activate lactation in animals in humans, though these remedies have not been proven through scientific study (Arung et al. 2006). Jackfruit trees are often farmed together with coffee, black pepper, pineapple, and other crops for shade and support. Jackfruit wood chips are used to make a dye, which is used to give the famous orange-red color to the robes of Buddhist priests. Because many of these products are consumed locally, it is difficult to estimate the true economic value of jackfruit worldwide.

Jackfruit Biology

Jackfruit grows in tropical and subtropical lowlands, thriving below altitudes of 1000 m. It is sensitive to frost and drought and prefers a wet environment, as it is native to monsoon climates of Southeast Asia. It grows well in almost any type of soil. Jackfruit is a medium sized tree, approximately 15 to 20 meters high. It is evergreen, with glossy, dark green, alternate leaves. Sticky white latex is found throughout all parts of the tree, including the fruits, though latex-free cultivars are being researched (Sarian 2001). Jackfruit is monoecious; male flowers are produced in the leaf axils, typically above the female flowers, which develop on short twigs from the trunk, branches, and sometimes

from below the soil level at the base of older trees (California Rare Fruit Growers, Inc. 1996, Elevitch and Manner 2006). The compound fruits (these will be referred to simply as fruits throughout the rest of the thesis) represent the largest tree-borne fruit structure in the world, reaching sizes up to 100 cm x 50 cm and weighing over 35 kg (Jarrett 1959). The edible parts of the fruit are called bulbs, and are the fully developed fleshy perianths. Each bulb encloses one "seed" (actually an achene fruit) covered with a thin white exocarp. The term 'flake' is used to represent the edible part of each jackfruit bulb. Each fruit contains about 100-500 seeds. The greenish yellow fruit rind is spiky on the outside, though the color and texture of the rind and flesh vary between cultivars and individuals.

While not self-incompatible, seed set and many fruit characteristics such as size, flesh percentage, and edible percentage are greatly enhanced with cross-pollination, and jackfruit is known as an outbreeding plant (El-Sawa 1998). The pollinator of jackfruit is unknown. Research investigating whether it is wind or insect pollinated, using germplasm collections in Florida and Australia, was inconclusive (El-Sawa 1998, Moncur 1985). Other research has suggested insect-assisted wind pollination, since insects are not often seen visiting female flowers and pollen is not free for easy wind pollination but must first be loosened from male flowers (Haq 2006). Hand pollination is possible but is not regularly practiced. Research into jackfruit's most closely related species, *Artocarpus integer*, has uncovered a possible mutualism involving gall midges and a fungus (Sakai et al. 2000). Hybridization between *Artocarpus heterophyllus* and *Artocarpus integer* does occur naturally, but is promoted for certain cultivars (Campbell and Ledesma, 2003).

Jackfruit Propagation

Traditionally, jackfruit is mostly seed propagated. Trees raised from seed germinate approximately 10 days after planting and start flowering in three to eight years (Gunasena et al 1996). Jackfruit is a low-input crop, growing in most soil types and requiring little to no fertilizer. It is also known to be relatively free of serious disease (Gunasena et al 1996). Seeds are recalcitrant, sensitive to both drying and freezing, and cannot be stored for more than a few weeks, making *in situ* preservation of genetic resources especially important for jackfruit conservation. Because it is seed propagated and seldom hand-pollinated, offspring can be unreliable. Even so, cultivars have been developed from seed, though their reliability for producing offspring that are true to type has not been studied.

In recent years, vegetative propagation methods have greatly improved, including grafting, budding, and tissue culture. Although vegetative propagation has been used for millennia for other Asian tree crops, including *Artocarpus altilis*, and although there is mention of jackfruit grafting as early as 500 A.D. (Varahamihira 1884), jackfruit has remained a seed-propagated crop. The new efforts to produce more reliable cultivars through clonal propagation are quite recent (see Haq 2006 for a good summary of clonal propagation trials in jackfruit since the mid-twentieth century) but grafting is now the dominant form of propagation on jackfruit plantations in Thailand, Australia, Indonesia, and elsewhere where jackfruit was introduced (Campbell and Ledesma 2003), leading to new, more uniform cultivars. In countries closer to the probable center of origin and

domestication, seed propagation still dominates; the reasons for this distinction have not been studied.

In all Asian countries where jackfruit is grown, two types of ripe fruits are usually identified – soft and crispy – with different jackfruit consumers preferring one texture over the other and different types being more appropriate for processed products (Haq 2006). It is unknown if this characteristic is due to genetics, as it could also be influenced by environmental conditions, tree age, time in fruiting season, or a combination of factors (see Appendix 1 for a more thorough discussion of fruit texture). In countries that graft jackfruit trees, a few dozen cultivars are regularly produced. These cultivars are characterized by traits such as fruit color, flavor, aroma, texture, and canopy size (Campbell and Ledesma, 2003). In countries where seed propagation is prevalent, trees with certain desirable characteristics (good scent, extraordinarily high yield of fruits, biannual fruiting) have been given names, but they are not cultivars in the sense that they are not reliably reproduced – offspring may or may not carry the desirable trait.

To assist farmers and scientists with their breeding programs, germplasm with known diversity must be preserved throughout jackfruit's range. Because seeds are recalcitrant, genes must be preserved through live germplasm. Currently, field genebanks at universities, botanic gardens, or research sites are the preferred method of conservation for this crop. At least twenty countries have collected germplasm for preservation or research (Haq 2006) with three sites in the United States: the Fairchild Tropical Botanic Garden in Miami, and Agricultural Research Stations in Hawaii and Puerto Rico. These accessions only represent field genebanks of plants moved from their original location; jackfruit is not currently being protected in its natural habitat (*in situ*).

Jackfruit Diversity

Morphological diversity has recently been studied in India (Jagadeesh et al. 2007) and Bangladesh (Azad et al. 2007, Khan et al. 2010), as well as surveyed in Florida (Schnell et al. 2001), where the Fairchild Tropical Botanic Garden keeps a large jackfruit collection of individuals collected throughout the tropics. Great variation was found in India and Bangladesh for quantitative traits, such as fruit size, but little phenotypic variation was observed in the Fairchild collection, which is mostly composed of accessions from outside jackfruit's likely center of origin, with only two trees from India and none from Bangladesh. There are several possible explanations for low phenotypic diversity in the Florida collection: morphological diversity could be greatly influenced by environmental factors, diversity could be greater in the center of origin, or the diversity could simply reflect the reasons why those individuals were selected for collection – if the collector was searching for fruits of a particular type for use in crop breeding, all of the fruits would likely be similar.

At least four studies have conducted assessments of genetic diversity among jackfruit trees. Genetic research was done in the U.S. at the same time as the morphological survey at the Fairchild Tropical Botanic Garden (FTG) in Florida. Using 12 primer pairs, 187 AFLP (Amplified Fragment Length Polymorphism) markers were scored for 26 jackfruit

accessions (Schnell et al. 2001). Jackfruit cultivars studied were originally developed in Indonesia, Australia, Thailand, Malaysia, India, Cambodia, Florida, and Singapore. They only found one real division in the genotypes, with the Indian varieties clustering separately from all of the other cultivars. Overall, they learned that the FTG germplasm contained very little genetic diversity, which matched their assessment of morphological diversity. No individuals from Bangladesh were available for analysis.

Studies assessing diversity between habitat and environmental conditions were conducted in Bangladesh and India. In the Bangladeshi study, isozyme analysis was conducted on 50 second-generation accessions taken from home gardens in five different agro-ecological regions (Bogra, Gazipur, Moulovibazar, Ramgarh, Khulna). These included three areas with distinct rainfall patterns: low, medium, and high, as well as a saline belt and a hill. Accessions were compared using electrophoretic patterns of four enzymes at a time. Their results clustered the Bangladeshi jackfruit into four groups, but these groupings did not match the habitat-based groupings they found in the corresponding morphological study (Azad et al. 2007). They concluded that patterns of germplasm clustering suggest, as expected, that both genetic and environmental components result in the observed diversity, however they suggest using first generation samples in a repeat study (Azad et al. 2007).

Shyamalamma et al. (2008) used AFLP markers (like in the U.S. study) to analyze 50 accessions from across southern India with 8 primers, giving a total of 5,796 unique markers. The results created three clusters of jackfruit in southern India, with some possible environmental relationships (rainfall), and some relationships with morphological characteristics (fruit shape, leaf shape, etc.). The authors classify the genetic diversity of Indian jackfruit as “moderate.” For future study, they suggest comparing a larger list of morphological traits to molecular markers.

Most recently, AFLP markers were used to analyze 50 jackfruit accessions in China. The trees were housed in a germplasm, but were originally collected from throughout three Chinese provinces to represent the greatest possible range of morphological diversity. Eight primers yielded 320 bands that were scored and analyzed for genetic diversity. Results indicated that diversity was slightly higher than what was found in India. Cluster analysis revealed seven groups of jackfruit trees, though the individuals did not group by geographic region, fruit texture, or by the special characteristics of biannual fruiting or low latex content. Three individuals of poor quality (did not produce fruit) did cluster alone into three separate groups (Li Ying-zhi et al. 2010).

Microsatellite markers have not yet been used to analyze jackfruit, despite their increasing use in crop diversity studies. Crop research comparing the utility of AFLP and microsatellite analysis has found that microsatellites detect higher levels of polymorphisms than AFLPs, improving cultivar differentiation (Jakše et al. 2001, Powell et al. 1996). Furthermore, microsatellite analysis and sequencing are the preferred techniques for answering questions of domestication, as AFLPs are dominant markers and results can underestimate the chance of identifying multiple domestications (Burger et al. 2008).

Future of Jackfruit

Despite the importance of jackfruit as a staple crop in Southeast Asia and the large range it now occupies, it is underrepresented in the scientific literature. Not much is known about it's origins, domestication, pollination, or genetic diversity. Jackfruit is listed as an underutilized crop by several international organizations (Global Facilitation Unit for Underutilized Species, International Centre for Underutilized Crops, International Plant Genetic Resources Institute) (Jaenicke 2006, ICUC 2003, Hossain 1996). Because of this recognition, and because it is high yield, low input, and nutritious (jackfruit can be used as a significant source of carbohydrates and cartenoids (Rahman et al. 1999, deFaria et al. 2009), especially in countries concerned with malnutrition and vitamin A deficiency (Chandrika et. al 2005)), jackfruit is being promoted internationally for the various causes of food security, rural development, health and nutrition, poverty alleviation, and women's empowerment.

Bangladesh

Jackfruit is the national fruit of Bangladesh. Bangladesh had a population of 156 million in 2009, ranking 7th amongst the world's countries. In area, however, Bangladesh covers only 55,598 square miles, making it the 5th most densely populated country in the world, behind 4 small island and city-state nations. Seventy-three percent of Bangladeshis live in rural areas (CIA World Factbook 2009), and 62% of the workforce is employed in agriculture (Alam 2005). The 2002 per capita income was US$370, with 36% of the population living in poverty and one quarter of the country's GDP coming from agriculture (World Bank 2002). Within that, 57% of the agricultural GDP comes from crops, with forestry, fisheries, and livestock making up the remainder. Seventy-five percent of Bangladesh's cropland is devoted to rice (Alam 2005), but fruit crops are usually grown in private homegardens. Homegardens provide approximately 80% of total fruits and 85% of fuel wood and timber grown in Bangladesh (Rahim 1994).

Bangladeshi weather is extreme, and is predicted to grow more unpredictable with rising sea levels and precipitation changes as a result of climate change. Bangladesh is a delta; at least one-fifth of the country floods every year. Pre-monsoon season temperatures reach an average high of 36.7 degrees Celsius; monsoon season lasts four months, followed by a cooler, drier winter. The topography and geographic location of Bangladesh make it susceptible to cyclones, tornadoes, and, of course, flooding. Climate models predict that rising sea levels, melting Himalayan glaciers, increased precipitation, and drier winters will increase incidences of floods, cyclones, and droughts (Christensen et al. 2007). In addition, increased salinity is already changing coastal habitat and agricultural fields (Agrawala et al. 2003).

Another issue of environmental concern for Bangladesh is a decrease in forest cover. Before Great Britain divided its Southeast Asian colonies in 1947, a border did not separate Bangladesh from India in the north, and Bangladeshis could access forests in West Bengal and Assam. After the borders were set, pressure for timber and land increased, resulting in a decrease of the little forest that remained. Tree cover of

Bangladesh is estimated at 9-10%, with 35 of 64 districts having no forest cover, making it impossible for Bangladesh to meet its own demands for timber, firewood, and fodder (Ahmed 2011). Much of the existing forestland is left over from private forests of the colonial period. In addition, public forests are still managed for restoration and use of forest products, and are home for indigenous tribes (pers. obs.). The lack of forestland and the history of management of these forests means it is unlikely that any wild jackfruit trees will be found in Bangladesh, if they ever existed.

Jackfruit production covered an estimated 25,000 acres of land in Bangladesh in 2010, and produced over one million metric tons of fruit, ranking fifth in land cover among fruits, after banana, mango, melon, and pineapple, and ranking a very close second in tons of fruit produced, after mango (Bangladesh Bureau of Statistics 2010). Bangladesh exports only a minimal amount of the fresh fruit and does not have processing facilities to export other jackfruit products; most jackfruit is sold within the country.

Almost every family in Bangladesh has access to a homegarden, and jackfruit can be found in almost every homegarden, where it is the second most common homegarden tree, after mango (Ahmed 2011, Hocking et al. 1996, Khan et al. 2010). It is also planted between rows in coffee, pineapple, and other crop plantations, and occasionally in jackfruit and mixed-fruit orchards (Haq 2006, pers. obs.). It can be seen growing along streets, on school and university campuses, and in city courtyards (pers. obs.). At some institutions, particular trees are designated for particular employees, dorms, or grade levels, who may collect the fruit for their own use (pers. comm.).

In Bangladesh, jackfruit is harvested from mid April to mid July (Bangladesh Bureau of Statistics 2010). Jackfruit growers usually sell some portion of their crop and keep some for personal use. The current marketing system for fresh fruit in Bangladesh does not benefit the local grower; several layers of middlemen usually come between the farmer's profit and the final profit that is gained from sale to the consumer (Rahman et al. 2006, Haq 2006, Haq and Hughes 2002). Because fruit quality is almost impossible to ascertain without opening each fruit, and because consumer demand has not yet pressured wholesalers or farmers for higher quality fruits, farmers are able to sell poor quality fruits for market (pers. obs. and comm.). Jackfruit timber is very valuable, and most homegarden farmers expect each tree to provide profit from both fruit and timber.

Farmers plant trees from seed or from saplings (which are also seed propagated) purchased at local markets and traveling sapling sales. Saplings have increased in popularity since the emergence of jackfruit nurseries in the 1980s. Saplings are easier to protect from damage (such as grazing of domestic animals) and they fruit earlier because they are usually around 2 years old at the time of purchase, making them seem like a good investment for homegarden owners (Haq 2006). Fruit quality and consistency are not listed among the advantages of saplings presented in the literature, and a range of morphological diversity is present in both seed- and sapling-propagated trees. Morphological diversity can be seen in fruit size, color, texture, taste, season, and yield, and in other characteristics of the tree, including leaf size, leaf color, leaf shape, canopy shape, and location of fruits on the tree (e.g. roots, trunk, primary branches, secondary

branches, etc.) (Azad et al. 2007, Haq 2006, Khan et al. 2010, pers. obs.). In addition, trees can be observed across Bangladesh that possess undesirable traits that make fruits inedible, such as fruits made up partially or completely of unfertilized flowers, fruits that split before ripe, or fruits that drop off the tree before ripe (pers. obs.). These trees are usually not culled in Bangladesh due to the value of jackfruit timber (pers. comm.).

Bangladeshis see the possible profits of developing stronger jackfruit processing and export markets, as is seen in Thailand and some other Southeast Asian countries. To achieve success in the processing and export markets, more uniform cultivars are needed. Currently, Bangladeshi farmers do not grow specific jackfruit cultivars, though cultivars are common for some other fruits, like mango and guava (Rahman et al. 2006, Rahman et al 2007). Bangladeshi research stations and universities are using vegetative propagation methods, including grafting and tissue culture, to produce reliable jackfruit cultivars with desirable characteristics (Haq 2006, Haq and Hughes 2002). However, these clones are rarely available to jackfruit growers outside the institutions (pers. comm.). Germplasm is also being collected at Bangladeshi research stations as a way of accessing diverse traits for breeding. Bangladesh is also collaborating with international research organizations such as the Underutilized Tropical Fruits in Asia Network (UTFANET). UTFANET was formed in 1995 to promote the development of jackfruit, pummelo, and mangosteen in nine Asian countries (Bangladesh, India, Indonesia, Nepal, Pakistan, Philippines, Sri Lanka, Thailand, and Vietnam). The network's goals for jackfruit include the development of commercial cultivars and promotion of the fruit to growers and consumers. UTFANET helped to identify desirable individual genotypes for cloning in Bangladesh and the other countries, though it did not measure existing genetic diversity in the populations. Selected material was then used to produce "mother trees" for future vegetative propagation by local researchers (Haq and Hughes 2002).

Jackfruit promotion in Bangladesh

Since the early 1980s, jackfruit has been promoted throughout Bangladesh for a variety of reasons and by several different organizations. Jackfruit has been included as one species promoted for forest restoration efforts in initiatives such as the Community Forestry Project in north Bengal, which began in 1982 (Ahmed 2011). Jackfruit has also been promoted in projects that are attempting to improve the yield of homegardens through multi-story agroforestry (Rahim 1994).

In addition to the benefits of increased forest cover and more efficient land use, jackfruit is promoted as a good source of extra income for the poor and a way of empowering women, who often tend to the trees (Ahmed 2011, Ali 2005). These goals, along with a need for planting material for forest restoration projects, have led to the promotion of jackfruit nurseries. Beginning in the early 1980s, several government and NGO initiatives began promoting and assisting in the development of tree nurseries. Nurseries provide income, business experience, and leadership opportunities for local villagers. Participants are given either materials or low interest loans, as well as minimal training in nursery management and sapling care (Ali 2005).

The Village and Farm Forestry Program (VFFP), sponsored by the Swiss Agency for Development and Cooperation, began training local Bangladeshi villagers as nursery growers in 1986. Between 1986 and 1995, almost 6 million trees were planted in several regions of Bangladesh through this program. Although participants could choose from dozens of tree species, jackfruit was the fifth most planted tree, after mahogany, mango, eucalyptus, and coconut (Hocking et al. 1996). Jackfruit saplings in VFFP-sponsored nurseries are planted from seed and then sold to other villagers.

In 1985, the Proshika Center for Human Development, a Bangladeshi NGO, began its Social Forestry Program (SFP). It promoted the planting of trees on public and private land throughout the country and the development of village nurseries. By 2005, Proshika's SFP program had reached 1.3 million homegardens, planted 13.78 million saplings, and planted trees at 552 government institutions and schools. Of the trees planted, 12% were jackfruit (Ali 2005). It is unknown how seeds are selected for the planting of saplings in Proshika-sponsored nurseries or how saplings are chosen for transplanting.

Similarly, another NGO, the Rangpur Dinajpur Rural Development Service (RDRS), started a Social Forestry Program in 1977 in northwest Bangladesh. The goal of the RDRS program was to empower local villagers to plant trees along roadsides by granting them rights to the products from their assigned trees. In 2007, the RDRS SFP involved 13,586 households, 6,108 km of roadsides, and 277 hectares of plantations. Jackfruit represents 4% of total trees planted (Ali 2005). RDRS reports that a large number of local tree nurseries have developed in northwest Bangladesh to handle the SFP's demand for saplings (RDRS Bangladesh 2003).

In addition to privately owned nurseries that were established either independently or with NGO/government support, there are also tree nurseries managed by government agencies, such as the Forest Department or the National Parks (pers. obs.), and NGOs. For example, Heed Bangladesh, a local NGO, runs a nursery that produces saplings including mango, jackfruit, guava, papaya, and coconut in the Moulavibazar district (Heed Bangladesh 2011). The promotion of jackfruit nurseries has succeeded in increasing the popularity of nursery-raised, seed-propagated saplings in Bangladesh.

These projects were put in place without an initial measurement of genetic diversity. They do not include strategies for monitoring genetic diversity. Training of nursery owners is limited and does not include instructions for selecting seeds or mitigating possible diversity loss through conservation of germplasm. Some agencies claim that nurseries provide higher quality planting material to tree farmers (Ahmed 2011, SDC 2011), but there is no evidence in the literature that nurseries monitor or assess the quality of either seed source or sapling material. In practice, most nursery owners do not select or sort seeds for any particular reason (Quddus 2011, pers. comm.) Although there does not appear to be intentional selection of seeds for nurseries, there could be unintentional selection occurring due to large numbers of saplings emerging from each nursery and being distributed throughout the country, or for reasons not yet understood. There may also be selection pressures on the selection of saplings, and there is some suggestion that

nursery saplings are of poor genetic quality, as nursery owners choose tall saplings with shallow roots. Farmers also tend to choose tall saplings from the market (Quddus 2011).

As more jackfruit growers choose to buy saplings rather than select their own seed, the genetic makeup of the entire Bangladeshi jackfruit population could be in transition. The bottlenecking of genetic material through selection by nursery owners (whether intentional or unintentional) could lead to a slow loss of genetic variation. Alternatively, nurseries could be exercising less selection than direct-seed farmers and assisting the dispersal of previously unselected alleles and genotypes, leading to increased genetic variation of the total jackfruit crop. Furthermore, it is the recommendation of many researchers that genetic diversity be quantified now, before the development of uniform cultivars in Bangladesh, and that germplasm be preserved for the conservation of Bangladesh's national fruit and the benefit of future jackfruit breeders.

Research Objectives

Loss of crop diversity, especially plant genetic resources that farmers use when breeding and improving crops, is an issue of international concern. A diverse gene pool is essential for ensuring the capability of crops to adapt in the future. Wild and unimproved populations often contain important traits for improving agricultural production and maintaining sustainable agroecosystems, including resistance to pests and diseases; tolerance of drought, salinity and other abiotic stresses; and the ability to achieve higher yields and quality. The demand for genetic material for breeding new adapted cultivars is ongoing (Heywood et al. 2007). In addition to concerns about meeting the needs of current and future crop breeders, decreases in genetic diversity within a population can result in crop failures that negatively affect farmers, consumers, and the economy, as we have seen previously in wheat, potato, rice, corn, coffee, and tobacco (Heal et al. 2004).

