

Dwarfing of breadfruit (*Artocarpus altilis*) trees: Opportunities and challenges

ABSTRACT

Breadfruit [*Artocarpus altilis* (Parkinson) Fosberg)] is a traditional staple crop grown for its starchy fruit throughout the tropics. It has long been recognized for its potential to alleviate hunger in the region. However, being a tree of 10 – 30 m, breadfruit is vulnerable to wind damage. Owing to the continuing trend of global climate change, the success of the species as a sustainable crop for delivering local food security is compromised by the likelihood of more intense tropical windstorms in the island nations. Tree height also forms a major constraint to disease management and fruit harvesting. These imperatives have driven an increasing interest in developing breadfruit varieties with short stature. While a great diversity of breadfruit cultivars with varying nutritional and agronomic characteristics exists, the genetic resource showing dwarfing traits is largely uncharacterised. Historically, there has been no intentional breeding for breadfruit cultivars. The long growth cycle, predominantly vegetative propagation and lack of genome information create challenge for crop improvement through traditional breeding. In this review, we highlight the current knowledge of plant dwarfism and its application in agricultural practices and genetic improvement for dwarf phenotype, and present options and tools for breadfruit dwarfing with special reference to natural genetic variability for dwarfing rootstocks, plant growth regulators, potential of mutagenesis and its combination with the currently established *in vitro* propagation protocol in breadfruit. The role of genetic transformation, high-throughput mutant detection by using Targeting-Induced Local Lesions IN Genomes (TILLING) and tools of next generation sequencing is also discussed.

Keywords: *dwarfism, Artocarpus, breadfruit, genetic variability, plant growth regulator, mutagenesis, mutation breeding, genetic transformation*

17 1. INTRODUCTION

18
19 Breadfruit [*Artocarpus altilis* (Parkinson) Fosberg)] is a staple tree crop in the Oceania and
20 throughout the tropics [1]. The millennia of selective breeding by the indigenous peoples of
21 Oceania has resulted in great diversity in morphological, agronomic, and nutritional
22 characteristics among cultivars [2], resulting in hundreds of cultivars [3], some of which have
23 been globally distributed including Central and South America, Africa, India, Southeast Asia,
24 Indonesia, Sri Lanka, northern Australia, as well as Madagascar, the Seychelles, the
25 Maldives and Mauritius [4]. Breadfruit is regarded as an energy food, a source of complex
26 carbohydrates, vitamins and minerals [5]. Breadfruit bears fruit with edible dry mass up to 6
27 t/ha, comparing favourably with other common staple crops, and has been recognized for its
28 potential to alleviate hunger in the tropics [6]. Breadfruit makes a significant contribution to
29 the local food security, often a major tree crop within an indigenous agroforestry system
30 which can be grown sustainably with relatively low agricultural inputs [3].

31
32 Despite its importance as a traditional food security crop and its emergence as a commercial
33 crop [7], breadfruit cultivation encounters several constraints in the tropical regions. These
34 include susceptibility to natural disasters, such as cyclones and hurricanes and prolonged
35 drought [8]. Breadfruit is also susceptible to some pests and diseases, namely the fungi,
36 *Phytophthora palmivora* and *Phellinus noxius* which cause rots. *P. palmivora* affects the fruit
37 and *P. noxius* the trunk and root, eventually killing the tree [9]. *Bactrocera frauenfeldi* can
38 also attack breadfruit and in combination with *B. umbrosa* affected 75% of fruits in Papua
39 New Guinea [10]. Climate change is likely to exacerbate the impact from extreme weather
40 and natural disasters according to current projections [11]. How global climate change will
41 affect breadfruit pests and diseases is unclear.

42
43 Being an evergreen tree from 15-30 m, breadfruit is prone to wind damage [1,12]. In the
44 past decades, many atolls and high islands have experienced destructive cyclones which
45 can have a devastating impact on islands that rely heavily on breadfruit for a staple food.
46 For example, in 1990, Cyclone 'Ofa' destroyed 100% of the breadfruit crop in Samoa, and
47 50 - 90% of the big mature trees were blown over [13]. Almost entire breadfruit crops were
48 lost in Upolu after Cyclone 'Evan' hit Samoa in 2012 [14]. Cyclone-related tree loss was
49 also responsible for a reduced number of fruiting trees in Fiji [9]. Similarly in the Caribbean,
50 a major breadfruit producing region, hurricanes in the 1990s resulted in the loss of and
51 damage to a significant number of breadfruit trees [13]. In the 1980s an estimated 50% of
52 the breadfruit trees in Jamaica were killed or damaged by windstorms [15]. The continuing

trend of global climate change with more intense hurricane-force storms will have serious implications for island nations throughout the Pacific and Caribbean [8,11].