Decreases in levels of genetic variation can be caused by changes in propagation; a transition to intensive breeding that leads to uniform cultivars is associated with diversity loss. In traditional agroecosystems, changes in seed and vegetative material exchange practices have led to changes in genetic diversity, as seen in crops like cassava and maize (Dyer et al. 2011, Dyer and Taylor 2008). The intentional or unintentional introduction of transgenic individuals into an environment can alter the level of genetic variation present in the target species, its wild relatives, and the associated pests (Andow and Zwahlen 2006). A shift in rice propagation from seedling transplantation to direct seeding may have contributed to the evolution of weedy rice genotypes (Chen et al. 2004). Vegetative propagation has also been associated with diversity loss in some species (Pissard et al. 2006). Propagation methods have influenced crop evolution through both random and systematic changes in farmer practices, and can lead to both losses of genetic diversity through population bottlenecks and increases in diversity through the introduction of non-local germplasm (Dyer and Taylor 2008). In addition to changes in propagation, deforestation, which may have been caused by increased agriculture, war, natural disaster, or other reasons, leads to a loss of fallow crop populations. Fallow crop populations are acted on by natural selection and often contain traits that contribute to

crop improvement, such as resistance to pests, disease, and abiotic stress (Smith and Schultes 1990).

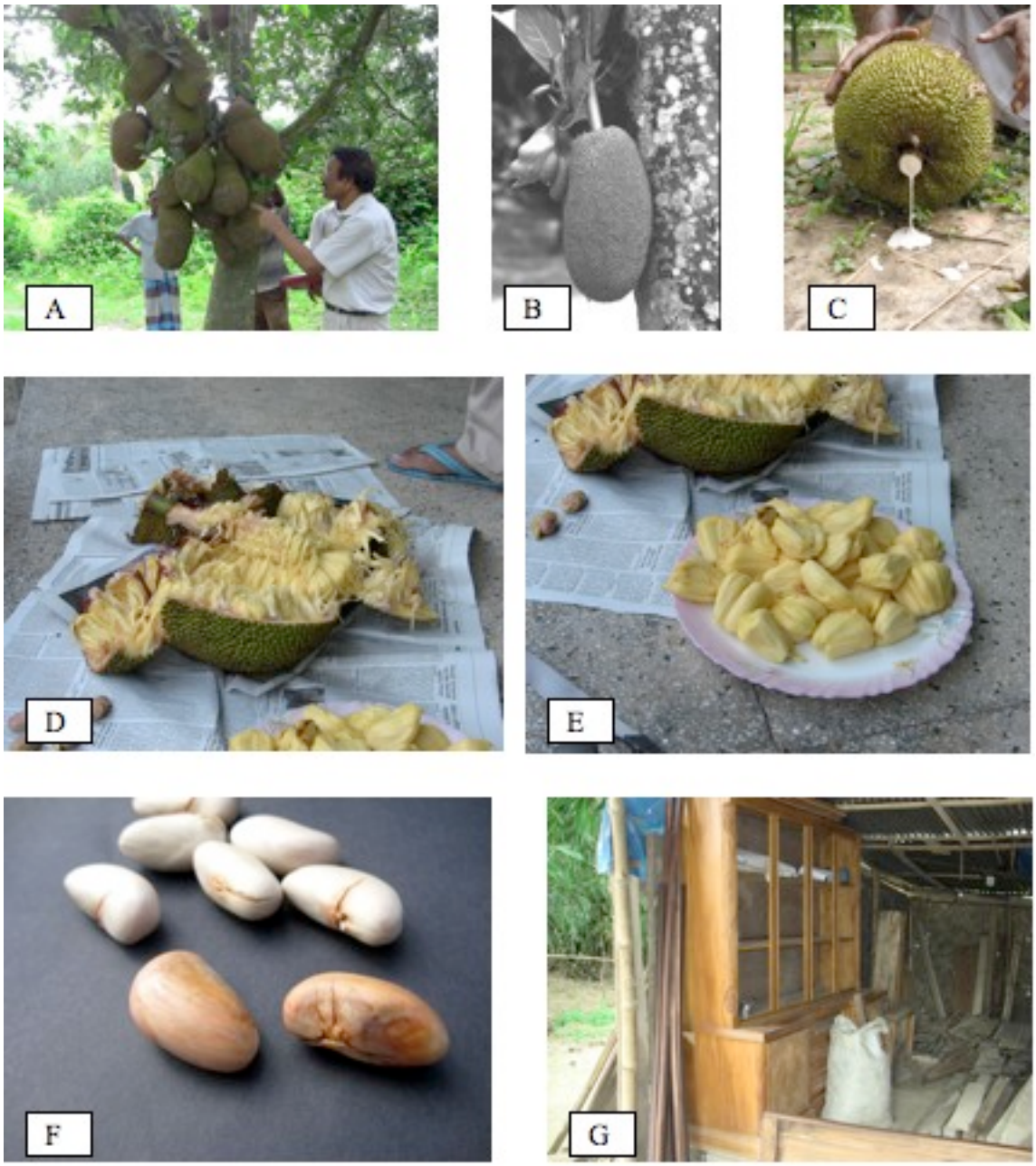
Bangladesh could be a prime source of genetic material for future jackfruit improvement due to the presence of great morphological diversity and undesirable "wild" phenotypes that could harbor useful traits. These traits may not be present in countries with more selective cultivation for several reasons. Currently, Bangladeshis do not practice hand pollination or vegetative propagation, resulting in a cross-pollinated, seed-propagated crop. Trees producing poor quality fruits are kept in the gene pool due to the value of jackfruit timber and the ability to sell poor quality fruits at market. Bangladesh may also possess more jackfruit diversity because it may be near the possible center of origin, making it less likely to have been affected by historical bottlenecks.

There are signs, including the popularity of seed-propagated nursery-raised saplings and new research by Bangladeshi scientists into vegetative propagation, that jackfruit cultivation in Bangladesh is headed toward more uniform cultivars. These cultivars would improve the quality of the fruit, making it easier for Bangladeshi jackfruit growers to market, export, and profit from their crop. Unfortunately, the development of uniform cultivars could also decrease the existing level of genetic variation, as we have seen in wheat, potato, rice, corn, coffee, and tobacco (Heal et al. 2004). Bangladesh is in a unique position to address the loss of diversity in one of its more important crops before the shift to uniform cultivars occurs. Now is the time to organize efforts to conserve jackfruit genetic resources by getting a baseline of diversity to measure future losses or gains, identifying patterns of genetic structure to shed light on the evolution and domestication history of the crop, measuring changes in diversity, identifying causes of possible diversity loss, and developing strategies for conserving diversity while meeting the goals of crop improvement.

Images

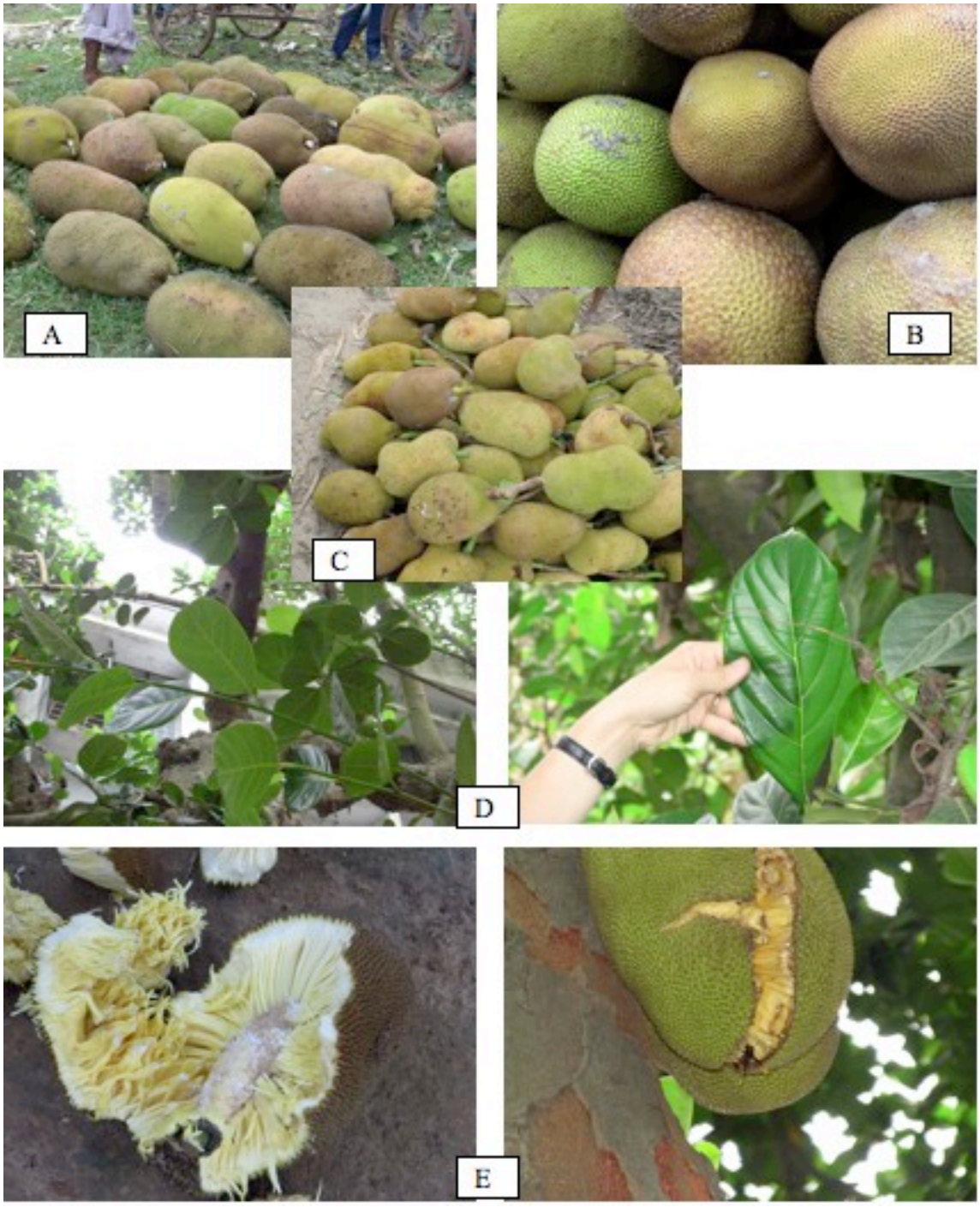
Jackfruit morphology

A. Jackfruit tree showing cauliflorous fruits. B. Male and female inflorescences; scale bar is 5 cm. C. Latex from harvested fruit. D. Open fruit showing pollinated bulbs and unpollinated flowers. E. Edible ripe jackfruit bulbs. F. Jackfruit seeds; seeds may be roasted for eating. G. Jackfruit wood used for furniture. All photos by Colby Witherup or Nyree Zerega, except jackfruit seeds by http://tenthousandtastebuds.wordpress.com.



Morphological diversity of Bangladeshi jackfruit

A. Variation in rind color of large fruits. B. Variation in rind texture. C. Various fruit shapes. D. Various leaf shapes and sizes. E. Undesirable fruit traits; unpollinated fruits, fruits with split rinds.



Jackfruit propagation and distribution in Bangladesh

A. Fruits displayed for sale to middlemen at a local village. B. Fruits for sale in Dhaka. C. Saplings raised in Lawachara National Park in Sylhet, for planting within the park. D. Saplings growing at Hortus Nursery in Savar, a commercial nursery. E. Tree growing in a homegarden. F. Jackfruit trees growing in a mixed-fruit orchard in Sylhet district.

Development and characterization of new microsatellite loci for the jackfruit tree, *Artocarpus heterophyllus* (Moraceae)

Abstract

- *Premise of the study:* Jackfruit, *Artocarpus heterophyllus* (Moraceae) is a cultivated tropical fruit tree that is native to Southeast Asia but grown throughout much of the tropics. Microsatellite primers that were developed for *Artocarpus altilis* were tested and characterized for *A. heterophyllus*. These primers provide new tools for further studies in jackfruit cultivar identification and germplasm diversity assessment.
- *Methods and Results*: Ten microsatellite primers were characterized using 394 *A. heterophyllus* individuals collected from Bangladesh and 19 individuals representing cultivars from Thailand, Indonesia, Malaysia, Jamaica, Singapore, Australia, India, and Miami. The primers amplify 13 microsatellite regions, and the number of alleles for each region ranges from 4-16. All of the regions are polymorphic and the average number of alleles per microsatellite region is 9.1.
- *Conclusions*: Results indicate that the primers will be useful in future studies of jackfruit diversity and genetic structure, and in understanding jackfruit domestication.

The genus *Artocarpus* J. R. Forst. and G. Forst. (Moraceae) contains several species of economic and agricultural significance throughout the tropics. While many produce timber or fruits of only regional significance, two species are cultivated throughout the tropics: breadfruit (*A. altilis* (Parkinson) Fosberg) and jackfruit (*A. heteropyhllus* Lam.) (Zerega et al. 2010). *Artocarpus heteropyhllus* is a monoecious tree that is cultivated throughout Southeast Asia for its fruit and timber. It has been introduced in the Philippines, Australia, and throughout Africa and the Neotropics. It produces the world's largest tree-borne fruit structure and is listed as an underutilized crop by several international organizations (Jaenicke, 2006). Its origins and domestication history are unclear. In some countries, jackfruit propagation involves grafting superior cultivars onto rootstock (Campbell and Ledesma, 2003), but in other places, like Bangladesh, propagation is still predominantly by seed (Witherup et al. unpublished). Despite the prominence of jackfruit in Southeast Asian diets, little is known about the diversity of jackfruit germplasm, the effects of modern propagation methods and environmental factors on genetic diversity, or how to differentiate between cultivars (Azad et al., 2007, Schnell et al., 2001, Shyamalamma et al., 2008). Microsatellite primers that were originally developed for *A. altilis* (Zerega et al. unpublished) were tested on 394 *A. heteropyhllus* samples collected in Bangladesh, as well as 19 samples from germplasm collections in the USA, Puerto Rico, and Jamaica. Thirteen polymorphic loci were identified that will aid in future studies of *A. heterophyllus* diversity and cultivar identification.

Methods and results

To construct the genomic library, leaf tissue was obtained from a single *Artocarpus altilis* individual (voucher deposited at PTBG) collected from Viti Levu, Fiji (Living Accession 900260002 at the Breadfruit Institute, Hawaii). DNA from the leaf tissue was extracted using Qiagen DNeasy (Qiagen, Valencia, CA) extraction kits following standard protocol. The microsatellite libraries were developed by Genetic Identification Services (Chatsworth, CA) following the methods of Jones et al. (2002) and from approximately 100 ng of genomic DNA. The libraries were enriched for four repeat motifs—(GA)*n*, (CA)*n*, (ATG)*n*, and (TAGA)*n*. One hundred clones were sequenced, and primer pairs were designed for 38 unique microsatellite sequences using DesignerPCR version 1.03 (Research Genetics, Huntsville, AL).

Twelve *A. heterophyllus* individuals were used to initially screen 75 microsatellite primer pairs to test for amplification and levels of polymorphism among those 38 unique sequences. DNA was extracted from silica-dried leaf tissue using Qiagen DNeasy (Qiagen, Valencia, CA) extraction kits and protocols. For PCR reactions, each forward primer had a M13 tail (5′- CACGACGTTGTAAAA -3′), and M13 primer labeled with Wellred Dye D2, D3, or D4 was added to each reaction, following Schuelke (2000). PCR reactions for each microsatellite locus utilized a two-step process. First, 10 ul reactions contained 5 ul of Master Mix (Promega, Madison, WI), 0.5 ul of 10mg/ml BSA, 0.25 ul of 10uM forward primer with the M13 tail, 0.25 ul of 10uM reverse primer, 3 ul of H_2O, and 1 ul of template DNA. PCR conditions for the first step were 94ºC for 3 min, 13 cycles at 94ºC for 30 s, 59.8ºC for 30 s, 72ºC for 1 min and a final extension of 72ºC for 10 min. Then, to each 10 ul reaction was added 2.5 ul Master Mix (Promega, Madison, WI), 0.25 ul of 10 mg/ml BSA, 0.125 ul of 2.5uM $MgCl_2$, 0.25 of 10 uM labeled M13 primer, and 1.875 ul of H_2O for a total reaction of 15 ul. PCR conditions for the second step were 94ºC for 3 min, 27 cycles at 94ºC for 30 s, 55ºC for 30 s, 72ºC for 1 min and a final extension of 72ºC for 10 min. PCR products were run on a 1% agarose gel stained with SYBR Green (Proligo, Hamburg, Germany) and UV light was used to visualize bands and check for amplification. Thirty-eight primer pairs yielded a single band or two bands that were consistently different sizes, and the PCR products were analyzed on a Beckman Coulter CEQ 8000 Genetic Analysis System. Before adding samples to the CEQ, PCR product (0.5 ul of Wellred Dye D4-labeled product, 1 ul of Wellred Dye D3-labeled product, or 2.5 ul of Wellred Dye D2-labeled product) was added to 30 ul of HiDi formamide (Azco Biotech., San Diego, CA) and 3.3 ul 400bp size standard ladder (Beckman Coulter, Brea, CA). Alleles were scored using the CEQ 8000 software v9.0. Ten primer pairs showing reliable amplification and allelic polymorphism were chosen to run on 394 *A. heteropyhllus* individuals from Bangladesh, as well as 19 additional trees growing in germplasm collections in Miami (Fairchild Tropical Botanic Garden), Puerto Rico (USDA TARS), Hawaii (USDA ARS), and Jamaica (private collection), representing original collections from Thailand, Indonesia, Malaysia, Jamaica, Singapore, Australia, India, and Miami. PCR products were analyzed on a Beckman Coulter CEQ 8000 as described above. Three primer pairs showed amplification and polymorphism in two unlinked regions of differing lengths. These regions were sequenced for both *A. altilis* and *A. heterophyllus* and likely represent historical duplications that have since diverged (see Karhu et al., 1999 for an example in *Pinus*). Since these regions were straightforward to score with no ambiguities, and since *A. heterophyllus* is known to be

diploid, the regions have been treated separately for analysis.

GenAlEx v.6.4 (Peakall and Smouse, 2006) was used to evaluate observed and expected heterozygosity of the 13 microsatellite regions. Number of alleles per region ranged from 4 to 16 (Table 1). Because these primers were tested for use in *A. heterophyllus* across several disparate populations, and because selection pressures on this agricultural crop are not yet well understood, deviation from Hardy-Weinberg equilibrium may not be unexpected.

Conclusions

Thirteen microsatellite regions were identified for *A. heterophyllus* using 10 primer pairs originally designed for *A. altilis*. These primer pairs will be useful in future studies aimed at measuring and comparing jackfruit crop diversity, differentiating cultivars, and understanding jackfruit origins and dispersal. The primers have proven reliable for at least three other species in the genus (Zerega et al., unpublished), and they may be useful for additional *Artocarpus* species.

TABLE 1. Characteristics of 13 microsatellite loci amplified in *Artocarpus heterophyllus*.

Primer	Sequences[1]	Repeat[2]	T_a (ºC)[3]	GenBank Accession Number	Allele Size (bp)	N_a	H_e	H_o
MAA26	F: CATGAATGAAACAACATCAGAC R: CGCAGGGCTTTATGACTAT	$(GT)_9$	59.8/55.0	JQ952762	273-297	10	0.704	0.659
MAA54a	F: AACCTCCAAACACTAGGACAAC R: AGCTACTTCCAAAACGTGACA	$(CA)_5,(AT)_4$	59.8/55.0	JQ952763	181-187	4	0.095	0.039
MAA54b	F: AACCTCCAAACACTAGGACAAC R: AGCTACTTCCAAAACGTGACA	$(AT)_9,(CA)_6,(AT)_4$	59.8/55.0	JQ952764	211-239	9	0.783	0.531
MAA105	F: GTTGGGACACTGTGAACTATTC R: AAAAGCTAGTGGATTAGATGCA	$(GT)_{11}$	59.8/55.0	JQ952765	265-293	13	0.627	0.286
MAA122	F: CTGGCCTTCAGTTTTGTCAAC R: CACCAGGCTTCAAGATGAAA	$(GT)_{11}(GA)_4,(GA)_{11}$	59.8/55.0	JQ952766	254-312	16	0.265	0.206
MAA140	F: CCATCCCCCATCTTTCCT R: TCCTCGTTTGCCACAGTG	$(CT)_{25}$	59.8/55.0	JQ952767	142-160	8	0.634	0.507
MAA145	F: CCAACGCATAGCCAAATC R: AAATCCCAAACCCAACGT	$(CTT)_9,(GA)_{14},(GA)_8$	59.8/55.0	JQ952768	275-299	8	0.715	0.321
MAA156	F: CTGGTGCTTCAGCCTAATG R: TCAGCGTCAAAGATAACTCG	$(GA)_3,(GA)_5,(GA)_8,(GA)_{13}$	59.8/55.0	JQ952769	283-307	6	0.279	0.293
MAA178a	F: GATGGAGACACTTTGAACTAGC R: CACCAGGGTTTAAGATGAAAC	$(GT)_3,(GT)_6,(GT)_3,(GA)_3,(GA)_{10}$	59.8/55.0	JQ952770	250-258	4	0.201	0.153
MAA178b	F: GATGGAGACACTTTGAACTAGC R: CACCAGGGTTTAAGATGAAAC	$(GT)_3,(GT)_3,(GA)_3,(GA)_{11}$	59.8/55.0	JQ952771	268-284	8	0.418	0.363
MAA182	F: TACTGGGTCTGAAAAGATGTCT R: CGTTTGCGTTTGGATAAAT	$(CT)_{19}$	59.8/55.0	JQ952772	186-216	13	0.338	0.293
MAA196a	F: GGAATGTGGTAGATGAAACTCC R: CGACAAAAAAACAAAGGAAGAC	$(CT)_{11},(GA)_4$	59.8/55.0	JQ952773	283-315	7	0.335	0.167
MAA196b	F: GAATGTGAGAGATAAATCTCC R: CGACAAAAAAACAAAGGAAGAC	$(CT)_{12}$	59.8/55.0	JQ952774	337-377	12	0.508	0.093

Notes: T_a = annealing temperature; N_a = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity [1]All forward primers were tagged; [2]Commas indicate presence of non-repeating nucleotides between repeats; [3]PCR was done using a two-step process (see methods).

APPENDIX 1. Location data for *Artocarpus heterophyllus* sample trees used to characterize microsatellite loci.

TABLE 1. Samples from Bangladesh. 394 individuals were collected from Bangladesh by the authors. A representative herbarium voucher was made for most sites. A photo voucher exists for all samples analyzed. Voucher specimens are deposited at the Nancy Poole Rich Herbarium at the Chicago Botanic Garden (CHIC). N = number of samples analyzed from each site.

N	Date collected	Site	District	GPS Lat	GPS Long	Accuracy	Voucher
12	5-Jul-10	Madhupur village	Tangail	N24.61475	E090.03149	10 m	CW12
21	6-Jul-10	Mohismara village	Tangail	N24.59060	E90.12699	16 m	CW24
30	6-Jul-10	GachaBari village	Tangail	N24.67237	E090.07662	14 m	
27	8-Jul-10	Bangladeshi Tea Research Institute, Srymangal	Sylhet	N24.29532	E091.74686	15 m	CW65
29	9-Jul-10	Ashidu orchard and village	Sylhet	N24.28276	E091.76509	11 m	CW92
36	9-Jul-10	Lawachara National Park	Sylhet	N24.31972	E091.78361	20 m	CW125
23	10-Jul-10	Bangladesh Agricultural Research Institute	Sylhet	N25.13075	N92.118263	20m	CW160
13	11/19-Jul-10	National Botanic Garden of Bangladesh	Dhaka	N23.81300	E90.34690	20 m	CW288
13	12-Jul-10	Bangladesh Agricultural Research Institute	Gazipur	N23.99420	E090.41130	9 m	
16	12-Jul-10	Bagabazar village	Gazipur	N24.16302	E090.43024	62 m	CW205
19	14-Jul-10	Leather Research Institute	Savar	N23.91534	E090.23549	20 m	CW221
28	16-Jul-10	Madan hati village	Rajshahi	N24.48321	E088.59244	10 m	CW240
17	17-Jul-10	Nimtoli village	Jessore	N23.16448	E089.29896	12 m	CW274
31	20-Jul-10	Jahangirnagar University	Savar	N23.88113	E090.26915	12 m	CW294
11	20-Jul-10	Gono University	Savar	N23.91812	E90.24538	14 m	
30	21-Jul-10	Khula Pater village	Comilla	N23.67553	E091.17191	11 m	CW333
20	23-Jul-10	Council of Scientific and Industrial Research	Dhaka	N23.74027	E090.38531	19 m	CW368
18	20-Jul-10	Hortus Nursery	Savar	N23.91812	E90.24538	14 m	

TABLE 2. *Artocarpus heterophyllus* samples from germplasm collections. Nineteen samples were acquired from living germplasm collections. Photo vouchers exist for all CW collections. CHIC = Nancy Poole Rich Herbarium at the Chicago Botanic Garden

Sample name	Tree owner	Tree location	Cultivar origin	Voucher
HART27	USDA Agricultural Research Station	Hilo, Hawaii	Thailand	
TARS1566-5	USDA Tropical Agricultural Research Station	Puerto Rico	Indonesia	
TARS1566-10	USDA Tropical Agricultural Research Station	Puerto Rico	Indonesia	
TARS18002-7	USDA Tropical Agricultural Research Station	Puerto Rico	Malaysia	
TARS18002-8	USDA Tropical Agricultural Research Station	Puerto Rico	Malaysia	
JM1	Private owner	Jamaica	Jamaica	
JM2	Private owner	Jamaica	Jamaica	
JM3	Private owner	Jamaica	Jamaica	
CW383	Fairchild Tropical Botanic Garden	Miami	Australia	CHIC
CW384	Fairchild Tropical Botanic Garden	Miami	Singapore	CHIC
CW385	Fairchild Tropical Botanic Garden	Miami	Thailand	CHIC
CW388	Fairchild Tropical Botanic Garden	Miami	Australia	
CW389	Fairchild Tropical Botanic Garden	Miami	Thailand	
CW395	Fairchild Tropical Botanic Garden	Miami	India	CHIC
CW396	Fairchild Tropical Botanic Garden	Miami	Malaysia	
CW403	Fairchild Tropical Botanic Garden	Miami	Malaysia	
CW405	Fairchild Tropical Botanic Garden	Miami	Malaysia	CHIC
CW406	Fairchild Tropical Botanic Garden	Miami	Australia	CHIC
CW410	Fairchild Tropical Botanic Garden	Miami	Miami	

Jackfruit (*Artocarpus heterophyllus* Lam., Moraceae) genetic diversity and structure in Bangladesh and beyond

Abstract
High levels of genetic diversity are required for crop improvement. Jackfruit (*Artocarpus heterophyllus*, Moraceae) is an important fruit crop in Southeast Asia. Little is known about its origins or levels of diversity throughout its range. Bangladesh could be a prime source of jackfruit genetic material due to the presence of diverse phenotypes and its proximity to the possible area of origin. As Bangladesh moves from seed propagation to the development of uniform cultivars, genetic diversity could be lost. Thirteen microsatellite markers were used to analyze 394 jackfruit individuals from throughout Bangladesh and 19 individuals from germplasm collected from throughout jackfruit's range. Overall diversity in Bangladesh showed a mean number of alleles of 8.31 ±1.015, expected heterozygosity of 0.443 ±0.063, and observed heterozygosity of 0.297 ±0.051. Diversity levels were spread relatively equally among 8 geographic districts of Bangladesh. Very weak correlation was found between genetic differentiation and geographic districts through pairwise comparison of Fst and Structure analysis. Microsatellites were effective for finding genetic structure (K=4) among a small number of jackfruit individuals from throughout its range and should be useful for a broader phylogenetic study.

Background
Loss of crop diversity, especially plant genetic resources that farmers use when breeding and improving crops, is an issue of international concern. A diverse gene pool is essential for ensuring the capability of cultivars to adapt in the future. Wild and unimproved populations often contain important traits for improving agricultural production and maintaining sustainable agroecosystems, including resistance to pests and diseases; tolerance of drought, salinity and other abiotic stresses; and the ability to achieve higher yields and quality. The demand for genetic material for breeding new adapted cultivars is ongoing (Heywood et al. 2007).

The genus *Artocarpus* J. R. Forst. and G. Forst. (Moraceae) contains several species of economic and agricultural significance throughout the tropics. While many produce timber or fruits of only regional significance, two species are cultivated throughout the tropics: breadfruit (*A. altilis* (Parkinson) Fosberg) and jackfruit (*A. heteropyhllus* Lam.) (Zerega et al. 2010). *Artocarpus heteropyhllus* is a monoecious tree that is cultivated throughout Southeast Asia for its fruit and timber. It produces the world's largest tree-borne fruit structure and is listed as an underutilized crop by several international organizations (Jaenicke, 2006). The pollinator of jackfruit is unknown. Exact numbers are not known for the economic value of the world's jackfruit crop, however, it is estimated that nine million metric tons of jackfruit are produced annually in Asia (Haq 2006). The fruit is increasing in popularity in Australia, Europe, the Middle East, and the United States, fueled by demand from Asian immigrants and migrant workers (Rahman and

Talukder 2001), with Thailand, China, and Malaysia being the dominant exporters of fresh jackfruit and jackfruit products (Haq 2006).

Jackfruit has been cultivated for millennia and was referred to as early as 300 B.C. by Theophrastus from his travels in India (Hort 1916). Today it can be found in cultivation at low elevations throughout the Indian subcontinent, Bangladesh, Myanmar, Sri Lanka, Southern China, Nepal, Laos, Vietnam, Cambodia, Malaysia, and has also been introduced in the Philippines, Indonesia, and throughout Africa, Australia, and the Neotropics, especially Jamaica and Brazil. It is now so widely cultivated that it is unclear in which region it is indigenous and which region holds its greatest diversity. The rainforests of the Western Ghats in India (where it is a commercially minor fruit and is seldom found in regular plantations) are generally cited as both the area of origin and center of diversity of jackfruit (Jagadeesh et al. 2007). This citation, however, is based in part on Wight (1843) who observed jackfruit trees growing in primary forest away from human habitation; no update has since been provided on the presence of wild trees in the Western Ghats. Barrau (1976) suggested that the point of origin could be Malaysia due to the large number of cultivars grown there, but no wild trees have been observed there. Jackfruit has also been reported in forests away from human habitation on the Andaman Islands (Bashar and Hossain 1993) and trees with "wild" characteristics have been reported in Bangladesh (Khan et al. 2010), though the status of these trees has not been confirmed. It is possible that jackfruit has a broader center of origin than previously proposed, or that the true point of origin will never be known (Jarrett 1959).

Jackfruit is the national fruit of Bangladesh, one of the poorest and most densely populated countries in the world. Bangladesh could be a prime source of genetic material for future jackfruit improvement due to the presence of great morphological diversity and undesirable "wild" phenotypes that could harbor useful traits (Azad et al. 2007, Khan et al. 2010, pers. obs). These traits may not be present in countries with more selective cultivation for several reasons. Currently, Bangladeshis do not practice hand pollination or vegetative propagation, resulting in a cross-pollinated, seed-propagated crop. Trees producing poor quality fruits are kept in the gene pool due to the value of jackfruit timber and the ability to sell poor quality fruits at market. Bangladesh may also possess more jackfruit diversity because it may be near the possible center of origin, making it less likely to have been affected by historical bottlenecks.

There are signs, including the popularity of seed-propagated nursery-raised saplings and new research by Bangladeshi scientists into vegetative propagation, that jackfruit cultivation in Bangladesh is headed toward more uniform cultivars. These cultivars would improve the quality of the fruit, making it easier for Bangladeshi jackfruit growers to market, export, and profit from their crop. Unfortunately, the development of uniform cultivars could also decrease the existing level of genetic variation over time, as we have seen in wheat, potato, rice, corn, coffee, and tobacco (Heal et al. 2004). Bangladesh is in a unique position to address the loss of diversity in one of its more important crops before the shift to uniform cultivars occurs. Now is the time to organize efforts to conserve jackfruit genetic resources by getting a baseline of diversity to measure future losses or gains, identifying patterns of genetic structure to shed light on the evolution and

domestication history of the crop, identifying causes of diversity loss, and developing strategies for conserving diversity while meeting the goals of crop improvement.

In this study, we use microsatellites to measure genetic diversity of jackfruit in Bangladesh. Four previous studies have looked at genetic structure in jackfruit (Table 1), though this is the first study to use microsatellite markers and to collect from such a broad number of trees *in situ*. We collected leaf material from 394 jackfruit individuals growing *in situ* across eight divisions in Bangladesh (Figure 1). We analyzed data from 13 microsatellite regions in order to establish a baseline measurement of overall genetic diversity in Bangladesh for future research comparing diversity levels between populations or monitoring changes over time. We also hypothesized that analysis would reveal genetic structure within the Bangladeshi population and that genetic groupings would correspond to the geographic districts of Bangladesh, as local differentiation has been seen at microsatellite loci in other tree crops in traditional agroecosystems (Achtak et al. 2010, Zhang et al. 2008). Finally, we acquired 19 samples from germplasm collections in the USA, Puerto Rico, and Jamaica, representing collections provenanced from Thailand, Indonesia, Malaysia, Jamaica, Singapore, Australia, India, and Miami. These individuals and a random selection of Bangladeshi samples were analyzed for genetic structure to determine the strength of microsatellite markers for discerning genetic differences within the international jackfruit population and to establish preliminary data for future phylogenetic studies of jackfruit dispersal.

Methods

Fieldwork was conducted in Bangladesh over three and a half weeks in July, 2010. Study locations were chosen to cover the maximum number of Bangladeshi districts in the time available to researchers. Districts were used as populations in order to establish geographic distance between populations, not as an attempt to collect evenly from within each district. Study sites were chosen to represent a broad variety of *Artocarpus heterophyllus* habitats, including private homesteads, a mixed-fruit orchard, a national park, public and private schools and universities, a city park, government agricultural research institutes, and urban and rural government land (Table 2). Individual trees were chosen with the help of tree owners to represent a range of ages, fruit qualities, and propagation methods. Leaf material was collected from each sample tree and stored in silica. DBH was recorded for each tree, along with additional data collected through interviews with tree owners (when present), including age, propagation method (purchased from seed-propagated nursery-raised sapling or self-sowed from seed), fruit quality, fruiting season, fruit size, fruit texture, number of fruits per tree, seed or sapling source, and fruit destination (consume at home or sell to market) (Table 3). Representative voucher specimens were collected from each site and deposited at CHIC. Additional leaf material was acquired from trees growing in germplasm collections in Miami (Fairchild Tropical Botanic Garden), Puerto Rico (USDA TARS), Hawaii (USDA ARS), and Jamaica (private collection), representing original collections provenanced from Thailand, Indonesia, Malaysia, Jamaica, Singapore, Australia, India, and Miami (Table 4).

Leaf material was brought back to the Chicago Botanic garden and stored in a -80 degree Celsius freezer. DNA was extracted using Qiagen DNeasy (Qiagen, Valencia, CA) extraction kits following standard protocol. PCR was conducted for 13 microsatellite loci (Witherup et al., submitted) using a two-step process. First, 10 ul reactions were used containing 5 ul of Master Mix (Promega, Madison, WI), 0.5 ul of 10mg/ml BSA, 0.25 ul of 10uM forward dye-labeled M13 primer, 0.25 ul of 10uM reverse primer, 3 ul of H_2O, and 1 ul of template DNA. PCR conditions for the first step were 94ºC for 3 min, 13 cycles at 94ºC for 30 s, 59.8ºC for 30 s, 72ºC for 1 min and a final extension of 72ºC for 10 min. Then, to each 10 ul reaction was added 2.5 ul Master Mix (Promega, Madison, WI), 0.25 ul of 10 mg/ml BSA, 0.125 ul of 2.5uM $MgCl_2$, 0.25 of 10 uM M13 dye (blue, green, or black), and 1.875 ul of H_2O for a total reaction of 15 ul. PCR conditions for the second step were 94ºC for 3 min, 27 cycles at 94ºC for 30 s, 55ºC for 30 s, 72ºC for 1 min and a final extension of 72ºC for 10 min. PCR product was analyzed on a Beckman Coulter CEQ 8000 Genetic Analysis System. Before adding samples to the CEQ, PCR product (0.5 ul of Wellred Dye D4-labeled product, 1 ul of Wellred Dye D3-labeled product, or 2.5 ul of Wellred Dye D2-labeled product) was added to 30 ul of HiDi formamide (Azco Biotech., San Diego, CA) and 3.3 ul 400bp size standard ladder (Beckman Coulter, Brea, CA). Alleles were scored using the CEQ 8000 software v9.0.

GenAlEx v.6.4 (Peakall and Smouse, 2006) was used to evaluate observed and expected heterozygosity, conduct AMOVA analysis, PCoA, Mantel tests, and to compute Nei's distance and F statistics for the 13 microsatellite regions. Structure (Pritchard 2000) was used to analyze genetic structure within populations, with help from Evanno (2005) for choosing the most likely K.

Results

Total diversity in Bangladesh

The mean number of alleles for all 394 Bangladeshi jackfruit individuals across the 13 microsatellite regions was 8.31 ±1.015, with an expected heterozygosity of 0.443 ±0.063 and observed heterozygosity of 0.297 ±0.051 (Table 5).

Bangladesh regional diversity and structure

Mean number of alleles ranged from 3.46 ±0.312 (N=17) to 5.69 ±0.728 (N=115) for the geographic districts, with Sylhet having the highest number and Jessore the lowest (Table 6, Appendix 1). Number of private alleles ranged from 0 (Jessore) to 12 (Sylhet). Expected heterozygosity ranged from 0.414 ±0.063 (N=17) (Jessore) to 0.439 ±0.062 (N=33) (Dhaka), and observed heterozygosity ranged from 0.245 ±0.063 (N=28) (Rajshahi) to 0.326 ±0.057 (N=33) (Dhaka). AMOVA results were significant and revealed that 96% of variation is distributed within the geographic populations, with only 4% of variation distributed between populations (p=0.010). Pairwise comparison of genetic distance shows a relationship between genetic distance and geographic distance (Figure 2). Dhaka, the capital of Bangladesh, is the economic and geographic center of the country. PCoA shows that Dhaka is also the center of jackfruit diversity, having genetic distances from the other seven populations in the range of 0.006 to 0.025. Jessore, located in Southwest Bangladesh, had the highest genetic distances from the other

populations, with a range of 0.025 to 0.046 (Table 7). However, these Fst values are quite low; for example, genetic differentiation in Central and South American beans showed a range of Fst values from 0.028 to 0.563 (Blair et al. 2009), and for Central African coffee, Fst values ranged from 0.04 to 0.63 (Musoli et al. 2009). Mantel tests of Euclidean distances between populations and Euclidean distances through Dhaka were both insignificant (Table 8). Structure analysis found the strongest likelihood for 4 groups following the method of Evanno (2005) (Figure 3).

International jackfruit structure

When 18 Bangladeshi samples (2 or 3 randomly chosen from each of 8 Bangladeshi regions) were analyzed with 19 samples originally collected from Thailand, Indonesia, Malaysia, Jamaica, Singapore, Australia, India, and Miami, using Structure and the Evanno method of determining K, 4 groups were found (Figure 4). The Bangladeshi samples, though there were more of them than from any other country, clustered together, separate from the samples of other countries.

Discussion

Total diversity in Bangladesh

While it is tempting to qualify diversity as "high" or "low" and to compare diversity measures across those found in other species, this does not necessarily provide meaningful data. Even if microsatellite markers were used in two different species, not all regions are created equally and may not accurately reflect species diversity. Even within species, if the same microsatellite regions are not compared, the results may be misleading. For example, this study finds an expected heterozygosity of 0.443 across 13 microsatellite regions, but if we reported findings from only the 8 regions showing the highest diversity, our expected heterozygosity would be 0.582. It is interesting to note, however, that the expected heterozygosity we found for jackfruit is lower than what has been reported in other perennial crops. In their review of perennial crop studies, Miller and Gross (2011) report a range of expected heterozygosity from 0.640 to 0.814 in studies using microsatellite analysis. Diversity measurements are useful when comparing the same species using the same microsatellite regions, such as comparisons between different populations or within one population over time. It is our hope that other researchers will use these microsatellite regions to measure diversity in other parts of jackfruit's range to gain a clearer picture of how jackfruit genetic diversity is dispersed around the world. The data can also be considered temporally, and results presented here can be used in the future as a baseline of jackfruit genetic diversity in Bangladesh in 2010.

Bangladesh regional diversity and structure

From these results, we can see that diversity is spread relatively equally throughout the country. Private and mean allele numbers for each geographic region correspond to the sample size; further sampling from some locations could reveal more alleles.

Genetic distance evolves through patterns of movement of genetic material, which can be influenced by geographic distance, geographic barriers, or reproductive barriers. For

jackfruit, genetic material moves through seed dispersal, pollen dispersal, and the sale of saplings. Little is known about jackfruit pollen dispersal, but jackfruit saplings and most jackfruit seeds are dispersed by humans. The slight relationship between genetic and geographic distance as revealed by pairwise comparison may reflect Bangladeshi infrastructure and culture, as a Mantel test of Euclidean distance was insignificant (Table 8). Major highways to outer districts (Comilla, Sylhet, Tangail, Rajshahi, and Jessore) all meet in Dhaka, the capitol and center of commerce. This appears to be reflected in the PCoA analysis; for example, although Jessore and Rajshahi are closer on the geographic map than on the PCoA, it is possible that the fruit and sapling trade passes through Dhaka first before heading back out to other districts. A second Mantel test of Euclidean distance passing through Dhaka, however, was also insignificant. Future research could reveal more about the connection between jackfruit genetic structure and trade.

The four groups found by Structure are fairly evenly dispersed among the 8 geographic regions, showing only very weak differences between the geographic regions (Figure 5). There are a few possible explanations for these results. Pritchard suggests that if you see equal dispersal among your populations, the structure may not be real (i.e. K=1) (Pritchard 2000). K=1 is difficult to find using Structure. It is interesting, however, that Azad et al. also found 4 groupings when they conducted isozyme analysis on 50 Bangladeshi jackfruit individuals (2007). A second explanation is that the 4 groups are reflecting a characteristic other than geographic region that has mixed into the populations without being diluted. We checked this possibility against all of the data categories we collected for these trees, including age, DBH, propagation method, fruit quality, fruit texture, and fruiting season, but no connection was found to the four groups (see Figure 6 for an example). A third possibility is that the groups are lingering from introductions of jackfruit into Bangladesh – either there were four genotypes in the original introduction or there have been four introductions at various times in history. Analyzing DNA from jackfruit individuals in countries surrounding Bangladesh (India, China, Myanmar, Nepal), could help clarify the origins and dispersal history of jackfruit in this part of the world.

Low Fst values and the absence of geographic differentiation in the Structure analysis suggest that jackfruit trees in Bangladesh belong to one large interbreeding population. Miller and Gross (2011) report that researchers of long-lived perennial crops usually find only limited population structure when using neutral markers, which would make our results typical.

International jackfruit structure

These results show good preliminary evidence that there are genetic distinctions between populations in different nations and different parts of the world (Figure 7). Among the international samples, samples collected from USDA research stations and a private collection in Jamaica showed relatedness between individuals from the same country of origin. Samples collected from the Fairchild Tropical Botanic Garden did not group by country or with USDA samples from the same nation of origin. However, this could be due to trees being mislabeled, misidentified, or other human error. It is important that a

future phylogenetic study of jackfruit analyzes samples collected directly from the country of origin or from *ex situ* collections that have reliable provenance data.

Conclusions

Our results suggest that both jackfruit diversity levels and individuals of particular genotypic groups are relatively evenly dispersed throughout Bangladesh, although there is evidence of a weak correlation between genetic differentiation and geographical district. Although Sylhet district showed a higher number of alleles and private alleles, it also included the largest sample size. We suggest that, until further genetic research is conducted, *in situ* preservation should concentrate on a selection of trees with varied morphological characteristics in all Bangladeshi districts as a way of preserving existing diversity through the impending demand for vegetative propagation. The capital, Dhaka, exhibited the highest level of expected heterozygosity and the smallest amount of genetic differentiation from the other districts, reflecting the nature of internal commerce in a densely-populated, developing country. We suggest that Bangladeshi jackfruit be reanalyzed with the same microsatellite markers in subsequent years to track possible declines in genetic diversity. Structure analysis of individuals from throughout jackfruit's range shows promise for the use of microsatellite markers in a phylogeographic analysis of the species, which should be conducted using samples collected directly from an expanded number of jackfruit-growing countries.

TABLE 1. A comparison of 4 previous studies assessing jackfruit genetic diversity. N = number of samples analyzed; K = number of genetic clusters identified within the samples tested.