Tree height is also a major constraint to disease control and fruit harvesting. Fruit production costs increase as trees grow taller, due to the high labour cost for ongoing pruning, removal of dead or disease-affected branches, and challenging harvest. Mechanical harvesters have not been utilized for most of breadfruit cultivation, therefore labour intensive (frequent climbing) remains the only viable option [3]. This method of harvesting results in a high proportion of either damaged fruit, or fruit with a limited shelf-life after falling to the ground during harvest. It is estimated that over 50% of the fruit may be lost due to the difficulty of harvesting from large trees (Roberts-Nkrumah 2007).

Pruning is an option often used to reduce tree size, however it has its drawbacks. Severe branch pruning can reduce yield because the trees are stimulated to grow more vigorously in the subsequent season. Poor pruning practices may have severe effects on the health of a tree leading to wound injury and invasion of fungal pathogens [9]. Research on the agronomy, pruning and orchard management of breadfruit is currently limited [9].

In response to these constraints there is increasing interest in developing breadfruit varieties with short-stature [16]. The benefits of dwarfism in fruit-tree industry have been clearly demonstrated with the widespread use of dwarfing rootstocks in apple and peach [17,18]. Today, breeding efforts have resulted in selection of dwarf scions or dwarfing rootstock varieties in almost all of the main temperate and tropical fruit species [19]. These commercially acceptable dwarf varieties have revolutionised fruit production by allowing dense field cultivation, increasing harvest index and substantially decreasing production costs [20,21]. Building on a going body of research contributing to our understanding of plant dwarfism, the present review discusses the opportunities and challenges toward breadfruit dwarfing, with focus on issues related to strategies and prospects of natural and induced genetic variability, horticultural techniques, molecular breeding and the potential role of genomic tools for the development of dwarf phenotype.

2. MECHANISM OF DWARFISM

89

90 **2.1 Gibberellin**

91

92 Various factors cause dwarfism in plant, of which gibberellin (GA) and brassinosteroids
93 (BRs) are the most important factors in determining plant height [22,23]. Research from
94 rice, barley, and *Arabidopsis* mutants has demonstrated that dwarfism is commonly
95 associated with deficiencies in GA levels or signalling [24,25]. The level of bioactive GAs in
96 plant is controlled by several mechanisms, including transcriptional regulation of genes
97 encoding enzymes for GA biosynthetic and catabolic pathways. The GA biosynthetic genes
98 were negatively regulated by high GA levels whereas the GA catabolism genes were
99 positively regulated by the GA concentrations [26,27]. GA promotes plant growth by
100 inducing the degradation of DELLA proteins which act as GA signal repressors [24,28].
101 DELLA proteins consist of N-terminal amino acids, D-E-L-L-A (DELLA domain) essential for
102 perception of the GA signal and a C-terminal region for repressor function [24,28]. Shortly
103 after gibberellin stimulation, DELLA proteins are degraded in the plant. However, mutant
104 DELLA proteins are resistant to destruction and accumulate to cause dwarfing phenotype by
105 constitutive growth repression [29]. Therefore by modifying regulation of genes controlling
106 GA flux and GA response, it is possible to modify processes regulated by GA and, thus,
107 plant form [30]. These apply to dwarf or semi-dwarf varieties of the wheat reduced height-2
108 (*rht*), the rice semi-dwarf-1 (*sd1*), the maize dwarf-8 (*d8*), the rice *gai* and the barley
109 slender1 (*sln1*) [25].

110

111 Recently, it has found that jasmonic acid (JA) can antagonize the GA mediated response
112 through modulating the levels of DELLA proteins [31]. *Arabidopsis* mutants over-producing
113 JA have stunted stems, and a rice semi-dwarf mutant *rim1-1*, displaying resistance to rice
114 dwarf virus, has high expression level of genes encoding JA biosynthetic enzymes, leading
115 to a rapid accumulation of JA after wounding [32]. In this sense, interaction between GA
116 and JA signalling is used to make a balance between “growth” and “defence” in response to
117 various stimuli [33].

118

119 **2.2 Brassinosteroids (BRs)**

120

121 Brassinosteroids are a class of plant steroid hormones that promote plant growth and
122 regulate organ morphology through controlling cell elongation and division. They are also
123 important for vascular differentiation, flowering, light responses, and regulation of other
124 hormone signalling, particularly the auxin pathway [34]. BRs are produced from campesterol

by a network of reactions, the genes responsible for each reaction are not completely known [35]. BR-related mutants usually exhibit short and compact stature with deep green and erect leaves and delayed flowering [36,37]. The rice *Osdwarf4-1* mutant exhibits erect leaves and slight dwarfism without compromising grain yield [38]; this phenotype is due to loss of function of a cytochrome P450 involved in BR biosynthesis [39]. A dwarf brassinosteroid-deficient mutant of broad bean (*Vicia faba* L.) created by γ -ray irradiation was defective in sterol C-24 reduction [40]. The barley semi-dwarf mutant carries a mutant allele of a gene encoding a putative BR receptor [36]. Manipulating BR biosynthesis and signal transduction is a strategy for generating dwarf phenotypes [37].

3. GENETIC RESOURCES FOR DWARFING CLONES

3.1 Reproductive system

The genetic structure of a plant species is largely influenced by its reproductive system. Breadfruit is considered as an out-crossing species [1], but the species is monoecious, with self pollination prevented by a temporal separation due to the male inflorescences appearing earlier than female inflorescences [41]. Breadfruit comprises fertile and sterile diploids ($2n = 2x = 56$) and sterile triploids ($2n = 3x = 84$) [1,42]. Significant morphological variability exists including true seedless varieties, varieties with several aborted seeds, and those with numerous viable seeds [12]. Fruit development in seedless breadfruit is parthenocarpic and does not require pollen to be initiated [43] and in fact, little is known about pollination in seeded cultivars with both wind and insect pollination being suggested [3].

The seeded, out-crossing, fertile varieties are mostly found in the western South Pacific, while the seedless forms predominate in the eastern islands of Polynesia [42,44]. Molecular evidences have suggested that the Melanesian and Polynesian cultivars, *A. altilis*, may have been derived from *A. camansi*, whereas the Micronesian cultivars may be the product of interspecific hybridization between the *A. camansi* –derived cultivars and *A. mariannensis* and subsequent introgression [43,44]. Frequent recombination and segregation events have contributed to the genetic diversity of the domesticated breadfruit during thousands of years of evolution. At the same time, repeated vegetative propagation has played a role in fixing heterozygosity and maintaining the unique gene combinations that confer the specific phenotypes. This is evident by the high degree of morphological diversity and many distinct cultivars specific to particular island groups [43].

160

161 Vegetative propagation is required for seedless varieties and preferred for seeded varieties
162 [3]. Seeds are rarely used as true-to-type seedlings rarely occur, and it is difficult for viable
163 seeds to survive desiccation [1]. Clonal propagation is generally through root suckers, root
164 cuttings, or air layering [1,12]. Seedless varieties can be grafted onto seeded rootstock
165 using various techniques such as approach grafting or cleft grafting [3].

166

167 **3.2 Breeding**

168

169 Deliberate breeding of breadfruit has not been reported. Indigenous islanders have selected
170 seedlings or somatic variants from natural populations for desirable characters over
171 thousands of years [1], but selection has not been rigorous in most areas where breadfruit is
172 cultivated. Many of the Pacific Island cultivars have been present for generations. Generally
173 few new cultivars are recognized and selected, particularly where seedless and few-seeded
174 cultivars predominate – in these locations islanders typically rely on a group of preferred
175 cultivars, because they are well-adapted to that location and grow and fruit well [1].
176 Seedling trees are retained on occasion but rarely multiplied. In limited few areas, such as
177 Santa Cruz Islands, where breadfruit forms important part of traditional arboriculture systems
178 and most cultivars have seeds, seedlings are allowed to grow until they bear fruit. New
179 seedlings with desirable traits are selected and maintained by vegetative propagation [1,45].

180

181 **3.3 Genetic diversity and dwarfing rootstocks**

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183 Through horticultural practice, scions may be dwarfed by grafting onto dwarfing rootstocks.
184 The dwarfing effect of the rootstock may also be induced by a stem piece or interstock
185 (intermediate stock) grafted between a scion and rootstock [18]. Apart from greatly reducing
186 the vigour of the grafted scion, the practice has revolutionized the production of some
187 perennial tree crops by shortening the time to flowering (juvenile phase) [21]. Apple
188 seedlings have a long juvenile period and can take 4–8 years to flower and grafting the scion
189 onto dwarfing rootstocks shortens this period by several years [46]. Similarly, grafted
190 breadfruit trees can begin bearing in 2-3 years, while vegetatively propagated trees start
191 | fruiting in 3-6 years, and trees grown from seed may begin to produce fruit in 6-10 years [9].

192

193 The wealth of genetic diversity of breadfruit that exists is the source for future development
194 of dwarf rootstocks or scions. 132 cultivars from Vanuatu, 70 from Fiji, 50 from Pohnpei,
195 more than 30 from Tahiti and over 40 from Samoa have been documented [3]. The genetic

variability has resulted in enormous phenotypic variation in morphological, agronomic, and nutritional characteristics among cultivated varieties [43]. Research is being carried out to develop high polymorphism molecular markers, such as microsatellite loci to facilitate phylogenetic analyses, cultivar identification and germplasm conservation of breadfruit [47]. A dwarf variety of breadfruit was reported at the Pacific island of Niutao [48]. However, the agronomical characteristics of the variety are largely unknown. In addition, two other cultivars, 'Ma'afala' and 'Puou', popular in Samoa and Tonga, tend to be shorter and more compact than most other varieties. They are commonly seen as small trees up to 10 m with dense spreading canopy at their local regions [49]. Detail information on tree architecture of many traditional Pacific Island cultivars is currently limited and it may be possible that naturally dwarf cultivars with desirable agronomical traits can be identified through in-depth characterisation and vigour selection.