Country	N	Marker	Collected from:	Original source of samples:	K	Citation
USA (Miami)	26	AFLP	germplasm	Indonesia, Australia, Thailand, Malaysia, India, Cambodia, Florida, and Singapore	2	Schnell et al. 2001
Bangladesh	50	isozymes	germplasm	Bangladesh (Bogra, Gazipur, Moulovibazar, Ramgarh, Khulna)	4	Azad et al. 2007
India	50	AFLP	germplasm (30), other sites (20)	Southern India	3	Shyamalamma et al. 2008
China	50	AFLP	germplasm	China (Yunnan, Guangdong, Hainan)	7	Li Ying-zhi et al. 2010

TABLE 2. *Artocarpus heterophyllus* samples from Bangladesh. 394 individuals were collected from Bangladesh by the authors. A representative herbarium voucher was made for most sites. A photo voucher exists for all samples analyzed. Voucher specimens are deposited at the Nancy Poole Rich Herbarium at the Chicago Botanic Garden (CHIC). N = number of samples analyzed from each site.

N	Date collected	Site	District	Site type (# of trees sampled)
12	5-Jul-10	Madhupur village	Tangail	homegardens (9), primary school (3)
21	6-Jul-10	Mohismara village	Tangail	homegardens (19), Forestry Department plot (2)
30	6-Jul-10	GachaBari village	Tangail	homegardens (24), Forestry Department office (6)
27	8-Jul-10	Bangladeshi Tea Research Institute (BTRI), Srymangal	Sylhet	BTRI guest house (5), BTRI campus (22)
29	9-Jul-10	Ashidu orchard and village	Sylhet	fruit orchard (21), homegardens (3), Bugunbari Forest (5)
36	9-Jul-10	Lawachara National Park	Sylhet	national park (21), park tree nursery (15)
23	10-Jul-10	Bangladesh Agricultural Research Institute (BARI)	Sylhet	BARI research campus (18), germplasm from Chittagong region of Bangladesh (5)
13	11/19-Jul-10	National Botanic Garden of Bangladesh	Dhaka	public park (13)
13	12-Jul-10	Bangladesh Agricultural Research Institute	Gazipur	germplasm collected locally (9), BARI campus (4)
16	12-Jul-10	Bagabazar village	Gazipur	homegardens (16)
19	14-Jul-10	Leather Research Institute (LRI)	Savar	LRI campus (19)
28	16-Jul-10	Madan hati village	Rajshahi	homegardens (28)
17	17-Jul-10	Nimtoli village	Jessore	homegardens (17)
31	20-Jul-10	Jahangirnagar University	Savar	university campus (31)
11	20-Jul-10	Gono University	Savar	university campus (11)
30	21-Jul-10	Khula Pater village	Comilla	homegardens (30)
20	23-Jul-10	Council of Scientific and Industrial Research (CSIR)	Dhaka	CSIR campus (20)
18	20-Jul-10	Hortus Nursery	Savar	jackfruit nursery (18)

TABLE 3. Individual tree characteristics collected through interviews with Bangladeshi jackfruit tree owners and caretakers in July 2010. Responses are followed by the number of times they were given; only one response was allowed per category per tree. N= number of trees for which data were collected in a particular category

Category	**N (of 394 trees)**	**Responses**
Tree age	340	Range (1-100 years)
Planting intention	257	Intentional (229), Unintentional (28)
Propagation Method	243	Seed (99), Sapling (130), Graft (2)
Source of propagation material	102	Seed: own fruit (3), neighbor's fruit (1), family member's fruit (3), fruit bought at market (1); Sapling: direct from commercial nursery (26), market (22), tree fair (1), institutional nursery (32), own private nursery (12). Neighbor's private nursery (1)
Annual number of fruits	150	Range (2-350 fruits)
Fruiting season	97	Early (37), Mid (14), Mid/Late (5), Late (37), Baromashee (twice a year) (3), Alternate years (1)
Fruit destination	84	Eat (15), Sell (25), Eat and sell (30), Feed to cows (5), Eat and leave for wildlife (2), Eat, sell, and leave for wildlife (1)
Fruit texture	111	Soft (64), Crispy (32), In between (12), Mix of soft and crispy fruits on same tree (2)
Sweetness	116	Sweet (102), Not sweet (14)
Fruit size	109	Small (29), Medium (35), Large (44), Mix (1)
Fruit quality	142	Poor (35), Fair (19), Good (66), Very good (17), Excellent (5)

TABLE 4. *Artocarpus heterophyllus* samples from germplasm collections. Nineteen samples were acquired from living germplasm collections. Photo vouchers exist for all CW collections. CHIC = Nancy Poole Rich Herbarium at the Chicago Botanic Garden

Sample name	Tree owner	Tree location	Cultivar origin	Voucher
HART27	USDA Agricultural Research Station	Hilo, Hawaii	Thailand	
TARS1566-5	USDA Tropical Agricultural Research Station	Puerto Rico	Indonesia	
TARS1566-10	USDA Tropical Agricultural Research Station	Puerto Rico	Indonesia	
TARS18002-7	USDA Tropical Agricultural Research Station	Puerto Rico	Malaysia	
TARS18002-8	USDA Tropical Agricultural Research Station	Puerto Rico	Malaysia	
JM1	Private owner	Jamaica	Jamaica	
JM2	Private owner	Jamaica	Jamaica	
JM3	Private owner	Jamaica	Jamaica	
CW383	Fairchild Tropical Botanic Garden	Miami	Australia	CHIC
CW384	Fairchild Tropical Botanic Garden	Miami	Singapore	CHIC
CW385	Fairchild Tropical Botanic Garden	Miami	Thailand	CHIC
CW388	Fairchild Tropical Botanic Garden	Miami	Australia	
CW389	Fairchild Tropical Botanic Garden	Miami	Thailand	
CW395	Fairchild Tropical Botanic Garden	Miami	India	CHIC
CW396	Fairchild Tropical Botanic Garden	Miami	Malaysia	
CW403	Fairchild Tropical Botanic Garden	Miami	Malaysia	
CW405	Fairchild Tropical Botanic Garden	Miami	Malaysia	CHIC
CW406	Fairchild Tropical Botanic Garden	Miami	Australia	CHIC
CW410	Fairchild Tropical Botanic Garden	Miami	Miami	

TABLE 5. Genetic diversity of jackfruit in Bangladesh. Mean diversity measurements for 394 samples across 13 microsatellite loci and diversity measurements by locus. N = number of samples; N_a = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; SE = standard error.

	N	**N_a**	**H_e**	**H_o**	**F**
Mean	394	8.308	0.443	0.297	0.310
SE		1.015	0.063	0.051	0.069
Locus	**N**	**N_a**	**H_e**	**H_o**	**F**
MAA26	394	10	0.697	0.668	0.043
MAA54a	394	3	0.076	0.041	0.465
MAA54b	390	9	0.782	0.518	0.338
MAA105	394	13	0.615	0.282	0.542
MAA122	394	15	0.239	0.183	0.234
MAA140	394	7	0.634	0.515	0.187
MAA145	394	7	0.701	0.330	0.529
MAA156	394	5	0.278	0.292	-0.049
MAA178a	394	3	0.182	0.147	0.190
MAA178b	394	7	0.397	0.353	0.112
MAA182	394	10	0.325	0.287	0.116
MAA196a	360	7	0.323	0.158	0.509
MAA196b	357	12	0.507	0.092	0.818

TABLE 6. Genetic diversity of jackfruit in 8 districts of Bangladesh. Mean diversity measurements across 13 microsatellite loci. N = number of samples; N_a = number of alleles; SE = standard error; H_e = expected heterozygosity; H_o = observed heterozygosity; P_a = number of private alleles; F = inbreeding coefficient

Population	**N**	**N_a**	**SE**	**H_e**	**SE**	**H_o**	**SE**	**P_a**	**F**	**SE**
Comilla	30	4.077	0.400	0.421	0.068	0.312	0.057	4	0.199	0.079
Dhaka	33	4.231	0.508	0.439	0.062	0.326	0.057	2	0.215	0.074
Gazipur	29	4.538	0.462	0.436	0.068	0.307	0.054	4	0.287	0.111
Jessore	17	3.462	0.312	0.414	0.063	0.261	0.047	0	0.319	0.100
Rajshahi	28	4.308	0.548	0.423	0.070	0.245	0.063	3	0.364	0.109
Savar	79	5.077	0.560	0.425	0.056	0.309	0.043	5	0.240	0.068
Sylhet	115	5.692	0.728	0.433	0.066	0.280	0.055	13	0.371	0.079
Tangail	63	5.154	0.576	0.428	0.068	0.320	0.068	9	0.260	0.090

TABLE 7. Pairwise comparison of jackfruit genetic differentiation between 8 districts of Bangladesh based on F_{st}.

Population	Comilla	Dhaka	Gazipur	Jessore	Rajshahi	Savar	Sylhet	Tangail
Comilla	0.000							
Dhaka	0.015	0.000						
Gazipur	0.021	0.010	0.000					
Jessore	0.046	0.025	0.036	0.000				
Rajshahi	0.036	0.020	0.019	0.038	0.000			
Savar	0.015	0.006	0.014	0.034	0.023	0.000		
Sylhet	0.020	0.012	0.012	0.044	0.022	0.013	0.000	
Tangail	0.017	0.010	0.014	0.036	0.026	0.014	0.012	0.000

TABLE 8. Results of Mantel tests between geographic distance (Euclidean) and genetic distance (Fst). A second Mantel test was performed using the sum of Euclidean distances from each district to Dhaka and from Dhaka to each district. Neither test was significant.

	SSx	**SSy**	**SPxy**	**Rxy**	**P-value**
Direct	0.003	217085.201	11.895	0.444	0.190
Through Dhaka	0.003	315045.527	12.580	0.390	0.240

FIGURE 1. Locations of 8 Bangladeshi districts sampled for this study.

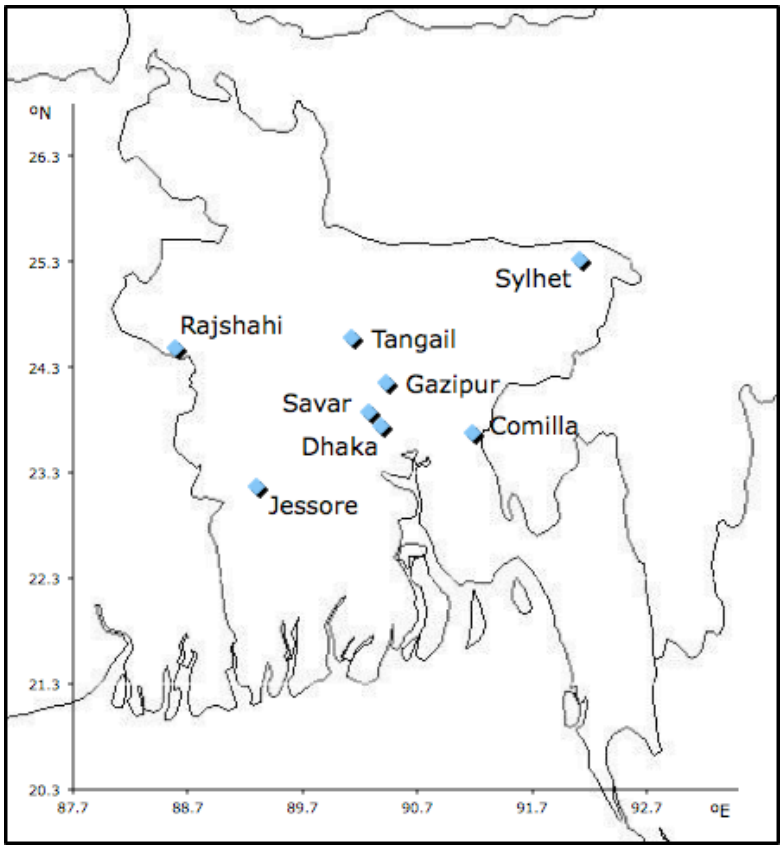
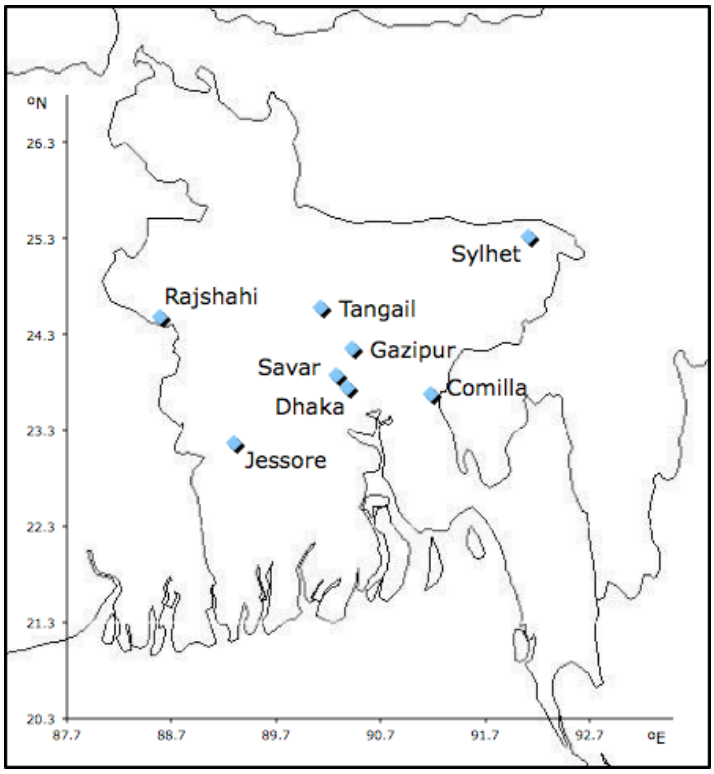


FIGURE 2. Geographic and genetic distances between 8 jackfruit-growing districts of Bangladesh. Top: Geographic mapping of Bangladeshi districts using longitude and latitude. Bottom: Principal coordinate analysis of pairwise comparison of jackfruit genetic differentiation of districts based on F_{st} (see Table 6 for data). Percent of variation explained by Axis 1 is 38.9%, Axis 2 is 24.5%.



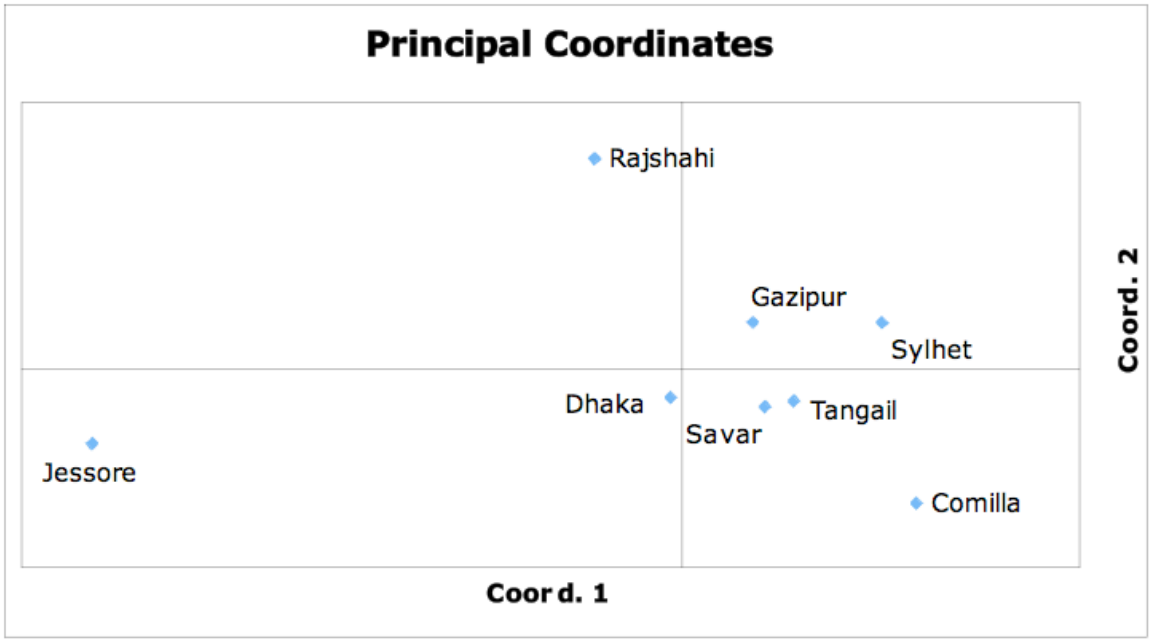


FIGURE 3. Determining K=4 for 394 jackfruit individuals in Bangladesh following Evanno 2005. Graph of MEAN L''(K) / STD DEV L(K) with peak at K=4.

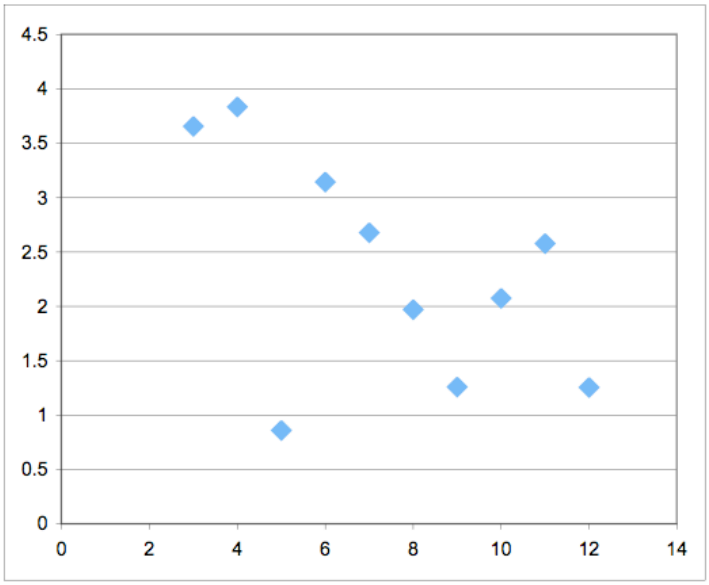


FIGURE 4. Determining K=4 for 37 international samples representing 9 countries of origin following Evanno 2005. Graph of MEAN L''(K) / STD DEV L(K) with peak at K=4.

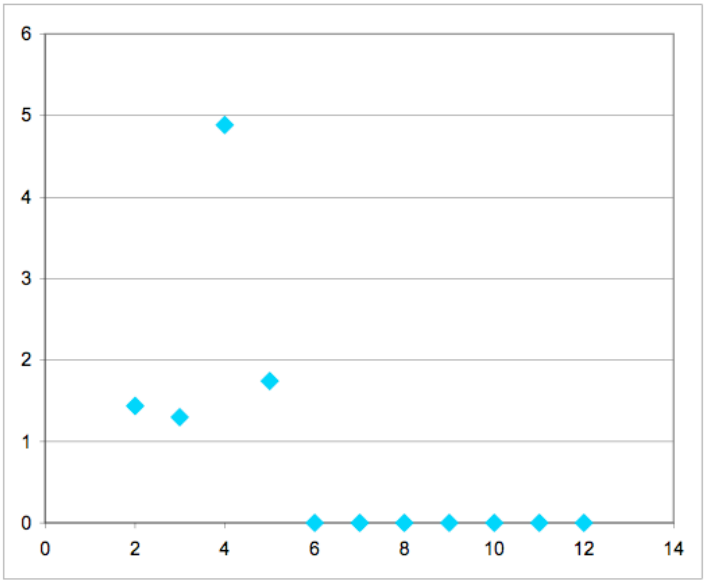
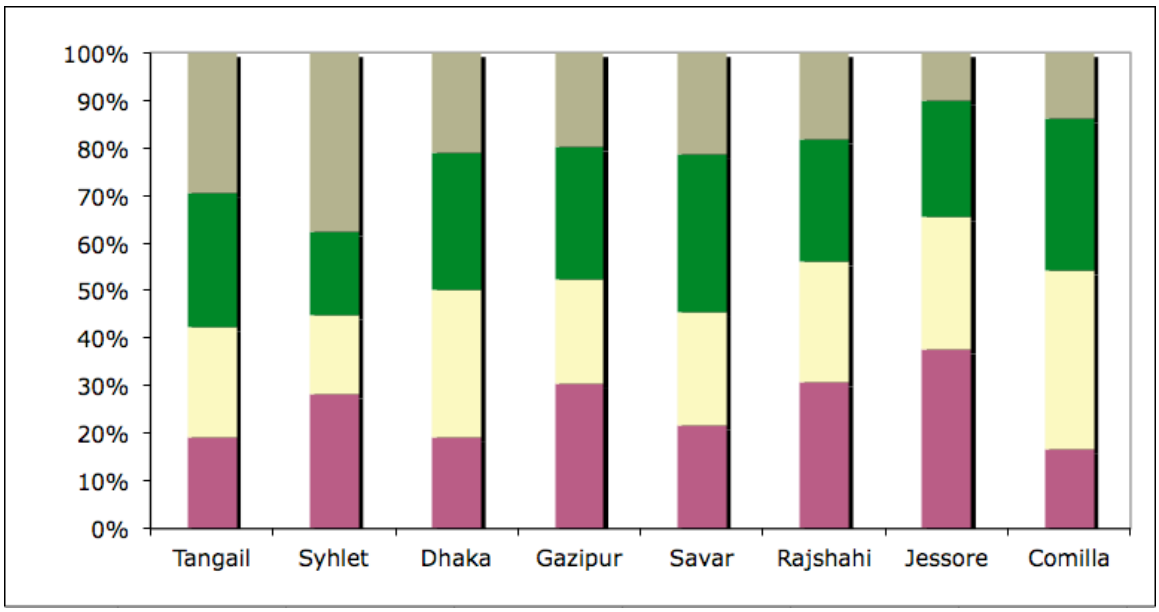


FIGURE 5. Genetic structure of Bangladeshi jackfruit samples as analyzed in Structure. A: Average percentages of 4 genetic groupings represented within each of 8 geographic districts. B: Genetic structure of 394 Bangladeshi jackfruit individuals, organized by geographic district: 1=Tangail, 2=Sylhet, 3=Dhaka, 4=Gazipur, 5=Savar, 6=Rajshahi, 7=Jessore, and 8=Comilla.

A



B

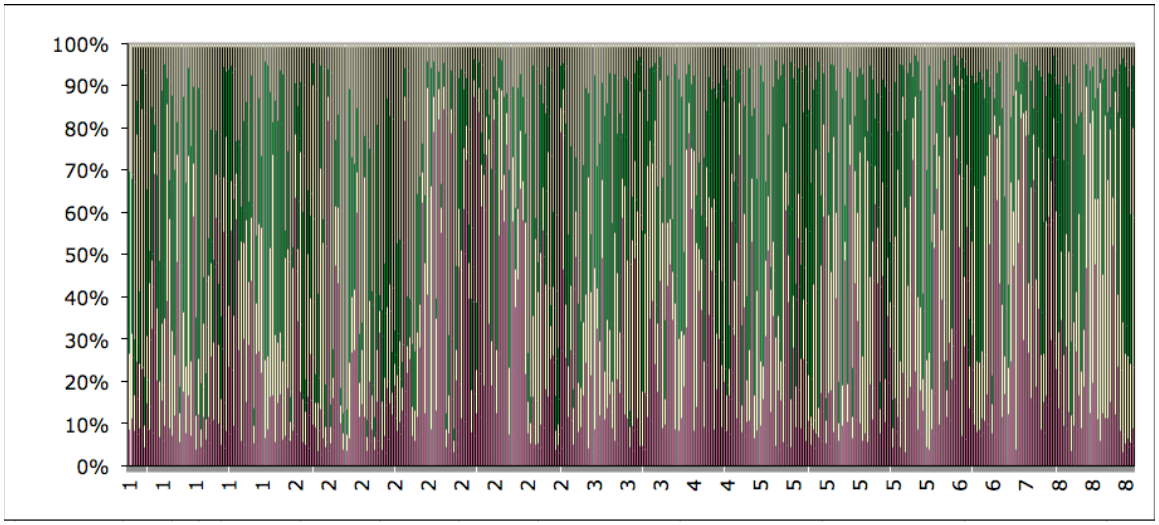
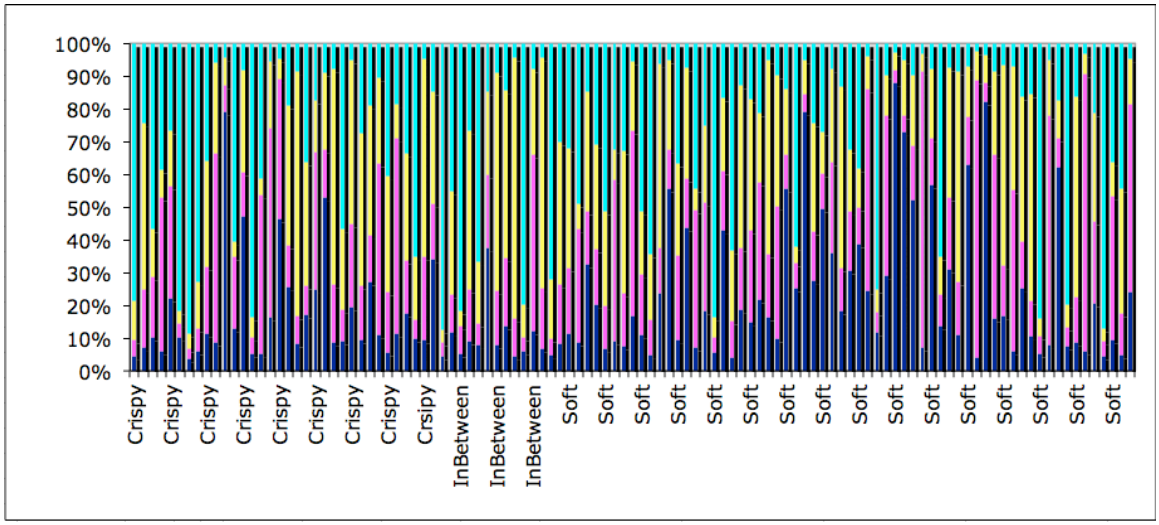


FIGURE 6. Examples of K=4 for Bangladeshi jackfruit sorted by characteristics other than geographic district. A: Sorted by fruit texture as reported by tree owners or caregivers. B. Sorted by fruiting season as reported by tree owners or caregivers.

A



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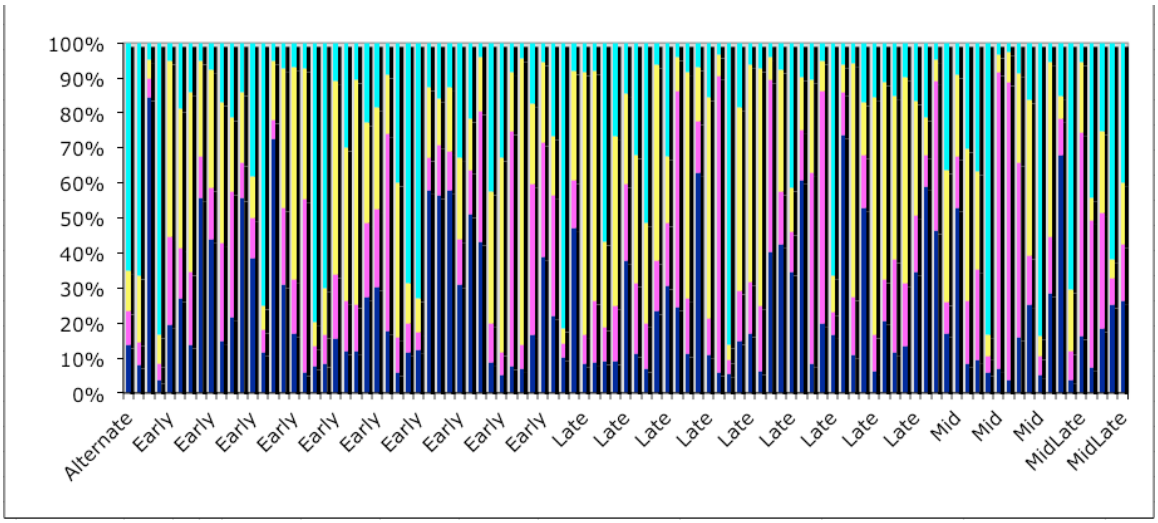
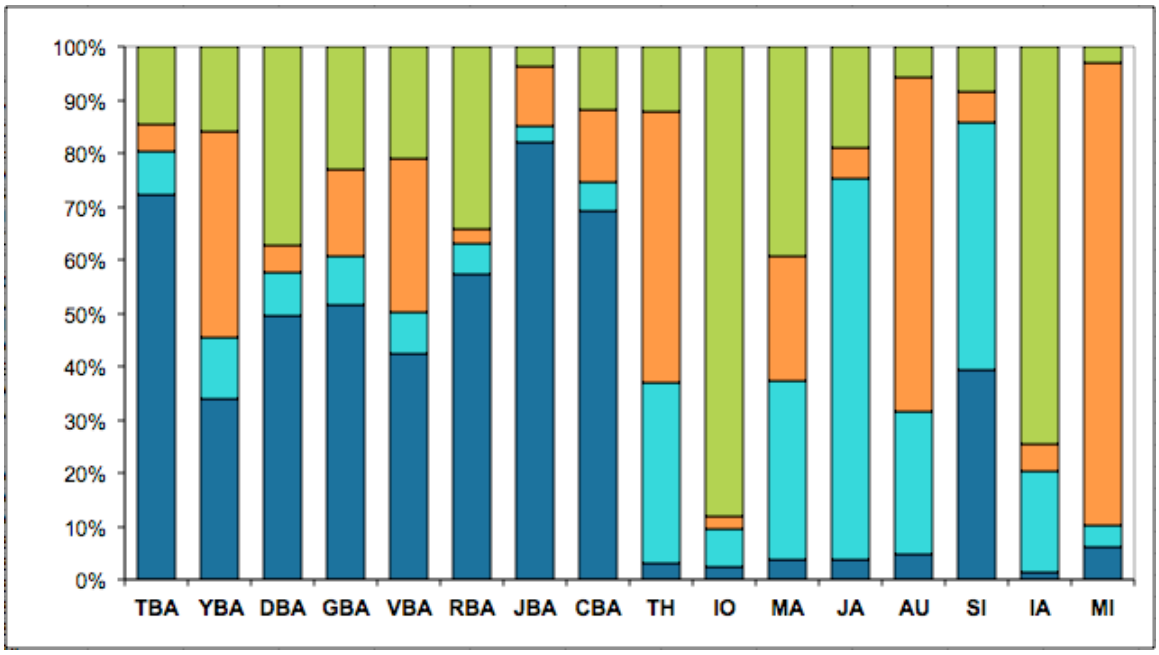
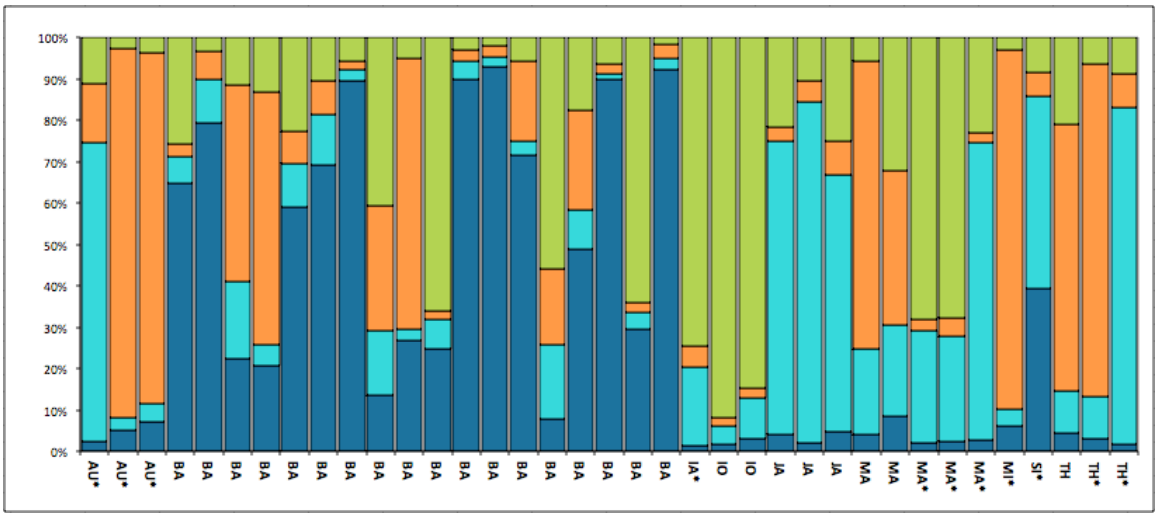


FIGURE 7. Genetic structure of international jackfruit samples as analyzed in Structure. A: Average percentages of 4 genetic groupings represented within each of 9 countries and 8 Bangladeshi districts. TH=Thailand, IO=Indonesia, MA=Malaysia, JA=Jamaica, AU=Australia, IA=India, MI=Miami, BA=Bangladesh. BA prefixes: T=Tangail, Y=Sylhet, D=Dhaka, G=Gazipur, V=Savar, R=Rajshahi, J=Jessore, C=Comilla. B: Genetic structure of 37 jackfruit individuals, organized by geographic district. *Indicates sample collected from Fairchild Tropical Botanic Garden in Miami, FL.

A



B



APPENDIX 1

TABLE 1. Genetic diversity statistics of jackfruit in 8 Bangladeshi regions across 13 microsatellite loci. N = number of samples; N_a = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; F = inbreeding coefficient.

	Locus	N	N_a	H_o	H_e	F
Comilla	**26a**	30	5.0	0.767	0.686	-0.118
	A54a	30	2.0	0.067	0.124	0.464
	B54a	28	7.0	0.321	0.759	0.576
	105a	30	5.0	0.333	0.656	0.492
	122a	30	3.0	0.100	0.096	-0.040
	140a	30	4.0	0.500	0.642	0.221
	145a	30	3.0	0.400	0.527	0.241
	156a	30	3.0	0.300	0.266	-0.127
	A178a	30	2.0	0.133	0.124	-0.071
	B178a	30	5.0	0.533	0.537	0.007
	182a	30	5.0	0.333	0.476	0.299
	A196a	25	4.0	0.120	0.115	-0.042
	B196a	27	5.0	0.148	0.470	0.685
Dhaka	**26a**	33	3.0	0.848	0.661	-0.284
	A54a	33	2.0	0.061	0.059	-0.031
	B54a	32	7.0	0.563	0.774	0.274
	105a	33	8.0	0.455	0.638	0.288
	122a	33	5.0	0.212	0.221	0.041
	140a	33	4.0	0.394	0.613	0.358
	145a	33	5.0	0.303	0.642	0.528
	156a	33	3.0	0.303	0.268	-0.130
	A178a	33	2.0	0.182	0.213	0.147
	B178a	33	5.0	0.273	0.319	0.145
	182a	33	3.0	0.242	0.355	0.317
	A196a	27	3.0	0.148	0.359	0.588
	B196a	27	5.0	0.259	0.582	0.554
Gazipur	**26a**	29	5.0	0.655	0.687	0.046
	A54a	29	2.0	0.000	0.067	1.000
	B54a	28	6.0	0.393	0.760	0.483
	105a	29	8.0	0.552	0.722	0.236
	122a	29	5.0	0.172	0.376	0.542
	140a	29	4.0	0.414	0.697	0.407
	145a	29	5.0	0.379	0.684	0.446
	156a	29	3.0	0.241	0.216	-0.118
	A178a	29	2.0	0.138	0.128	-0.074
	B178a	29	6.0	0.276	0.252	-0.094
	182a	29	5.0	0.379	0.327	-0.160
	A196a	28	4.0	0.393	0.402	0.024
	B196a	24	4.0	0.000	0.354	1.000
Jessore	**26a**	17	4.0	0.588	0.680	0.135
	A54a	17	2.0	0.000	0.111	1.000
	B54a	17	5.0	0.412	0.777	0.470

	105a	17	6.0	0.176	0.574	0.693
	122a	17	3.0	0.176	0.164	-0.074
	140a	17	3.0	0.471	0.512	0.081
	145a	17	3.0	0.176	0.304	0.420
	156a	17	3.0	0.176	0.164	-0.074
	A178a	17	2.0	0.176	0.161	-0.097
	B178a	17	4.0	0.235	0.310	0.240
	182a	17	3.0	0.353	0.431	0.181
	A196a	13	3.0	0.385	0.530	0.274
	B196a	15	4.0	0.067	0.664	0.900
Rajshahi	**26a**	28	6.0	0.607	0.736	0.175
	A54a	28	1.0	0.000	0.000	#N/A
	B54a	28	5.0	0.500	0.649	0.229
	105a	28	9.0	0.214	0.695	0.692
	122a	28	4.0	0.321	0.457	0.296
	140a	28	4.0	0.679	0.669	-0.014
	145a	28	5.0	0.107	0.510	0.790
	156a	28	3.0	0.179	0.166	-0.073
	A178a	28	3.0	0.286	0.281	-0.016
	B178a	28	3.0	0.071	0.135	0.472
	182a	28	4.0	0.179	0.167	-0.069
	A196a	24	3.0	0.042	0.378	0.890
	B196a	22	6.0	0.000	0.653	1.000
Savar	**26a**	79	6.0	0.633	0.694	0.088
	A54a	79	3.0	0.127	0.154	0.178
	B54a	79	8.0	0.468	0.785	0.403
	105a	79	9.0	0.278	0.565	0.507
	122a	79	4.0	0.253	0.285	0.112
	140a	79	4.0	0.468	0.517	0.094
	145a	79	6.0	0.342	0.626	0.454
	156a	79	5.0	0.392	0.348	-0.128
	A178a	79	2.0	0.228	0.240	0.049
	B178a	79	6.0	0.354	0.380	0.067
	182a	79	4.0	0.228	0.251	0.092
	A196a	74	3.0	0.108	0.215	0.498
	B196a	73	6.0	0.137	0.465	0.706
Sylhet	**26a**	115	7.0	0.617	0.686	0.101
	A54a	115	2.0	0.009	0.026	0.662
	B54a	115	6.0	0.591	0.740	0.201
	105a	115	11.0	0.217	0.625	0.652
	122a	115	8.0	0.148	0.200	0.260
	140a	115	3.0	0.443	0.633	0.299
	145a	115	5.0	0.365	0.766	0.523
	156a	115	3.0	0.296	0.289	-0.022
	A178a	115	3.0	0.096	0.196	0.511
	B178a	115	6.0	0.391	0.466	0.161
	182a	115	6.0	0.296	0.302	0.021
	A196a	111	5.0	0.144	0.300	0.520
	B196a	111	9.0	0.027	0.406	0.933

Tangail	**26a**	63	4.0	0.714	0.670	-0.067
	A54a	63	2.0	0.016	0.076	0.792
	B54a	63	7.0	0.603	0.749	0.195
	105a	63	10.0	0.222	0.433	0.487
	122a	63	5.0	0.127	0.150	0.152
	140a	63	6.0	0.762	0.675	-0.129
	145a	63	6.0	0.349	0.726	0.519
	156a	63	3.0	0.254	0.275	0.077
	A178a	63	3.0	0.063	0.062	-0.026
	B178a	63	5.0	0.429	0.412	-0.041
	182a	63	6.0	0.333	0.366	0.088
	A196a	58	4.0	0.155	0.356	0.564
	B196a	58	6.0	0.138	0.612	0.775

Changes in diversity, changes in propagation: Jackfruit, *Artocarpus heterophyllus* (Moraceae), cultivation in Bangladesh

Abstract
Crop genetic diversity is necessary for crop health and future adaptation. Changes in farmer propagation methods have been connected to diversity loss in many crops. Jackfruit (*Artocarpus heterophyllus*, Moraceae), valuable for producing the world's largest tree-borne fruit structure, is an important crop throughout Asia, including in Bangladesh, where deforestation has resulted in the loss of most natural areas and forests. In Bangladesh, jackfruit is traditionally propagated through the direct planting of seeds, but nursery-raised, seed-propagated saplings have been increasing in popularity since the 1980s. We analyzed 13 microsatellite regions to measure jackfruit genetic diversity loss over time and to study the effects of propagation changes on diversity levels. Our results identify a trend of diversity loss over time, measured by tree age (as reported by tree caretakers) and DBH, with mean number of alleles, expected heterozygosity, and observed heterozygosity all trending downward. Trees planted from seed showed higher numbers of alleles and private alleles than trees planted from sapling when all trees were included (~1-100 years). This result, however, is biased, since sapling trees have only been prevalent for approximately 30 years. When analyzed over only the last 30 years, sapling trees show slightly higher diversity, though not significantly higher, than seed trees. Our results suggest that the change from direct-seed to sapling propagation is not influencing the loss of diversity. Unique diversity may exist in older trees and should be preserved in live germplasm before old trees are lost to deforestation and age.

Background
Loss of crop diversity, especially plant genetic resources that farmers use when breeding and improving crops, is an issue of international concern. A diverse gene pool is essential for ensuring the capability of crops to adapt in the future. Wild and unimproved populations often contain important traits for improving agricultural production and maintaining sustainable agroecosystems, including resistance to pests and diseases; tolerance of drought, salinity and other abiotic stresses; and the ability to achieve higher yields and quality (Heywood et al. 2007, Smith and Schultes 1990). The demand for genetic material for breeding new adapted cultivars is ongoing (Heywood et al. 2007). In addition to concerns about meeting the needs of current and future crop breeders, decreases in genetic diversity within a population can result in crop failures that negatively affect farmers, consumers, and the economy, as we have seen previously in wheat, potato, rice, corn, coffee, and tobacco (Heal et al. 2004).

Decreases in levels of genetic variation can be caused by changes in propagation; a transition to intensive breeding that leads to uniform cultivars is associated with diversity loss. Changes in propagation can lead to unexpected and undesired outcomes. In traditional agroecosystems, changes in seed and vegetative material exchange practices have led to changes in genetic diversity, as seen in crops like cassava and maize (Dyer et al. 2011, Dyer and Taylor 2008). The intentional or unintentional introduction of

transgenic individuals into an environment can alter the level of genetic variation present in the target species, its wild relatives, and the associated pests (Andow and Zwahlen 2006). A shift in rice propagation from seedling transplantation to direct seeding may have contributed to the evolution of weedy rice genotypes (Chen et al. 2004). Vegetative propagation has also been associated with diversity loss in some species (Pissard et al. 2006). Propagation methods have influenced crop evolution through both random and systematic changes in farmer practices, and can lead to both losses of genetic diversity through population bottlenecks and increases in diversity through the introduction of non-local germplasm (Dyer and Taylor 2008). In addition to changes in propagation, changes in crop diversity are known to be caused by deforestation, which may have been caused by increased agriculture, war, natural disaster, or other reasons. (Smith and Schultes 1990).

Study System

The *Artocarpus* genus contains over 50 species, with at least 25% producing edible fruits (Zerega et al. 2010). The two most widely cultivated and economically important species are *Artocarpus altilis* (breadfruit), a staple food of many countries in its native Oceania and in the Caribbean, where it was introduced in the 18th century (Ragone 1997), and *Artocarpus heterophyllus* (jackfruit). Jackfruit is a major crop in Southeast Asia and is growing in popularity in Australia, Europe, the Middle East, and the United States, fueled by demand from Asian immigrants and migrant workers (Rahman and Talukder 2001). Jackfruit has been cultivated for millennia and was referred to as early as 300 B.C. by Theophrastus (Hort 1916). Today it can be found in cultivation at low elevations throughout the Indian subcontinent, Bangladesh, Myanmar, Sri Lanka, Southern China, Nepal, Laos, Vietnam, Cambodia, Malaysia, and has also been introduced in the Philippines, Indonesia, and throughout Africa, Australia, and the Neotropics, especially Jamaica and Brazil. It is now so widely cultivated that it is unclear in which region it is indigenous and which region holds its greatest diversity. The rainforests of the Western Ghats in India are generally cited as both the area of origin and center of diversity of jackfruit (Jagadeesh et al. 2007), but it is possible that jackfruit has a broader center of origin than previously proposed, or that the true point of origin will never be known (Jarrett 1959).

Artocarpus heteropyhllus is a monoecious tree that is cultivated for its fruit and timber. The compound fruit is the largest tree-borne fruit structure in the world, reaching sizes up to 100 cm x 50 cm and weighing over 35 kg (Jarrett 1959). Each tree can produce more than 100 fruits in a season. Ripe fruit flesh is eaten raw; unripe flesh is cooked or dried and eaten like a vegetable. Seeds are roasted and eaten or ground into flour. The fruit can be used as a significant source of carbohydrates and cartenoids (Rahman et al. 1999, deFaria et al. 2009). Although the large, heavy, and perishable fruit is not well suited for fresh fruit export, it is canned and processed into products that are exported to Asia, Australia, Europe, and the United States. Whole trees are harvested for timber for furniture, construction, and musical instruments. Jackfruit wood chips are used to make a dye, which gives the famous orange-red color to the robes of Buddhist priests. Because many of these products are consumed locally, it is difficult to estimate the true economic value of jackfruit worldwide. It is estimated that nine million metric tons of jackfruit are

produced annually in Asia (Haq 2006). Jackfruit is known as an outbreeding plant (El-Sawa 1998), though the pollinator is unknown.