Dwarfing rootstocks may also come from species related to breadfruit. Grafting breadfruit on *A. camansi* rootstock has been reported [50]. Though species of *Artocarpus* genus are mostly tall tree and rarely shrubs, they display great diversity in the tree stature. For example, *A. anisophyllus* and *A. hirsutus* are large rainforest trees up to 50 m [51,52], species like *A. camansi* (breadnut), *A. nitidus*, *A. mariannensis* (dugdug), *A. integer* (chempedak) and *A. heterophyllus* (jackfruit) tend to be a medium size tree to 25 m, with other species, such as *A. lakoocha* about 10 ~ 15 m, and *A. petelotii* up to 10 m [45,53-55]. Noticeably, species *A. xanthocarpus* is reported to be up to 8 m [53].

The vigor of shoot growth and eventual size of tree at maturity is usually controlled by the use of rootstocks while the scions also have a significant effect on final tree size. Grafted *A. heterophyllus* (jackfruit) trees were found to have a dwarfing tendency [56], with cultivar "Ziman Pink" marketed as a dwarf type of jackfruit [57]. The effect of grafting on tree vigor, particularly the choice of rootstocks on tree stature of breadfruit cultivars is worthy of experimental investigation.

4. DWARFING BY CHEMICAL TREATMENT

Plant growth retardants are widely used in agricultural industry. Many species, including cereals, grasses, fruit trees and ornamentals, are regularly treated with chemicals to control plant stature [58]. Most of these growth regulators act as GA biosynthesis inhibitors. To date, four different types of GA inhibitors are known: 1) onium compounds including chlormequat chloride, chlorphonium and AMO-1618 (2-isopropyl-4-dimethylamino-5-

232 methylphenyl-1-piperidinecarboxylate methyl chloride); 2) N-containing heterocyclic
 233 compounds including hexaconazole (HX), ancymidol, flurprimidol, tetcy-clasis and
 234 paclobutrazol; 3) acylcyclohexanediones including prohexadione-calcium (Pro-Ca),
 235 trinexapac-ethyl (TNE) and daminozide; 4) 16, 17-dihydro-GA5 and related structures [59].
 236 Some chemicals such as daminozide, ethephon and paclobutrazol are persistent in the plant
 237 as an un-metabolized form and therefore have raised concern due to the residue toxicity and
 238 health risk [60]. Recently, chemicals like Pro-Ca, TNE and HX represent a novel class of
 239 plant growth regulators that show a lack of persistence in plant. The short-term effect of
 240 these chemicals provides a flexible tool for vegetative growth management that can be
 241 applied at different times and growth strategies [61].

242

243 Pro-Ca has gained attention not only for its specific inhibitory effect on seedling height and
 244 shoot length without any residual problems in the plant and soil, but also for increasing
 245 yield, fruit quality and fruit set of some species such as tomato, strawberry, pear and
 246 avocado [62]. Pro-Ca is a structural mimic of 2-oxoglutaric acid, a co-substrate of
 247 dioxygenases that catalyze late steps of GA biosynthesis, therefore blocking 3 β -
 248 hydroxylation and inhibiting the formation of active GAs which leads to the suppression of
 249 shoot elongation and a more compact canopy [59]. The chemical was shown to have a
 250 similar effect to another GA biosynthesis inhibitor, chlormequat chloride (CCC), but with low
 251 toxicity and limited persistence [63,64].

252

253 Pro-Ca has been registered in the U.S.A and Europe for use on apple [64], rice [61], petunia
 254 and okra plants [65], Camarosa strawberry [66], sorghum [67] and peanut [68]. Application
 255 of Pro-Ca reduces the length of stem internode and vegetative growth of fruit trees including
 256 apple [69], pear [60] and cherry [70]. Generally, the rate needed for effective vegetative
 257 control has to be raised as the vegetative vigour of the trees increases [61]. The compound
 258 has been reported to have no negative effect on yield, fruit quality, fruit set and flower
 259 initiation [60,69,71], although delayed initiation of flowering or fruit set has been reported in
 260 several studies [65]. Pro-Ca significantly shortened the annual shoots of a walnut cultivar
 261 [72], and greatly suppressed the stem length of peanut [68] and sweet sorghum crops [73].

262 Pro-Ca does not persist in the plant therefore does not directly affect vegetative growth in
 263 the following season [61]. In higher plants, the chemical degrades to a natural product
 264 through deacylation and ring cleavage with a half life of a few weeks, whereas in the soil,
 265 Pro-Ca decomposes mostly to carbon dioxide, with a half life of <7 d. No negative effects
 266 have been reported on birds, fish, honey bees (*Apis mellifera* L.) or soil micro-organisms
 267 [61].

268

269 The use of plant growth regulators in breadfruit trees has not been reported. There is a
270 need to investigate the response and sensitivity of these chemicals to the tree stature of
271 breadfruit cultivars and their persistence. Given that GA affects many developmental
272 processes including shoot elongation, flowering and fruit set [26,74], fruit size and even
273 postharvest fruit quality [75-77], the use of GA inhibitor has showed different response
274 depending on the timing, cultivars and rate used [78]. Further commercial use of these
275 chemicals in breadfruit trees should also consider the tropical climate, fertilization and the
276 multi-cropping system used in the cultivation. There would also be concern that the use of
277 these chemicals would conflict with the general move in the Pacific for produce to be organic
278 or as chemical free as possible.