Despite the importance of jackfruit in Southeast Asia and the large range it now occupies, it is underrepresented in the scientific literature. Not much is known about its origins, domestication, pollination, or genetic diversity. Jackfruit is listed as an underutilized crop by several international organizations (Global Facilitation Unit for Underutilized Species, International Centre for Underutilized Crops, International Plant Genetic Resources Institute) (Jaenicke 2006, ICUC 2003, Hossain 1996). Because of this recognition, and because it is high yield, low input, and nutritious, jackfruit is being promoted internationally for the various causes of food security, rural development, health and nutrition, poverty alleviation, and women's empowerment.

Jackfruit in Bangladesh

Jackfruit is the national fruit of Bangladesh. Bangladesh had a population of 156 million in 2009, ranking 7th amongst the world's countries. In area, however, Bangladesh covers only 55,598 square miles, making it the 5th most densely populated country in the world. Seventy-three percent of Bangladeshis live in rural areas (CIA World Factbook 2009), and 62% of the workforce is employed in agriculture (Alam 2005). Homegardens provide approximately 80% of total fruits and 85% of fuel wood and timber grown in Bangladesh (Rahim 1994). Bangladeshi weather is extreme, its topography and geographic location make it susceptible to cyclones, tornadoes, and flooding, and is predicted to grow more unpredictable as a result of climate change (Christensen et al. 2007). Increased salinity is already changing coastal habitat and agricultural fields (Agrawala et al. 2003). Tree cover of Bangladesh is estimated at 9-10%, with 35 of 64 districts having no forest cover, making it impossible for Bangladesh to meet its own demands for timber, firewood, and fodder (Ahmed 2011).

Jackfruit production covered an estimated 25,000 acres of land in Bangladesh in 2010, and produced over one million metric tons of fruit, ranking fifth in land cover among fruits, after banana, mango, melon, and pineapple, and ranking a very close second in tons of fruit produced, after mango (Bangladesh Bureau of Statistics 2010). Bangladesh exports only a minimal amount of the fresh fruit and does not have processing facilities to export other jackfruit products; most jackfruit is sold within the country. Almost every rural family in Bangladesh has access to a homegarden, and jackfruit can be found in almost every homegarden, where it is the second most common homegarden tree, after mango (Ahmed 2005, Khan et al. 2010, Hocking et al. 1996, pers. obs.). It is also planted between rows in coffee, pineapple, and other crop plantations, and occasionally in jackfruit and mixed-fruit orchards (Haq 2006, pers. obs.). It can be seen growing along streets, on school and university campuses, and in city courtyards (pers. obs.). Jackfruit growers usually sell some portion of their crop and keep some for personal use. Jackfruit timber is very valuable, and most homegarden farmers expect each tree to eventually provide profit from both fruit and timber.

Recently, a decline in jackfruit morphological variation has raised concerns about a possible decline in genetic diversity, though causes have not been studied or identified

(Khan et al. 2006). Direct seed planting was the predominant method of propagation until nursery-raised, seed-propagated saplings gained popularity in the 1980s. Great morphological diversity has been reported in Bangladesh and can be seen in fruit size, color, texture, taste, season, and yield, and in other characteristics of the tree, including leaf size, leaf color, leaf shape, canopy shape, and location of fruits on the tree (e.g. roots, trunk, primary branches, secondary branches, etc.) (Azad et al. 2007, Haq 2006, Khan et al. 2010, pers. obs.). Trees can be observed across Bangladesh that possess undesirable traits that make fruits inedible, such as fruits made up partially or completely of unfertilized flowers or fruits that drop off the tree before ripe (pers. obs.). These trees are usually not culled due to the value of jackfruit timber (pers. comm.). Currently, Bangladeshis do not practice hand pollination or vegetative propagation, resulting in a cross-pollinated, seed-propagated crop. Trees producing poor quality fruits are kept in the gene pool due to the value of jackfruit timber and the ability to sell poor quality fruits at market.

Bangladeshis see the potential profits of developing stronger jackfruit processing and export markets, as is seen in Thailand, China, and Malaysia. To achieve success in the processing and export markets, more uniform cultivars are needed. Currently, Bangladeshi farmers do not grow specific jackfruit cultivars, though cultivars are common for some other fruits, like mango and guava (Rahman et al. 2006, Rahman et al. 2007). Bangladeshi research stations and universities are beginning to use vegetative propagation methods, including grafting and tissue culture, to produce true-to-type jackfruit cultivars with desirable characteristics (Haq 2006, Haq and Hughes 2002, pers. comm.). However, these clones are rarely available to jackfruit growers outside the institutions, and non-vegetative propagation is still the principal method used.

Jackfruit has been promoted for forest restoration efforts in initiatives such as the Community Forestry Project in north Bengal, which began in 1982 (Ahmed 2011), and for projects that are attempting to improve the yield of homegardens through multi-story agroforestry (Rahim 1994). In addition to the benefits of increased forest cover and more efficient land use, jackfruit is promoted as a good source of extra income for the poor and a way of empowering women, who often tend to the trees (Ahmed 2011, Ali 2005). These goals, along with a need for planting material for forest restoration projects, have led to the promotion of jackfruit nurseries. Beginning around the early 1980s, several government and NGO initiatives began promoting and assisting in the development of tree nurseries to provide income, business experience, and leadership opportunities for local villagers. Participants are given either materials or low interest loans, as well as minimal training in nursery management and sapling care (Ali 2005). Initiatives that included jackfruit include the Village and Farm Forestry Program (VFFP) sponsored by the Swiss Agency for Development and Cooperation (1986), Social Forestry Program of the Proshika Center for Human Development, a Bangladeshi NGO (1985), and the Social Forestry Program of the Rangpur Dinajpur Rural Development Service, a Bangladeshi NGO (1977), as well as tree nurseries directly managed by government agencies, such as the Forest Department, Agricultural Research Institute, or the National Parks (Hocking et al. 2006, Ali 2005).

The promotion of jackfruit nurseries has succeeded in increasing the popularity of nursery-raised, seed-propagated saplings in Bangladesh. Easy availability of saplings, along with the benefits of raising trees from saplings instead of directly from seed (saplings are usually around 2 years old at the time of purchase, making them fruit sooner and easier to protect from the damage of grazing animals), make saplings seem like a good investment for homegarden owners and other jackfruit growers (Haq 2006). Promotion initiatives were put in place without an initial assessment of jackfruit genetic diversity, and do not include strategies for monitoring genetic diversity. Training of nursery owners is limited and does not include instructions for selecting seeds or mitigating possible diversity loss through conservation of germplasm. There is no evidence in the literature that nurseries monitor or assess the quality of either seed source or sapling material. In practice, most nursery owners do not select or sort seeds for any particular reason (Quddus 2011, pers. comm.) Although there does not appear to be intentional selection of seeds for nurseries, there could be unintentional selection occurring due to the large numbers of saplings emerging from each nursery and being distributed throughout the country, or for reasons not yet understood. There may also be selection pressures coming from how nursery owners and farmers choose which saplings to sell and purchase, respectively (Quddus 2011).

As more jackfruit growers choose to buy saplings rather than select their own seed, the bottlenecking of genetic material through selection by nursery owners (whether intentional or unintentional) could lead to a slow loss of genetic variation. Alternatively, nurseries could be exercising less selection than direct-seed farmers and assisting the dispersal of previously unselected alleles and genotypes. Additionally, deforestation over the past 100 years has decreased wild areas in Bangladesh to almost nothing in the areas of the country where jackfruit grows (Ahmed 2011). This loss of forest may have decreased the genetic diversity of jackfruit by reducing the number of old and unimproved trees that may harbor unique traits (Heywood et al. 2007).

In this study, we use microsatellite markers to examine changes in *Artocarpus heterophyllus* genetic diversity in Bangladesh over the past 100 years. We hypothesize:

1. Levels of genetic diversity of jackfruit in Bangladesh will vary over the past 100 years.
2. Diversity levels will vary between trees planted directly from seed and trees planted from saplings.
3. If we observe a loss of diversity over time, we will investigate the contribution of the recent increase in the popularity of nursery-raised saplings.

By testing these hypotheses, we seek to better understand how jackfruit genetic diversity has changed over time and if older trees harbor diversity that should be preserved for the future. By analyzing the genotypes of trees aged approximately 1-100 years, we also investigate diversity levels before and during the increase in sapling propagation. By measuring diversity in individuals planted from seed and individuals planted from saplings, we seek to identify the effects of propagation method on genetic variation. Our goal is to clarify the role of changes in propagation on changes in diversity so that strategies may be developed that preserve genetic diversity while also meeting the goals of crop improvement.

Methods

Fieldwork was conducted in Bangladesh over three and a half weeks in July 2010. Study locations were chosen to cover the maximum number of Bangladeshi districts in the time available to researchers. Study sites were chosen to represent a broad variety of *Artocarpus heterophyllus* habitats, including private homesteads, a mixed-fruit orchard, a national park, public and private schools and universities, a city park, government agricultural research institutes, and urban and rural government land (Table 1). Eight districts were sampled: Comilla, Dhaka, Gazipur, Jessore, Rajshahi, Savar, Sylhet, and Tangail (Figure 1). Individual trees were chosen with the help of tree owners to represent a range of ages, fruit qualities, and propagation methods. Leaf material was collected from each sample tree and stored in silica. Diameter at breast height (DBH) was recorded for each tree. When possible, informal interviews were conducted with the owner of each tree sampled. Owners or caretakers were present for interviews for 96.2% of trees. Interviews collected a variety of categorical, quantitative, and qualitative data as it applied to each individual tree. The most consistent data collected through interviews included tree age and propagation method (sapling, seed, or graft), as these data were necessary for our research questions. Less consistent data collected included fruit quality, fruiting season, fruit size, fruit texture, number of fruits per tree, seed or sapling source, and fruit destination (consume at home or sell to market). The volume of data collected in each interview was limited by time constraints of the researchers, communication with the translators, and knowledge of the interview subject about the sample tree. All collections and interviews were recorded with consent on a video camera, and photos were taken of each tree sampled. Translation was conducted in the field by our Bangladeshi collaborators. Representative voucher specimens were taken from each site and deposited at CHIC.

Leaf material was brought back to the Chicago Botanic Garden and stored in a -80 degree Celsius freezer. DNA was extracted using Qiagen DNeasy (Qiagen, Valencia, CA) extraction kits following standard protocol. PCR was conducted for 13 microsatellite loci (Witherup et al., submitted) using a two-step process. First, 10 ul reactions were used containing 5 ul of Master Mix (Promega, Madison, WI), 0.5 ul of 10mg/ml BSA, 0.25 ul of 10uM forward dye-labeled M13 primer, 0.25 ul of 10uM reverse primer, 3 ul of H_2O, and 1 ul of template DNA. PCR conditions for the first step were 94ºC for 3 min, 13 cycles at 94ºC for 30 s, 59.8ºC for 30 s, 72ºC for 1 min and a final extension of 72ºC for 10 min. Then, to each 10 ul reaction was added 2.5 ul Master Mix (Promega, Madison, WI), 0.25 ul of 10 mg/ml BSA, 0.125 ul of 2.5uM $MgCl_2$, 0.25 of 10 uM M13 dye (blue, green, or black), and 1.875 ul of H_2O for a total reaction of 15 ul. PCR conditions for the second step were 94ºC for 3 min, 27 cycles at 94ºC for 30 s, 55ºC for 30 s, 72ºC for 1 min and a final extension of 72ºC for 10 min. PCR product was analyzed on a Beckman Coulter CEQ 8000 Genetic Analysis System. Before adding samples to the CEQ, PCR product (0.5 ul of Wellred Dye D4-labeled product, 1 ul of Wellred Dye D3-labeled product, or 2.5 ul of Wellred Dye D2-labeled product) was added to 30 ul of HiDi formamide (Azco Biotech., San Diego, CA) and 3.3 ul 400bp size standard ladder (Beckman Coulter, Brea, CA). Alleles were scored using the CEQ 8000 software v9.0.

GenAlEx v.6.4 (Peakall and Smouse, 2006) was used to evaluate observed and expected heterozygosity of the 13 microsatellite regions. Jackfruit in Bangladesh is a tropical tree that does not produce reliable tree rings. To measure time, data from 13 microsatellite regions were analyzed in two different ways: DBH as measured in the field, and tree age as reported by the tree's owner. DBH is frequently used as a proxy for age (Lieberman et al. 1985, Lukaszkiewicz at al. 2005), but it can be affected by environmental factors. Reported age, which was often given as an estimate, is also not a completely reliable measurement of the tree's true age. By using both approaches to analyze the data and comparing results, we will be able to reduce the effect of error. In addition, DBH and age data were divided into classes for analysis to reduce the effect of error (Tables 2 and 3). Age classes were divided to be as equal in number as possible while keeping trees of the same age or DBH in the same class. Ages are reported here as they were reported in the field by tree owners or caretakers. For some trees, an age range was reported in the field instead of a specific number of years; when this occurred, the lower number was used for all calculations. Most ranges were reported as 2 or 5 year spreads, with some of the oldest trees given as a spread of 10 years. Linear regression was computed in R (R Development Core Team 2009) using mean number of alleles, expected heterozygosity, observed heterozygosity, and F as factors of median age or median DBH of each class. Jackknife analysis was also computed in R. It is important to note that jackfruit trees in Bangladesh effectively represent one large, inter-mixing population; previous genetic structure analysis showed very little differentiation between the geographic populations studied here (Witherup et al. unpublished, see chapter 2 above).

Results

Changes over time

Results reveal a downward trend for all diversity measurements tested except F statistics, though most were insignificant (Tables 4 and 5). For age class, mean number of alleles declined at a rate of 0.7 alleles per 100 years ($p=0.204$) (Figure 2). Mean expected heterozygosity decreased at a rate of 3 percent per 100 ($p=0.105$), and mean observed heterozygosity significantly decreased at a rate of 6 percent per 100 years ($p=0.029$). For size class, mean number of alleles declined ($p=0.078$) (Figure 3). Mean expected heterozygosity decreased ($p=0.654$), and mean observed heterozygosity significantly decreased ($p=0.028$). A range of private alleles from 0 to 7 were found across all age and size classes, unrelated to age or DBH. F statistics did not show a relationship with age ($p=0.619$) or size ($p=0.684$). Jackknife analysis was conducted of all 8 linear regressions to test for the influence of outlying age and size classes; all slopes were negative except for F statistics (Table 6).

Changes in propagation

When measured using all trees sampled for which propagation data were available (N=268), seed trees had a mean number of alleles of 7.000 ±0.760 (N=159) and 29 private alleles (Table 7). Sapling-propagated trees had a mean number of alleles of 5.615 ±0.665 (N=109) and 7 private alleles. Both showed similar levels of expected and observed heterozygosity. However, saplings have only been prevalent since about 1980,

and when results were recalculated to only include trees estimated to be 30 years old and younger, the wide discrepancy between allele numbers and private allele numbers was eliminated (mean number of alleles of 5.538 ±0.694 (N=74) for seed trees, 5.462 ±0.637 (N=97) for sapling trees; 9 private alleles for seed trees, 8 for sapling trees). In the more recent seed and sapling trees (20 years and less, 10 years and less), there is a trend toward higher genetic variation in younger sapling trees than in younger seed trees (Figure 4).

Discussion

Changes over time

These results represent trees from one to approximately 100 years in age. In that time, Bangladesh transitioned from being part of a larger British colony, being structured into the country of East Pakistan, fighting a war for independence, and struggling with balancing the demands of development and modernization in a country that is included in the "Next Eleven", a list of 11 nations that are thought to have the most potential for becoming some of the world's largest economies in the 21st century (O'Neill et al. 2005). While this research does not investigate how Bangladesh's political and economic upheavals have influenced changes in jackfruit evolution and propagation methods over the studied time period, such events likely affected the gene pool in various ways at various times. However, because all three diversity measurements show some trend of decrease for both age and DBH, it appears that some decline in diversity has occurred in the past 100 years. The decline in mean number of alleles indicates that as older trees are harvested or die off, alleles could be lost from the population. The trend of decline in expected heterozygosity signifies that uncommon genes are becoming more rare; trees are becoming more homozygous over time and the genetic makeup of more recent generations is more uniform than that of older generations.

The decline in diversity over time could be caused by any one of several factors or combinations of factors. Decreased numbers of alleles could result from deforestation and the harvesting of jackfruit trees for timber. Decreased access to remaining old trees, for both seed dispersers (usually humans) and pollinators, could lead to unintentional selection. A decline in forest cover or wild spaces could also provide less habitat for unintentional (escaped) trees to germinate, reach maturity, and enter the gene pool. Economic pressures could lead toward a decline in the average age at which a tree is harvested for timber, removing older trees from the gene pool and unintentionally intensifying other changes in selection. A shift in intentional selection patterns could decrease allele numbers and expected heterozygosity, such as a new desire for certain traits. However, other than breeding efforts at a few universities and research stations, no such shift has been reported in Bangladesh. Increased transport between geographic regions could lead to a more uniform crop, but should not lead to a decline in allele numbers. Changes in propagation could lead to intentional or unintentional bottlenecks, and one such change has been reported over the past 30 years – a shift from direct seed propagation to the planting of seed-propagated saplings purchased from nurseries. Finally, natural disaster or war, both of which have touched Bangladesh in the past 100 years, could reduce the size of the population or restrict access to portions of the gene pool.

If the decline in genetic diversity continues, Bangladesh's jackfruit crop could become more uniform, making it more susceptible to pests and extreme weather. A loss of alleles means a loss of genetic material that could be useful to future jackfruit breeders in Bangladesh and throughout the world, including tolerance of pests, flood, drought, salinity, and other important traits that will be needed as jackfruit adapts to climate change and grows in popularity in more countries outside of its native range.

The observed decline in diversity should be monitored for changes in intensity or direction. If the cause is historical, such as war or a natural disaster, it is possible that genetic diversity levels could rebound with timely intervention through conservation of existing diversity. If the cause of the decline is ongoing, such as propagation changes, climate change, or deforestation, the cause will continue to affect levels until it is addressed. Furthermore, if Bangladeshi farmers adopt vegetative reproduction or more commercial breeding on a larger scale, the downward trend could intensify. The trend could also reverse in the future, if offspring of older trees are preserved now or if new material is introduced from another part of jackfruit's range.

Changes in propagation

Although fewer alleles are found in the entire population of sapling-propagated trees compared to the entire population of seed-propagated trees, this comparison is biased because sapling trees have only been propagated in Bangladesh for about 30 years. The higher diversity observed in the total seed trees is due to the effect of the larger decline in diversity over time reported above. When restricting analysis to only trees propagated within the last 30 years, we see that sapling propagation does not appear to have negatively affected the genetic variation of Bangladesh's jackfruit crop. In fact, in more recent years, it appears that sapling-propagated trees may possess higher levels of heterozygosity and higher numbers of alleles and private alleles than seed trees. The reasons for this are not known, although lack of selection by nursery growers could be one influence.

Conclusions

The promotion of tree nurseries by government agencies and NGOs has succeeded in enhancing the livelihoods of rural villagers and planting new trees in deforested areas (Ali 2005). Our results indicate that the resulting change in jackfruit propagation is not likely causing a decline in diversity over time. However, diversity has declined in the past 100 years for other unknown or untested reasons. If Bangladesh's goal is to develop and sustain more uniform commercial jackfruit varieties, it will most likely rely on the allelic diversity that currently exists. It is possible that saplings may become more uniform in the future to meet the demands of farmers and Bangladesh's growing economy, but diversity can be conserved. Jackfruit preservation in the wild is almost impossible, since there are few wild areas left in the country and almost all of Bangladesh's public and private land is managed. Seeds should be collected from diverse individuals, including old trees, and planted in living germplasm collections. Bangladeshi jackfruit is currently not represented in any international germplasm collections, and Bangladesh's current collections do not represent the range of diversity present in the country (Azad et al.

2007, Schnell et al. 2001, pers. obs.). Jackfruit seeds are recalcitrant, sensitive to both drying and freezing, and cannot be stored for more than a few weeks, making conservation difficult. Not only is live germplasm the only option for conservation, but conservation will have to begin now before old trees die or are harvested and genetic diversity is lost. In the future, periodic assessment of jackfruit diversity in Bangladesh should be undertaken to continue to track and understand any changes that may be occurring.

The preservation of crop plant genetic resources is often promoted to reduce losses associated with the intentional development of uniform cultivars and a switch to vegetative propagation. The development of uniform jackfruit cultivars has not yet occurred in Bangladesh, and this study demonstrates that other changes can also influence genetic variation. Crop evolution is a dynamic process that is often unpredictable, and it can be difficult to identify the causes of change, which can be natural, anthropogenic, or a complicated combination of causes. For jackfruit in Bangladesh, efforts to preserve genetic diversity may be required to begin before the causes of diversity loss are fully understood.

TABLE 1. *Artocarpus heterophyllus* samples from Bangladesh. Individuals (N=361) were collected from Bangladesh by the authors. A representative herbarium voucher was made for most sites. A photo voucher exists for all samples analyzed. Voucher specimens are deposited at the Nancy Poole Rich Herbarium at the Chicago Botanic Garden (CHIC). N = number of samples analyzed from each site.

N	Date collected	Site	District	Site type (# of trees sampled)
12	5-Jul-10	Madhupur village	Tangail	homegardens (9), primary school (3)
21	6-Jul-10	Mohismara village	Tangail	homegardens (19), Forestry Department plot (2)
30	6-Jul-10	GachaBari village	Tangail	homegardens (24), Forestry Department office (6)
27	8-Jul-10	Bangladeshi Tea Research Institute (BTRI), Srymangal	Sylhet	BTRI guest house (5), BTRI campus (22)
29	9-Jul-10	Ashidu orchard and village	Sylhet	fruit orchard (21), homegardens (3), Bugunbari Forest (5)
21	9-Jul-10	Lawachara National Park	Sylhet	national park (21)
23	10-Jul-10	Bangladesh Agricultural Research Institute (BARI)	Sylhet	BARI research campus (18), germplasm from Chittagong region of Bangladesh (5)
13	11/19-Jul-10	National Botanic Garden of Bangladesh	Dhaka	public park (13)
13	12-Jul-10	Bangladesh Agricultural Research Institute	Gazipur	germplasm collected locally (9), BARI campus (4)
16	12-Jul-10	Bagabazar village	Gazipur	homegardens (16)
19	14-Jul-10	Leather Research Institute (LRI)	Savar	LRI campus (19)
28	16-Jul-10	Madan hati village	Rajshahi	homegardens (28)
17	17-Jul-10	Nimtoli village	Jessore	homegardens (17)
31	20-Jul-10	Jahangirnagar University	Savar	university campus (31)
11	20-Jul-10	Gono University	Savar	university campus (11)
30	21-Jul-10	Khula Pater village	Comilla	homegardens (30)
20	23-Jul-10	Council of Scientific and Industrial Research (CSIR)	Dhaka	CSIR campus (20)

TABLE 2. Jackfruit age classes used for linear regressions. Age range and median age given in years. N = number of individuals in each class; N districts = number of Bangladeshi districts represented in each class; N sites = number of sites represented in each class; Se = number of individuals propagated by seed; Sa = number of individuals propagated by sapling; Gr = number of individuals propagated by graft; Un = number of individuals of unknown propagation method.