279

280 **5. DWARFING BY INDUCED MUTATION**

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282 Genetic resources showing dwarfing characteristics are rare in some fruit trees, including
283 breadfruit, and breeding dwarf rootstocks in such species is difficult. Genetic improvement
284 of breadfruit cultivars by conventional breeding can be a very slow process due to their long
285 juvenile phase and heterogeneous genetic background. Alternatively, genetic variability can
286 be induced by mutagenizing agents, such as chemical and physical mutagens, and
287 transposon insertion [79-81]. Induced mutations are random changes in the nuclear DNA or
288 cytoplasmic organ, resulting in chromosomal or genomic mutations that enable plant
289 breeders to select desirable traits [82]. The technique has potential for modifying existing
290 traits or creating new valuable traits within the cultivated varieties [83]. It is widely used for
291 crop improvement in both seed and vegetatively propagated crops, with currently more than
292 3200 officially released mutant varieties from 214 different plant species throughout the
293 world [84]. Mutation breeding is being investigated in tropical fruit trees, such as litchi,
294 guava, cherimoya, pitanga, jaboticaba and carambola [85]. These species are all
295 characterised by long juvenile phases which make conventional breeding programmes slow
296 and costly [85]. In this context, the techniques can be used in breadfruit to overcome the
297 long growth cycle and triploidy problems and produce materials that can be screened for
298 advantageous traits such as dwarf phenotype. The putative dwarf mutants identified by
299 mutagenesis can be further used in breeding programs as parental germplasm materials or
300 rootstocks for grafting.

301

302 **5.1 *In vitro* mutagenesis**

303

304 The combination of *in vitro* culture with mutagenesis (*in vitro* mutagenesis) offers an efficient
 305 method for handling large populations, therefore increasing the efficiency of mutation
 306 induction, mutant recovery and selection [83]. *In vitro* mutagenesis in breadfruit is now
 307 feasible due to a robust *in vitro* culture system established for the species [86-89]. At least
 308 17 cultivars of breadfruit have been successfully *in-vitro* propagated [3]. The protocol
 309 involves shoot proliferation, rooting of regenerated shoots and the establishment of rooted
 310 plantlets in the greenhouse [87,88]. During the procedure, small pieces of breadfruit plant
 311 tissues, such as auxiliary shoot tips and small buds are surface sterilized and cultured on a
 312 growth medium under aseptic conditions. Optimization of the type and concentration of
 313 plant growth regulators in the culture medium induces the proliferation of shoots or roots.
 314 After root differentiation, whole plantlets are potted and acclimatized in growth chamber
 315 before transferring to greenhouse [87,88]. *In vitro* culture has the advantage of rapid
 316 multiplication and distribution of true-to-type planting material on a large scale and all year
 317 round [89,90].

318

319 The procedure of *in vitro* mutagenesis is rather straightforward: isolated explant tissues are
 320 either soaked in a chemical mutagen for a prescribed amount of time, or exposed to a pre-
 321 determined dosage of ionizing radiation. After mutagen treatments, tissues can be
 322 subjected to standard regeneration procedures [91]. It is important to note that in
 323 comparison with approaches associated with single cell origin procedures, such as somatic
 324 embryogenesis, mutagenesis on multicellular tissues including meristem tissues, offshoots
 325 and seeds leads to chimaeras composed of non-mutated cells and cells with different
 326 mutations. Repeated rounds of vegetative propagation must be carried out to dissociate
 327 chimeras [82,92,93]. The mutagenesized tissues (M1V1 generation) are typically required to
 328 undergo at least three culture cycles (M1V3) to eliminate chimeras [94,95].

329

330 *In vitro* mutagenesis was used to induce mutation for selection of reduced-stature and flower
 331 colour in *Rosa spp.* [96-98], streptomycin-resistance in *Solanum surattense* [99], salt
 332 tolerance in *Chrysanthemum morifolium* [100] and various morphological characters in
 333 *Lilium longiflorum* [101]. The techniques have successfully been used to improve agronomic
 334 characters such as tree stature, fruit yield and disease resistance of a variety of fruit crops
 335 (Table 1), inducing selection of 98 dwarf clones from 10,000 mutant banana plants [102].

336

337

338 Different mutagens can produce different spectrums of mutations [103]. The most
 339 commonly reported chemical mutagen, ethyl methanesulphonate (EMS), causes primarily

GC-AT base pair transition mutations in many species, leading to single nucleotide changes [104]. While the majority of induced point mutations are predicted to be functionally silent, other nucleotide changes such as non-sense, mis-sense, RNA splicing defects and regulatory alterations can have varying effects on gene expression and protein function [105]. On the other hand, physical mutagens have shown potential for application in fruit breeding. Among these, ionizing radiations (X-rays and gamma-rays) have been widely used in fruit trees [83]. Ionizing radiation can induce a series of mutations ranging from point mutation to mitotic recombination, or gene conversion and chromosome aberration, therefore generate many kinds of phenotypes including homozygosity [106,107]. Although point mutation rate with gamma rays can be lower than that with EMS, the rate of knockout mutations is usually higher [108].

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Table 1. Examples of *in vitro* mutagenesis for improvement of fruit trees

Species	Mutagens	Selected traits	Explants	References
<i>Pyrus communis</i>	gamma rays	compact trees, fruit traits	<i>in vitro</i> shoots	[109,110]
<i>Prunus salicina</i>	gamma rays	vegetative and fruit traits	<i>in vitro</i> shoots	[95]
<i>Phoenix dactylifera</i>	gamma rays	disease resistant	somatic, embryogenic cells	[82]
<i>Citrus</i>	gamma rays	seedless fruit	Shoot	[111,112]
<i>Psidium guajava</i>	gamma rays	disease resistance, Fruit characteristics	shoot tips	[85,113,114]
<i>Ziziphus jujuba</i>	gamma ray		<i>in vitro</i> shoot	[115] [95]
<i>Musa spp.</i>	EMS	disease-resistant,	shoot tips, axillary	[94,102,116]
	gamma rays	reduced height	buds, microsections	
<i>Beta vulgaris</i>	gamma ray	drought-tolerant	<i>in vitro</i> shoot tips	[117]
<i>strawberry</i>	gamma rays	morphological characters	<i>in vitro</i> buds	[118]
<i>Solanum lycopersicum</i>	EMS	various traits	<i>in vitro</i> cuttings	[119]

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357
358

5.2 Mutation induction on *in vivo* plant materials

Mutagenesis can be carried out on seeds and vegetative materials. Due to the chimeric nature of mutation on multi-cellular tissues, the phenotypic changes in the treated generation (M1) are potentially not heritable as only a subset of cells are involved in gametogenesis [120]. This issue can be overcome by performing at least one sexual cross prior to analysis. Mutagenesis on seeds is therefore relatively straightforward for seed-propagated plants that are capable of self-fertilization, as any chimerism is eliminated through sexual reproduction [92]. In this manner, seeds treated with different dose of EMS are planted (M1 generation), and a total of ~ 2000 M1 plants are self-fertilized to grow M2 generation. Most mutant alleles are recessive, so successful mutants would not be seen until the M2 generation [121], however, it might take a long time to grow out M1 generation. Lethal mutation and infertility will often significantly affect the population. Using gamma ray irradiation on seeds, Badigannavar and Suvendu (2009) identified dwarf mutants of groundnut (*Arachis hypogaea* L.) where the plant height was reduced by 24.5% to 41.0%. Progenies from a selected dwarf clone were consistently segregated into dwarf, extreme dwarf and parental types [122].

While seed mutagenesis can be applied to some fertile diploid breadfruit cultivars, the long growth cycle can make the process slow and costly. The out-crossing nature of breadfruit can also compound the difficulty of mutant identification, and the self-fertilizations necessary to identify mutants in the population can result in reduced plant vigour as a result of the genetic background and not necessarily the mutations.

Mutagenesis directly performed on shoot cuttings applies to both seed and vegetatively propagated crops. Irradiation is more widely used as a mutagen [83], after irradiation treatment, plant materials may be sub-cultured by cutting or used as scions and immediately grafted on other mature rootstocks [85]. This method was used for improving citrus [111,112] and guava [114]. Similar experiments have also been carried out on litchi, carambola, cherimoya, pitanga and jaboticaba [85].

5.3 Mutant detection

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396

397 **5.3.1 Phenotyping**

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399 The identification of desired mutants should be performed on nonchimaeric plants. An
400 affordable, high throughput phenotypic screening method for the traits of interest must be in
401 place to process the large number of individual lines. These include a fast analysis of plant
402 height, internode length and number, shoot and lateral shoot length and diameter for
403 detection of dwarf phenotypes in a population of several thousands or tens of thousands of
404 mutant plants [110].

405

406 Owing to lack of meiosis to assort mutation during vegetative propagation, induced
407 mutations are heterozygous, meaning only dominant or hemizygous alleles will likely yield
408 phenotypes of interest [103]. This is particularly challenging if the mutated cultivars are
409 polypoids. In these species, most genes are represented by multiple homoeologous copies,
410 and phenotypic effects in a single mutant may be masked by wild-type homoeologues
411 present in another genome. This not only raises doubts as to the effectiveness of the facile
412 screen of plant height for the outcome of mutagenesis, but also implies that in some cases,
413 combined mutations by developing double or triple mutants through genetic crosses is
414 required [123].