Age class	N	Age range	Median age	N districts	N sites	Source
1	34	50-100	60	5	8	Se=33, Sa=1
2	40	40-49	40	6	11	Se=36, Sa=4
3	32	28-39	30	7	11	Se=8, Sa=3, Un=21
4	37	17-27	20	8	13	Se=15, Sa=9, Un=13
5	37	11-16	15	8	14	Se=15, Sa=14, Un=9
6	39	8-10	10	8	15	Se=9, Sa=22, Gr=1, Un=7
7	29	6-7	7	7	10	Se=9, Sa=15, Un=5
8	33	4-5	5	8	13	Se=7, Sa=24, Gr=1, Un=1
9	26	2-3	2	5	8	Se=13, Sa=10, Un=3

TABLE 3. Jackfruit size classes used for linear regressions. Size range and median size are diameter at breast height (DBH) given in cm. N = number of individuals in each class; N districts = number of Bangladeshi districts represented in each class; N sites = number of sites represented in each class; Se = number of individuals propagated by seed; Sa = number of individuals propagated by sapling; Gr = number of individuals propagated by graft; Un = number of individuals of unknown propagation method.

Size class	N	Size range	Median size	N districts	N sites	Source
1	31	62.7-97.5	69.9	5	9	Se=28, Un=3
2	31	49.2-62.5	54.3	6	12	Se=25, Sa=2, Un=4
3	31	43.1-49.1	45.6	8	14	Se=13, Sa=3, Un=15
4	32	36.2-43.0	39.4	7	14	Se=14, Sa=6, Un=12
5	31	28.6-35.6	32.5	8	12	Se=14, Sa=6, Un=11
6	31	23.9-28.5	26.5	8	14	Se=10, Sa=12, Un=9
7	32	20.5-23.8	22	8	14	Se=11, Sa=13, Gr=1, Un=7
8	32	15.5-20.0	18.3	8	14	Se=5, Sa=17, Gr=1, Un=9
9	32	12.1-15.3	13.85	8	15	Se=11, Sa=17, Un=4
10	31	7.2-12.0	10	8	11	Se=11, Sap=17, Un=3
11	32	1.5-7.0	4.3	6	11	Se=12, Sa=13, Un=7

TABLE 4. Jackfruit age classes with genetic diversity statistics as calculated in GenAlEx. N = number of samples in age class; Median age is reported in years; N_a = mean number of alleles across 13 microsatellite loci; H_e = mean expected heterozygosity across 13 microsatellite loci; H_o = mean observed heterozygosity across 13 microsatellite loci; P_a = number of private alleles observed only in that age class; F = inbreeding coefficient.

Age class	N	Median age	Na	He	Ho	Pa	F
1	34	60	4.615	0.435	0.330	3	0.220
2	40	40	5.000	0.449	0.310	1	0.330
3	32	30	4.615	0.446	0.299	7	0.313
4	37	20	5.154	0.445	0.304	3	0.348
5	37	15	4.308	0.415	0.304	2	0.246
6	39	10	4.462	0.422	0.294	0	0.228
7	29	7	4.308	0.428	0.251	2	0.330
8	33	5	4.692	0.429	0.300	5	0.265
9	26	2	3.846	0.421	0.286	2	0.311

TABLE 5. Jackfruit size classes with genetic diversity statistics as calculated in GenAlEx. N = number of samples in size class; Median size is reported in cm DBH; N_a = mean number of alleles across 13 microsatellite loci; H_e = mean expected heterozygosity across 13 microsatellite loci; H_o = mean observed heterozygosity across 13 microsatellite loci; P_a = number of private alleles observed only in that size class; F = inbreeding coefficient.

Size class	N	Median size	Na	He	Ho	Pa	F
1	31	69.9	4.692	0.429	0.329	3	0.219
2	31	54.3	4.615	0.439	0.317	4	0.324
3	31	45.6	4.769	0.463	0.321	2	0.247
4	32	39.4	5.000	0.447	0.305	4	0.300
5	31	32.5	4.538	0.402	0.292	1	0.273
6	31	26.5	4.308	0.449	0.288	3	0.355
7	32	22	4.615	0.425	0.290	2	0.303
8	32	18.3	4.385	0.431	0.284	1	0.292
9	32	13.85	4.615	0.423	0.292	3	0.242
10	31	10	4.308	0.445	0.323	2	0.222
11	32	4.3	4.462	0.431	0.286	5	0.307

TABLE 6. Results of jackknife analysis of linear regressions. Linear regressions and jackknife analysis computed in R. P-value and slope refer to actual values using all age or size classes. Jackknife results report regression coefficients of resampled (N-1) age and size classes. Lowest = lowest value of range; Highest = highest value of range; Mean = mean of all resampled linear regressions. N_a = mean number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; F = inbreeding coefficient; * denotes significant regressions.

Age class regressions

	Reported	*Jackknife p-values*		
	P-value	Lowest	Highest	Mean
N_a	0.204	0.076	0.446	0.2463
H_e	0.105	0.019	0.226	0.136
H_o	0.029*	0.002	0.174	0.051
F	0.619	0.355	0.788	0.551
	Reported	*Jackknife slopes*		
	Slope	Lowest	Highest	Mean
N_a	-0.0095	-0.005	-0.021	-0.010
H_e	-0.0004	-0.0003	-0.0008	-0.0004
H_o	-0.0008	-0.0006	-0.0009	-0.0008
F	0.0005	-0.0013	0.0009	0.0004

Size class regressions

	Reported	*Jackknife p-values*		
	P-value	Lowest	Highest	Mean
N_a	0.078	0.053	0.162	0.097
H_e	0.654	0.391	0.969	0.672
H_o	0.028*	0.0002	0.147	0.051
F	0.684	0.331	0.874	0.638
	Reported	*Jackknife slopes*		
	Slope	Lowest	Highest	Mean
N_a	-0.0057	-0.0047	-0.0076	-0.0058
H_e	-0.0001	-9.61E-06	-0.0003	-0.0001
H_o	-0.0006	-0.0005	-0.0008	-0.0006
F	0.0003	-0.0006	0.0007	0.0003

TABLE 7. Genetic diversity of jackfruit trees propagated from seed and sapling in Bangladesh. N = sample size; N_a = mean number of alleles across 13 microsatellite loci; H_e = mean expected heterozygosity across 13 microsatellite loci; H_o = mean observed heterozygosity across 13 microsatellite loci; P_a = number of private alleles; F = inbreeding coefficient; SE = standard error.

	N	N_a	SE	H_e	SE	H_o	SE	P_a	F	SE
100 years and less										
Seed	159	7.000	0.760	0.438	0.063	0.304	0.054	29	0.311	0.049
Sapling	109	5.615	0.665	0.438	0.061	0.300	0.050	7	0.300	0.068
30 years and less										
Seed	74	5.538	0.694	0.422	0.067	0.282	0.055	9	0.323	0.074
Sapling	97	5.462	0.637	0.441	0.061	0.305	0.051	8	0.290	0.073
20 years and less										
Seed	58	4.923	0.674	0.420	0.068	0.290	0.056	7	0.279	0.075
Sapling	90	5.385	0.594	0.440	0.060	0.304	0.050	13	0.285	0.076
10 years and less										
Seed	38	4.846	0.659	0.415	0.066	0.279	0.053	6	0.277	0.080
Sapling	71	5.000	0.599	0.440	0.058	0.299	0.046	8	0.285	0.078

FIGURE 1. Locations of 8 Bangladeshi districts sampled for this study.

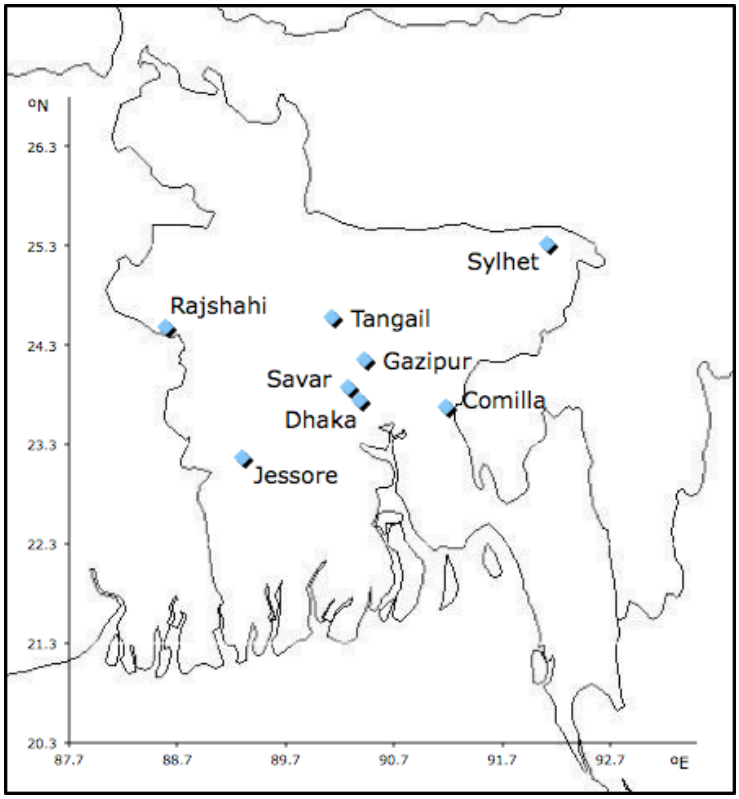
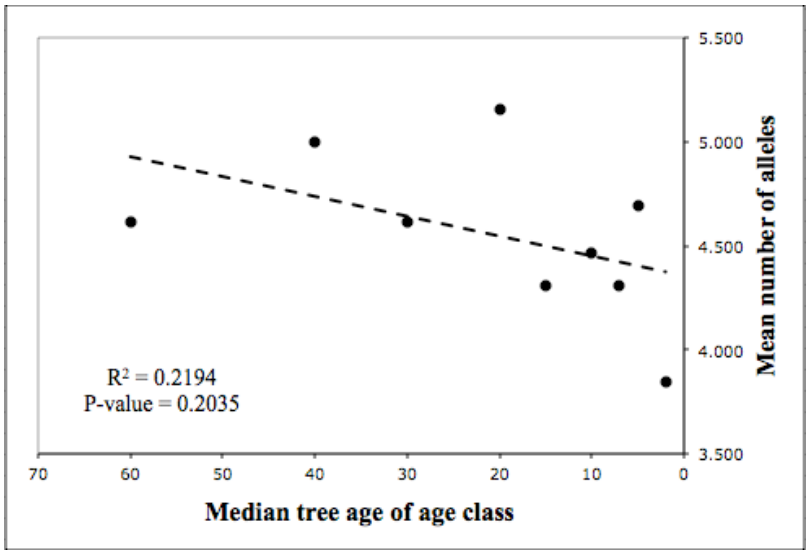
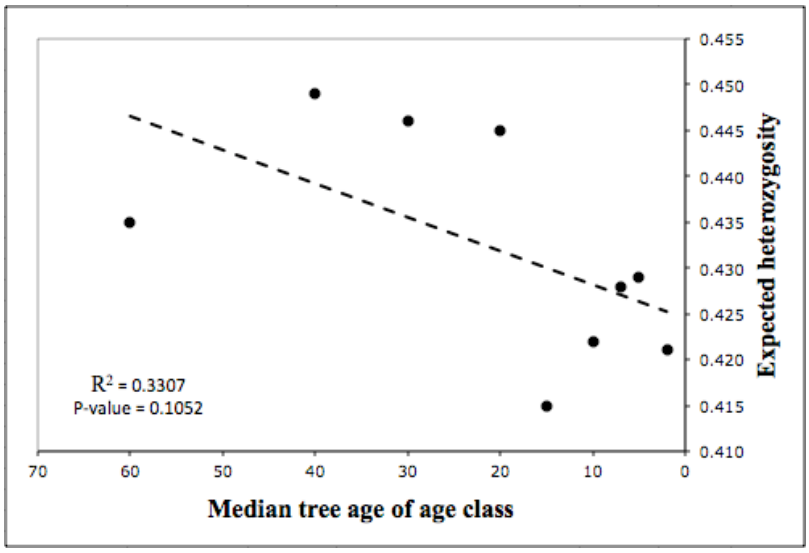


FIGURE 2. Linear regressions of measurements of genetic diversity as a function of age class for Bangladeshi jackfruit trees. A: mean number of alleles across 13 microsatellite loci, p-value = 0.204. B: mean expected heterozygosity across 13 microsatellite loci, p-value = 0.105. C: mean observed heterozygosity across 13 microsatellite loci, p-value = 0.029. D: inbreeding coefficient across 13 microsatellite loci, p-value = 0.619. Age classes are represented by the median age of all samples within a class. The X-axis is labeled by approximate year of germination.

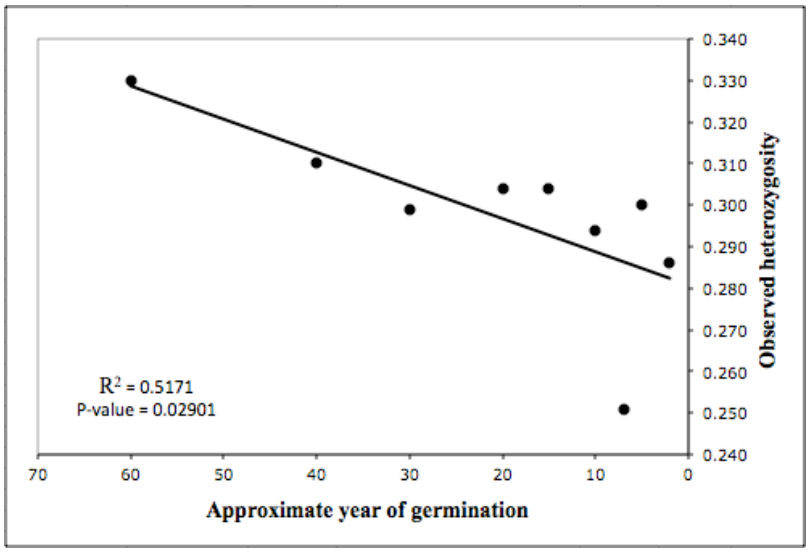
A



B



C


0.340
0.330
0.320
0.310
0.300
0.290
0.280
0.270
0.260
0.250
0.240
Observed heterozygosity
R² = 0.5171
P-value = 0.02901
70
60
50
40
30
20
10
0
Approximate year of germination

D

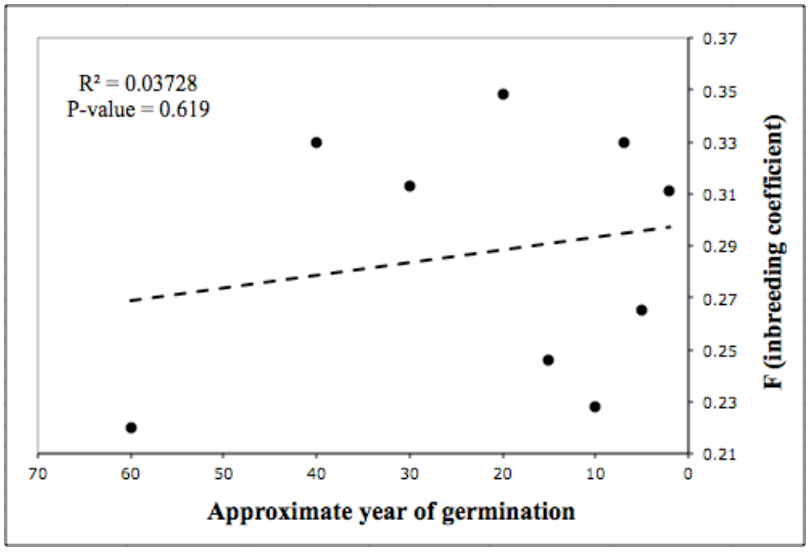
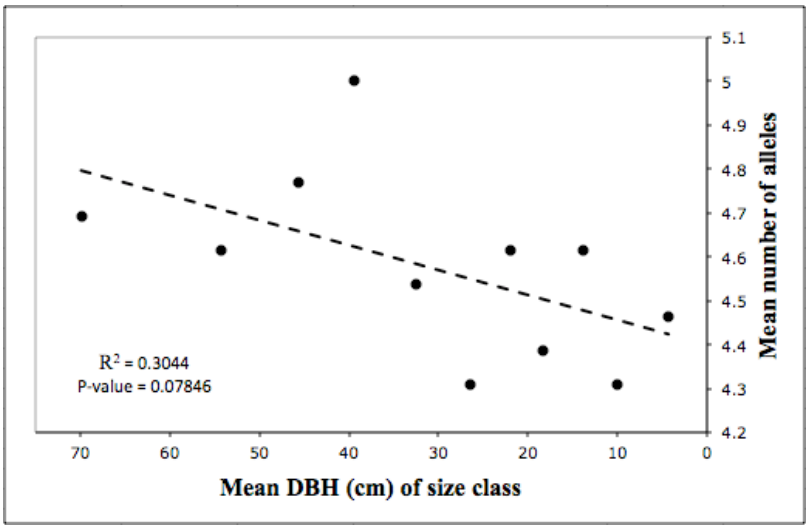
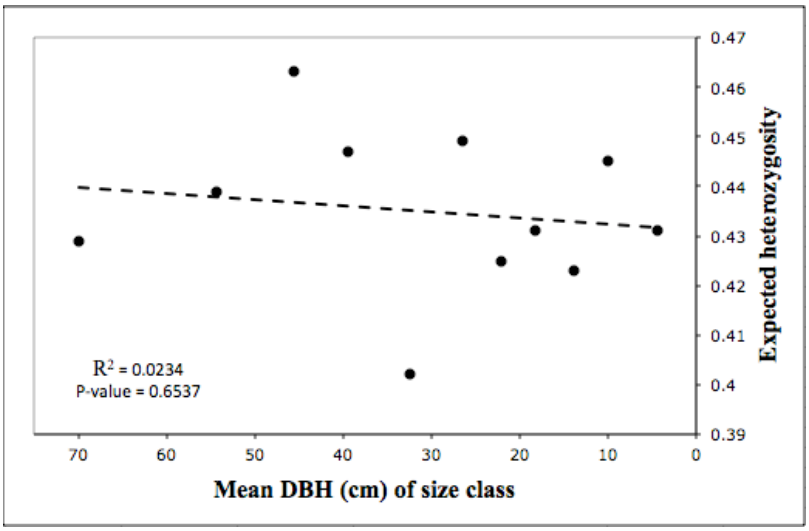

0.37
0.35
0.33
0.31
0.29
0.27
0.25
0.23
0.21
F (inbreeding coefficient)
R² = 0.03728
P-value = 0.619
70
60
50
40
30
20
10
0
Approximate year of germination

FIGURE 3. Linear regressions of measurements of genetic diversity as a function of size class for Bangladeshi jackfruit trees. A: mean number of alleles across 13 microsatellite loci, p-value = 0.078. B: mean expected heterozygosity across 13 microsatellite loci, p-value = 0.654. C: mean observed heterozygosity across 13 microsatellite loci, p-value = 0.028. D: inbreeding coefficient across 13 microsatellite loci, p-value = 0.684. Size classes are represented by the median DBH in cm of all samples within a class.