415

416 **5.3.2 High throughput genotyping**

417

418 Technologies allowing rapid and automated identification of mutations in targeted sequences
419 are emerging. One of these techniques is TILLING (targeting induced local lesions in
420 genomes) [124,125]. In this method, genomic DNA samples from a population are pooled,
421 arrayed on microtiter plates and subjected to gene-specific PCR with fluorescent-labelled
422 primers. The amplification products are incubated with an endonuclease that recognises
423 and cut mismatches in heteroduplexes. The cleavage products are electrophoresed and
424 visualized by fluorescence detection. The migration of cleaved products indicates the
425 approximate location of nucleotide polymorphisms. Upon detection of a mutation in a pool,
426 the individual DNA samples are similarly screened to identify the plant carrying the mutation
427 [124,126]. TILLING technology has been developed for efficient identification of induced
428 point mutations that are generated by chemical mutagens, such as EMS in a large
429 population, and has also shown great potential for screening population treated with gamma
430 ray and X-ray [108,127]. A variation of the TILLING method, known as EcoTILLING, has

also been developed for detection of natural (allelic) variation in the germplasm [126]. Techniques that do not rely on mismatch cleavage by endonucleases have also been developed. This alternative method employs the high-resolution melt analysis (HRM) to identify single-base mismatches in the mutant pools by analysing a shift in the melting temperature of the heteroduplex in the PCR products [128]. Recently, TILLING by sequencing, the application of next-generation sequencing (NGS) in TILLING, has provided a platform to screen mutant populations or germplasm collections in a high-throughput fashion. Rigola et al [129] first reported the KeyPoint technology which employed amplicon sequencing with multidimensional sample pooling and barcoding, and identified two mutants in a 287 bp targeted region from 15,000 plants representing 3008 families of M2 tomato plants [129]. Obviously, the success of the technology in identifying dwarf mutants will depend on the right selection of target genes. Numerous studies on plant dwarfism have led to the discovery of a few dwarfing candidate genes for crop improvement. These include orthologs of the wheat with reduced height-1 *rht* gene and the rice semi-dwarf *sd1* gene, both encoding DELLA proteins, an important components of GA signalling, and those of *sd1* encoding key enzymes in the GA biosynthesis pathway [24,28]. Using this high throughput platform, Zhu et al (2012) recently performed screen at these dwarfing candidate genes to identify semi-dwarf mutants in Tef (*Eragrostis tef*), an under-studied species [123]. Following sequenced with Roche 454 sequencing technology, they identified several mutant lines with mutation in the target regions from an EMS-mutagenized population consisting of 21,210 individuals [123].

The dwarf traits of the breadfruit species and its genetic basis have not been characterized. As there is limited genome sequence for the species, sequences of all homoeologues related to dwarfing candidate genes have to be obtained and annotated before the TILLING screen can begin. Strategies used to obtain these target sequences include screening and sequencing cDNA or genomic library, and gene cloning by degenerate PCR. Once sequences information and genomic structures of the dwarfing candidate genes are available, efficient gene specific primers can be designed for TILLING screening of indels or inter-homoeologue SNPs for target loci [130].

6. DWARFING BY TRANSFORMATION

Plant transformation is a technique by which a segment of DNA is introduced and stably expressed in a plant [131]. It usually involves the integration of DNA sequences into the genome of cells capable of giving rise to a whole plant [131,132]. Owing to the fact that

467 natural selection will act to remove dominant alleles that result in dwarf stature in the face of
468 competition for light, healthy dwarf genotypes are expected to be rare, and difficult to obtain
469 through traditional tree breeding [19]. Therefore, insertion of dominant transgenes may be
470 an alternative method for obtaining dwarf phenotypes in many species [58]. Increasing or
471 decreasing GA levels has been explored in several species, by over-expressing or silencing
472 genes involved in GA biosynthesis pathway [58]. Over-expression of GA2ox, a GA
473 catabolism enzyme caused dwarfism in tobacco and poplar [133,134]. In poplar, over-
474 expressing a gene encoding DELLA protein, a repressor of GA signalling caused severe
475 dwarfism and increased root proliferation [134,135]. Transgenic kiwifruit plants over-
476 expressing a isopentenyl transferase gene to control endogenous cytokinin levels were also
477 used as a rootstock directly grafted onto wild-type seedlings to produce dwarf phenotypes in
478 the grafted plants [136].

479

480 There are various methods available for gene transferring, such as *Agrobacterium*-mediated
481 transformation, particle bombardment, electroporation and viral transformation [131]. In all
482 case, single cells are transformed and regenerated into whole plants by *in vitro* culture
483 procedures [131]. The first requirement for transformation is an efficient regeneration
484 protocol. As previously discussed organogenesis has been developed for many breadfruit
485 cultivars providing efficient regeneration from auxiliary shoot tips and small buds [88]. There
486 is currently no report on somatic embryogenesis of breadfruit. Somatic embryogenesis is
487 usually preferred for transformation owing to its possible single cell origin, and less chance
488 of getting chimeric plants [131]. However, multiple rounds of shoot regenerations as
489 demonstrated by previous experiments [131,137] is a feasible strategy to achieve a
490 significant reduction of chimeras and purify isogenic transgenic lines.

491

492 While the technique for *in vitro* culture of breadfruit is available, an efficient gene transfer
493 protocol through *Agrobacterium*-mediated transformation or particle bombardment needs to
494 be established. This process can create a substantial barrier to the application of
495 transformation in some crops [132]. Compared to annual crops, woody trees such as fruit
496 trees were considered recalcitrant to genetic transformation and regeneration for a long
497 time, however through persistent efforts, they now begin to show enormous progress in
498 many species including apple, oranges and grapefruit [138,139].

499

500 Recently, “intragenic or cisgenic plant” represents a concept, wherein the gene, promoter
501 and terminator sequences derives from the same crop or from sexually compatible species
502 to generate designer crops with more public acceptability [140]. For this to happen, the

indigenous genes of the target species and their regulatory sequences need to be characterized [141]. There is hope that the expanding of genome sequencing and proper annotation to many non-model plant species would help in gene isolation and utilization for the development of these new generation of transgenic crops.

7. CONCLUSION AND FUTURE PROSPECTS

Agricultural solutions are required to reduce breadfruit tree stature in order to withstand cyclones/hurricanes and improve harvesting efficiency. The wealth of genetic diversity among breadfruit cultivars provides an important reservoir of genetic variation for developing dwarfing rootstocks. Phenotypic studies facilitated by Next Generation Sequencing (NGS) are generating a large volume of genomic and transcriptomic information for use as functional markers or breeding targets in many species with limited genome resources [142]. Morphological diversity in tree height and canopy shape exists in breadfruit cultivars throughout Oceania, but quantitative analysis is required with a focus on the tree architecture, yield and the resistance to wind damage. Genome-wide surveys are needed to improve our understanding of the genetic capacity of the diversity that exists, and to develop sequence-based genetic markers to assist in breeding programmes and early detection of dwarf traits [143].

When suitable dwarf lines are not available, chemical treatment is commonly used as an alternative to the high labor cost of mechanical pruning. Chemical Pro-Ca has shown considerable promise for use on some fruit trees due to its low persistence in plant and soil. Its responses and sensitivity however have not been tested on breadfruit cultivars. While it may have potential for reduction in tree height in the short term, application of such chemicals to vigorous fruit trees every growth season can be costly, and may not be sustainable in the long term. The development of operations that incorporate the relatively intensive use of chemicals would risk reducing opportunities for Pacific farmers to capture niche markets based on chemical-free/organic production.

In vitro mutagenesis provides an attractive solution for the development of dwarf varieties. The method would preferably be applied in diploid cultivars since loss-of-function mutations in triploids are less likely to produce a phenotype due to gene redundancy and lack of meiosis in successive rounds of tissue culture. Mutation in dwarfing candidate genes can be screened by PCR markers for fast detection. The application of NGS and TILLING has

538 provided high-throughput screening tools for mutation discovery without the need for an
539 expressed phenotype.

540

541 Plant transformation has shown potential of introducing dominant dwarf traits into plant
542 species through manipulating GA-related genes. However, even though the transformation
543 techniques would work on breadfruit species, the method has a long way to go before
544 gaining acceptance from both local consumers and the export market.

545

546 There is lack of information about the breadfruit genome. The species has a relative small
547 genome estimated as ~ 880 Mbp/C for diploid cultivars, Puou and Maafala [87]. It is an
548 attractive candidate for genome analysis initiatives and development of genomic tools for
549 breeding programmes. With large number of genomes being sequenced or resequenced in
550 the last several years [144], there is hope that as new technologies are developed, assembly
551 of *de novo* genome sequences in many less-characterized species like breadfruit will
552 become possible. The availability of the complete genome sequence will rapidly increase
553 molecular marker resources and their application to breeding and selection for important
554 traits, such as dwarf characters, stress and pathogen resistance in breadfruit cultivars.

555

556 The recent IPCC report (2013) confirmed that climate change will have a negative impact on
557 global food staples [145]. For those countries in the Caribbean and Pacific regions which are
558 largely dependent on imported foods for their food security – these projections are delivering
559 a warning as to the likelihood of future increased costs and instability in supply. The time is
560 right therefore to invest in staple food crops such as breadfruit to substitute for imported
561 staples. Strengthening breadfruit production so that continuity of supply is linked to
562 processing developments is very important for the future food security of these island
563 regions. Export opportunities also exist, reinforcing the need for increased investment and
564 research into areas like dwarfism that would facilitate development of commercial systems.

565 This review has described several approaches that could be used to generate dwarf
566 breadfruit trees. Understanding and analysing the wealth of diversity that exists in the Pacific
567 is clearly the first step in the process – farmers, extensionist and scientists all have a role to
568 play in this process. However, successfully developing an efficient and effective tool for
569 breadfruit dwarfing can only be achieved through partnerships with institutes capable of
570 delivering that technology.

571

572

573

574 **COMPETING INTERESTS**

575

576 Authors have declared that no competing interests exist.

577

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