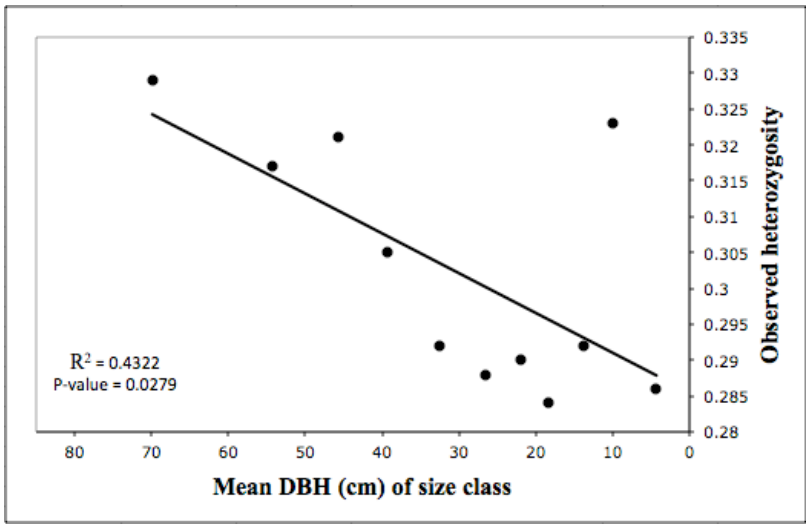
A



B



C


0.335
0.33
0.325
0.32
0.315
0.31
0.305
0.3
0.295
0.29
0.285
0.28
Observed heterozygosity
R² = 0.4322
P-value = 0.0279
80
70
60
50
40
30
20
10
0
Mean DBH (cm) of size class

D

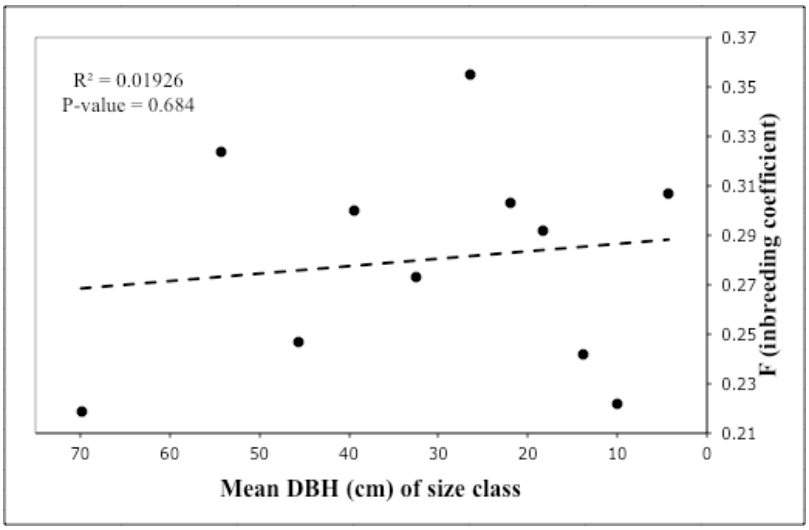
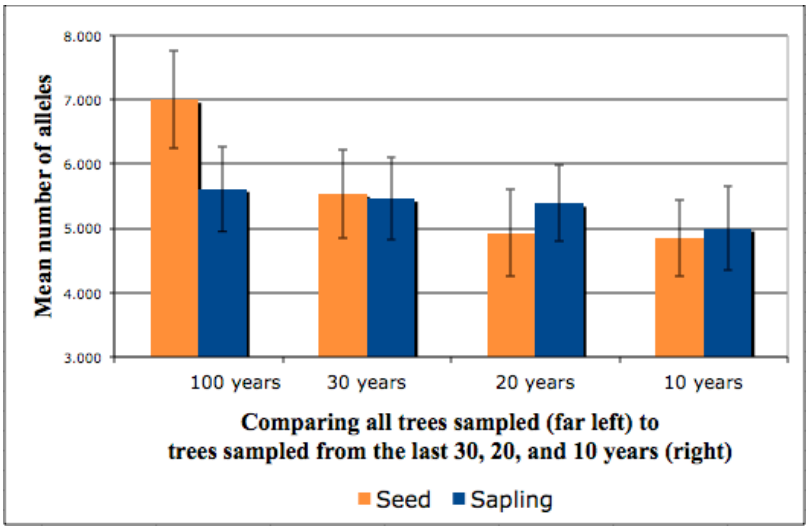
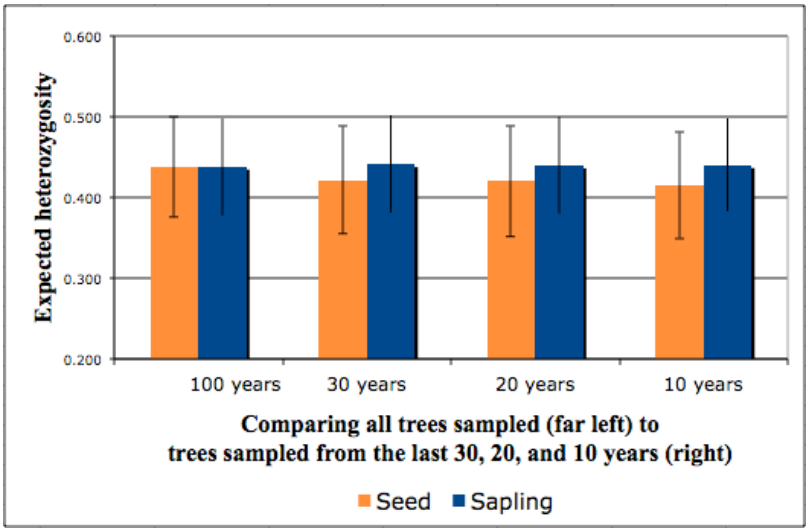

0.37
0.35
0.33
0.31
0.29
0.27
0.25
0.23
0.21
F (inbreeding coefficient)
R² = 0.01926
P-value = 0.684
70
60
50
40
30
20
10
0
Mean DBH (cm) of size class

FIGURE 4. A comparison of genetic diversity in Bangladeshi jackfruit trees propagated directly from seed and from saplings purchased at the market. A: mean number of alleles across 13 microsatellite loci. B: mean expected heterozygosity across 13 microsatellite loci. C: mean observed heterozygosity across 13 microsatellite loci. D: number of private alleles. E: inbreeding coefficient across 13 microsatellite loci. Error bars show standard error. For sample sizes, see Table 5.

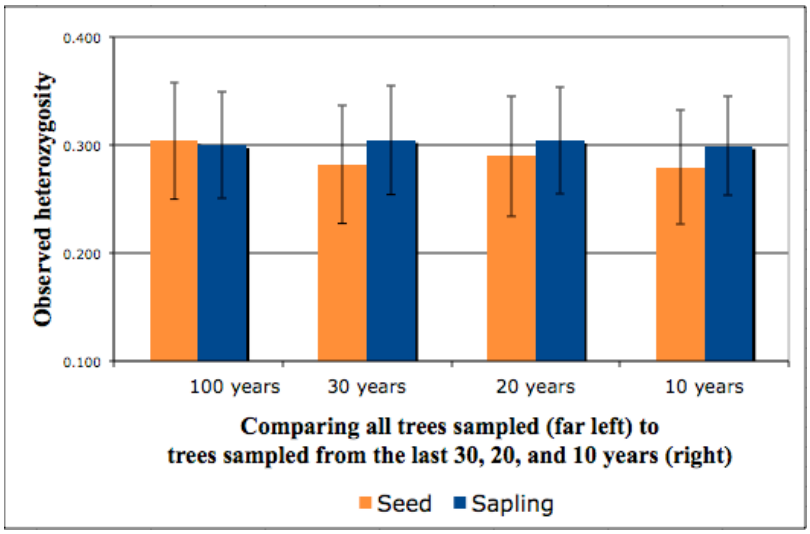
A



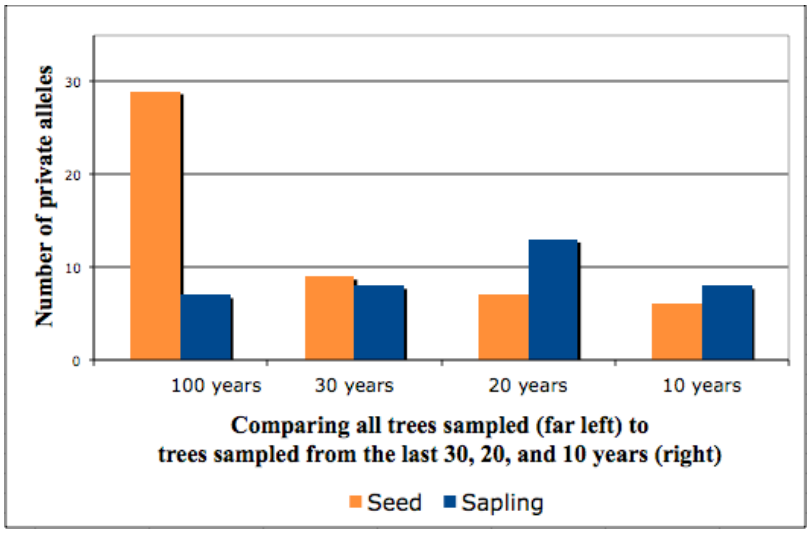
B



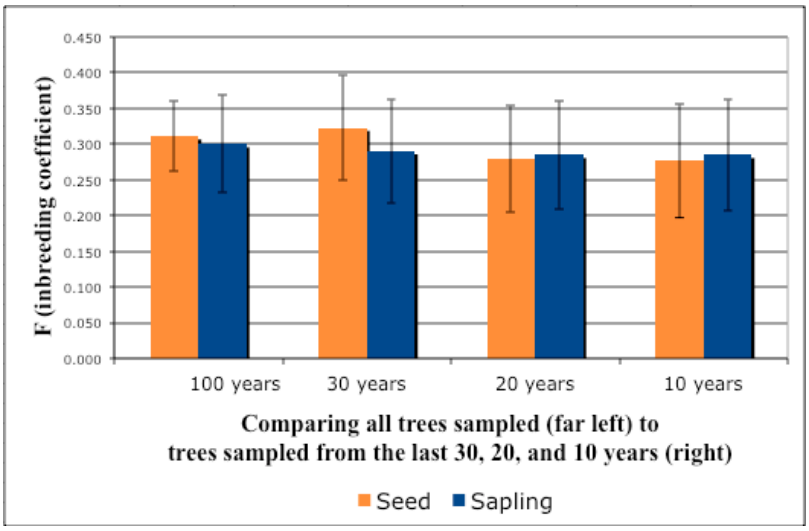
C


0.400
0.300
0.200
0.100
Observed heterozygosity
100 years
30 years
20 years
10 years
Comparing all trees sampled (far left) to
trees sampled from the last 30, 20, and 10 years (right)
Seed
Sapling

D


30
20
10
0
Number of private alleles
100 years
30 years
20 years
10 years
Comparing all trees sampled (far left) to
trees sampled from the last 30, 20, and 10 years (right)
Seed
Sapling

E


0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
F (inbreeding coefficient)
100 years
30 years
20 years
10 years
Comparing all trees sampled (far left) to
trees sampled from the last 30, 20, and 10 years (right)
Seed
Sapling

Appendix 1.

Observations

When possible, informal interviews were conducted with the owner of each tree sampled. Owners or caretakers were present for interviews for 96.2% of trees. Interviews collected a variety of categorical, quantitative, and qualitative data as it applied to each individual tree. The most consistent data collected through interviews included tree age and propagation method (sapling, seed, or graft), as these data were necessary for our research questions. Less consistent data collected included fruit quality, fruiting season, fruit size, fruit texture, number of fruits per tree, seed or sapling source, and fruit destination (consume at home or sell to market) (Table 1). The volume of data collected in each interview was limited by time constraints of the researchers, communication with the translators, and knowledge of the interview subject about the sample tree.

Anecdotal communications and observations were also made that, though not scientifically studied at this time, were both novel to the researchers (i.e. did not appear in the literature about either jackfruit in Bangladesh or jackfruit worldwide) and often unexpected. These observations warrant further study and could prompt researchers to view jackfruit diversity, genetics, domestication, and cultivation in a new light; at the very least, they will help future researchers develop appropriate research questions and better prepare for work in the field, and therefore merit documentation. However, these personal observations were made by the researchers during a three-and-a-half week field season, often through translation, and should not be taken as fact for all of Bangladesh or all jackfruit.

Wild trees

Although large trees with undesirable characteristics were reported to be growing in the Tangail region of Bangladesh, after discussion with local owners, it was found that the trees had been planted by the owner or the owner's parents. In our travels, we observed no trees growing away from human habitation in Bangladesh. In fact, we observed no wild areas of Bangladesh where any plant could be growing away from human habitation. (Remaining wild areas are mostly in the mangrove forests of the Sunderbans in the south, where jackfruit doesn't grow, and in the Chittagong Hill Tracts, which we were unable to visit due to both time and ongoing violence in the region.) In Lawachara National Park, we did observe old jackfruit trees. However, the largest trees of similar size appeared to be growing in a straight line through the other vegetation. Speaking with the park employees who were familiar with the park's history, we learned that the land had previously been privately owned by British individuals. The area was not officially made a national park until 1996. Park employees could identify several trees that were planted in 1968 and several others that were planted in the 1950s, when "many trees were planted". Indigenous tribes are allowed use of the park for their villages, hunting grounds, to collect firewood and other forest products, and for agriculture. In addition to the tribal people living in the park, the park runs a nursery inside the park for growing its own tree saplings for replanting into the forest (not for distribution outside the park), including

many jackfruit saplings. Park employees live within the park, and we observed livestock grazing near employee housing. Throughout the country, we did observe some trees that were not intentionally planted but had germinated from seeds discarded as trash or from fallen fruits. If these individuals survived the sapling stage, all homeowners we spoke with would allow the trees to grow to maturity for the benefits of fruit and timber, but there weren't large numbers of unintentional trees (28 of 257 surveyed, see Table 1), and statistically it is most likely that the unintentional offspring descended from intentional parents.

From our observations, it is unlikely that wild trees will be found in Bangladesh.

Cultivars

We observed no named cultivars being grown in Bangladesh, except for one (BARI One Kathal) that was recently developed at the Bangladesh Agricultural Research Institute (BARI) in Sylhet for within-park cultivation by vegetative grafting. Bangladeshi agroforesters are familiar with grafting and it is the most common way of propagating mango trees in the country. We did observe and discuss named cultivars of mango. In general, named cultivars are not available to jackfruit growers as seed or sapling. Some trees are named after specific characteristics they possess, such as baromashee (fruiting twice a year) and hazarikathal ("tree with a thousand fruits" – in reality usually over one or two hundred fruits per tree). These characteristics, however, were not known to be reliably passed on to offspring. We encountered several growers who planted seed from baromashee or hazarikathal trees and did not observe those characteristics in the offspring.

True-to-type

As described above, jackfruit growers often chose seeds from good quality fruits or trees with other desirable characteristics, but found that the offspring was often not true-to-type. This was reported by both homegarden owners and BARI researchers. It should be noted that almost every tree was reported to bear fruit that was homogeneous for fruit quality, fruit size, and fruit texture (see Table 1).

Propagation

Almost all jackfruit growers in Bangladesh propagate trees either directly from seed or from purchased seed-propagated saplings. Grafting, though reported to be the dominant propagation method in Thailand and Australia, was only observed at BARI, and only in the preliminary stages, the success rate was still quiet low. Tissue culture propagation research for jackfruit and other crops was observed at several Bangladeshi universities. Scientists mentioned that appropriate grafting techniques were very difficult to develop for jackfruit, though it is unclear what the reasons are for this. Vegetative grafting of mango is commonly practiced in Bangladesh.

Sapling vs. seed

When asked why they purchased saplings instead of planting from seed, jackfruit growers mentioned that saplings were easier to protect from livestock and children, fruited earlier, or had no response. Only one grower said that he bought a particular sapling because the

sapling seller claimed it would produce two crops in one year (baromashee); the tree was still too young to fruit at the time of our interview, so it is unclear if the tree will indeed be baromashee or if the grower will be satisfied with his purchase. Fruit quality was not quoted to us as a reason for buying saplings instead of planting from seed.

Abundance

Although we knew that jackfruit is the second most common fruit tree, after mango, and that it is the national fruit of Bangladesh, we were surprised by the abundance of jackfruit trees throughout the country. Except for the southern districts where we did not travel (because jackfruit is reportedly less common there due to precipitation and salinity), jackfruit trees were everywhere. Jackfruit trees are easy to spot from a distance because of their distinct shape (leaves usually point up to the sky), the large cauliflorous fruits, and the flatness of the land. Trees were seen in every homegarden, along every road, near shops, in city courtyards, on the campuses of government buildings in both rural and urban areas, at universities and schools, between rows on pineapple plantations, and throughout wooded areas. Fresh jackfruit was observed in every market in both rural and urban towns. Everyone we interviewed ate jackfruit, though some of the wealthier Bangladeshis professed that it wasn't their "favorite fruit", and both scientists and homegarden growers informed us that jackfruit is their national fruit.

Commercial orchards

We did not observe any commercial jackfruit orchards, and only one private orchard. Jackfruit is mostly planted as part of the homegarden alongside a mix of other crops, including other fruits (we observed mango, guava, lime, lemon, banana, longon, lotka, rambutan, litchi, blackberry, various melons, coconut, mangosteen, starfruit, and others). A man who was also employed as a cook at a nearby government institution owned the one orchard we did visit. A family of renters who lived on the orchard land oversaw his orchard.

Poor-quality fruits

We observed plenty of trees that produced fruits of poor quality. Again, most trees were reported to be homogeneous for fruit quality. Many trees produced only fruits that were unpollinated or only fruits that were semi-pollinated. Other trees produced only fruits that dropped off before ripening, fruits that rotted before ripening, or fruits that split on the tree. A few trees were reported to produce poor-quality fruits due to a lack of sweetness in the fruit, or fruit that was too soft or too watery. Poor-quality trees were not culled by owners because of the value of mature timber and the ability to sell some poor-quality fruits to middlemen if fruit quality could not be detected from the outside.

Fruit texture

Fruit texture was commonly described as either crispy or soft, with each tree known to produce fruit of one quality or the other. A dozen trees were described as producing fruit with a texture in between soft and crispy, but only two trees were described as producing separate soft and crispy fruits. Because growers identified two distinct textures, we first assumed that an individual genetic component must be responsible for fruit texture. We did speak to a few growers, however, who suggested fruit texture could change with tree

age, fruit ripeness, the time of fruiting within the year, or the time of fruiting on the particular tree (e.g. fruits produced early on the tree were soft and fruits produced at the end of that tree's fruiting season were crispy). In addition, there could be an environmental cause for fruit texture, or a combination of environmental and genetic factors.

Morphological diversity

A wide range of jackfruit morphological diversity was observed in Bangladesh. We observed variation in fruit size (see Table 1) and leaf size, as was reported in Khan et al. 2010. We also observed great variation in rind color, rind texture, leaf shape, location of fruits on the tree (trunk, roots, primary branches, secondary branches, or tertiary branches), and leaf color.

Pollination

Homegarden growers did not report any knowledge of the pollinator of jackfruit, and they did not distinguish between the male and female flowers. Neither local growers nor scientists conducted hand pollination. Employees at Lawachara National Park reported that they did not distinguish between male and female flowers. Bangladeshi scientists did provide us with results of studies they conducted in which they measured reproductive organs and seed set, but no pollinator was identified. We observed both ants and small flying insects on the trees, but we were in Bangladesh too late in the season to conduct any further investigations.

Seed dispersal

Other than intentional human dispersal, we observed dispersal of jackfruit seeds in human garbage. We also observed both dogs and monkeys (in Lawachara National Park) eating fresh jackfruit. However, due to population density in Bangladesh, any tree that germinated from animal dispersal would only be allowed to reach maturity because of human involvement.

Identifying good-quality jackfruit at the market

Jackfruit growers explained that fruit could be tested for ripeness by tapping the rind and listening for a hollow sound. Both rural and urban jackfruit consumers, however, reported no good way to identify the quality of a jackfruit being sold at market, until it was purchased, carried home, and opened. This characteristic allows growers to sell poor-quality fruits to middlemen. Due to the many layers of middlemen between growers and consumers, consumer pressure for higher quality fruits has not reached growers. It seems to be an accepted risk that a jackfruit purchased at market might be of poor quality.

Jackfruit transport

In the marketing process, each jackfruit exchanges hands several times. Jackfruit growers usually sell to local middlemen, who sell to other middlemen at village sales, who sell to transporters, who sell to shops and stalls in larger cities. During this process, jackfruit moves around the country fairly easily, carrying its seeds with it. The widespread exchange of jackfruit has led to the widespread dispersal of seeds, possibly contributing to the intermixing of jackfruit genes into one large Bangladeshi population. It is unclear

when this marketing process originated, or what effects it may have had on the genetic structure of the crop. It could be that the development or improvement of the national highway system greatly increased jackfruit dispersal in Bangladesh.

Commercial nursery selection

At Hortus Nursery, a commercial nursery in Savar (Dhaka Division) that sells saplings to the public, nursery workers informed us that they bought fruits from the local market, ate the fruits for lunch, and then planted the seeds from that fruit for the nursery saplings. They did not discriminate from using seeds from good- or poor-quality fruits. Although these nursery growers are not deliberately selecting seeds for any specific characteristics, there could be non-intentional selection occurring. For example, if they purchase their source fruit from the same market stand every day, it could be traced back to a limited geographic region or even a limited number of homegardens.

Chemical use

While no chemicals were reported to be used on jackfruit trees, chemicals are used on other crops, including pineapple (to promote biannual flowering). As jackfruit in Bangladesh is often grown alongside pineapple, at least one grower was concerned that the chemicals could be negatively affecting his jackfruit trees. His specific concern was a decreased lifespan in the jackfruit trees, but this and other effects have not been studied.

Jackfruit use in institutional spaces

Jackfruit trees on school, university, and government campuses are often assigned to particular individuals or groups for their exclusive use. Professors, employees, employee's families, dorms, grade levels, or employees of particular buildings or departments are assigned specific jackfruit trees. The fruit from the assigned trees could be eaten or given away by the designee. We observed some designees protecting their trees from grazing or theft by building fences or planting other small trees and vines around the jackfruit tree. In at least some cases, employees considered a designated jackfruit tree to be a perk of the job. Because jackfruit trees are so prevalent and high yielding, an added benefit of assigning public jackfruit trees to specific individuals could be the prevention of large fruits going unharvested and requiring cleanup.

The role of women

Although the literature has shown that women often tend to fruit trees in homegardens, most of the jackfruit owners and caretakers interviewed were male. In general, male tree owners presented themselves to be interviewed by our research team. There are several possible explanations for this. Women may have been shyer or reluctant to speak with strangers or with the male members of our team. The presence of American researchers in Bangladeshi villages often drew small crowds of interested onlookers, and it is possible that men may have enjoyed the attention and importance of being included in the study. If future researchers wish to do more in-depth ethnobotanical interviews about jackfruit growing practices, they will want to spend more time at each individual site to allow villagers to warm up to researchers and allow for the novelty of having researchers in the village to wear off.

Homegardens

Lastly, I will mention my impression of a high level of self-sufficiency observed in homegarden owners and their families. Homegardens are multi-crop agroecosystems that provide owners with food, timber, fuelwood, and income. Despite this impression, homegarden owners are farmers, and it is easy to see how one flood, cyclone, or drought could destroy their entire livelihood. As most of Bangladesh's almost 160 million residents rely in some part on a homegarden, it is critical that more attention be given to research on homegarden crops, agricultural methods, and economics.

TABLE 1. Individual tree characteristics collected through interviews with Bangladeshi jackfruit tree owners and caretakers in July 2010. Responses are followed by the number of times they were given; only one response was allowed per category per tree. N= number of trees for which data were collected in a particular category

Category	**N (of 394 trees)**	**Responses**
Tree age	340	Range (1-100 years)
Planting intention	257	Intentional (229), Unintentional (28)
Propagation Method	243	Seed (99), Sapling (130), Graft (2)
Source of propagation material	102	Seed: own fruit (3), neighbor's fruit (1), family member's fruit (3), fruit bought at market (1); Sapling: direct from commercial nursery (26), market (22), tree fair (1), institutional nursery (32), own private nursery (12). Neighbor's private nursery (1)
Annual number of fruits	150	Range (2-350 fruits)
Fruiting season	97	Early (37), Mid (14), Mid/Late (5), Late (37), Baromashee (twice a year) (3), Alternate years (1)
Fruit destination	84	Eat (15), Sell (25), Eat and sell (30), Feed to cows (5), Eat and leave for wildlife (2), Eat, sell, and leave for wildlife (1)
Fruit texture	111	Soft (64), Crispy (32), In between (12), Mix of soft and crispy fruits on same tree (2)
Sweetness	116	Sweet (102), Not sweet (14)
Fruit size	109	Small (29), Medium (35), Large (44), Mix (1)
Fruit quality	142	Poor (35), Fair (19), Good (66), Very good (17), Excellent (5)

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