INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 19, No. 2, p. 51-66, 2021
http://www.innspub.net

RESEARCH PAPER

OPEN ACCESS

Prioritizing analysis of forest genetic materials of seedlings of *Falcataria moluccana* (Miq.) Barneby and J. W. Grimes families in the secondary diffusion pathways

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Article published on August 26, 2021

Key words: Genetic analysis, Seedling growth parameters, Soil pH, Tree families, Water regime

Abstract

Tree breeding program requires expediting laboratory analysis of genetic materials of *Falcataria moluccana*. This study was conducted to prioritize genetic materials for analysis by identifying the differences of families'/provenances' seedling germination, diameter, root-shoot ratio, and biomass as influenced by growth stressors. Ten families from secondary diffusion pathways consisting of five provenances were used in the study. A nursery experiment laid out in completely randomized design was set up with four treatments: Treatment 1(acidic dry soil); Treatment 2(acidic wet soil); Treatment 3(alkaline dry soil); Treatment 4(alkaline wet soil). Ten seedlings in five replications were used for each treatment. Standardized protocol used pH of 4.2 acidic and 7.2 alkaline; every other day watering of 200ml/seedling for dry, and everyday watering of 200ml for wet. Results showed that soil pH had no significant effect to germination. The acidic wet soil treatment significantly resulted in the highest seedling height, diameter, and biomass. Root-shoot ratio was not significantly affected by the treatments. Significant variation among families was evident in seedling height and diameter, but not in root-shoot ratio and biomass. Difference among provenances was not significant indicating that families are possibly closely related with each other due to proximity of their locations. The genetic materials of Fm071, Fm078, Fm059, Fm085, and Davao Oriental and Compostela Valley provenances are priority for laboratory analyses. The protocol of the study is highly relevant to fast track and optimizes efficiency in genetic analysis.

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Introduction

Falcataria moluccana, a fast growing tree species which has been widely domesticated by local people in the Southern Philippines, is a major source of raw materials for the wood industry and has become a thriving business (Carandang *et al*., 2015). It is one of the tree species preferred for industrial forest plantations owing to its acceptable quality of wood for the panel and plywood industries (Krisnawati *et al*., 2011).The original population of the species came from Papua New Guinea and Solomon Island and diffused primarily to the provinces of Bukidnon and Surigao del Sur Philippines, respectively (Siladan, 2010). However, due to its limited adaptability to the island of Mindanao, the species merely diffused mainly to the other Mindanao provinces and some parts of the country. These provinces became the secondary diffusion pathways of *F.moluccana.*

The degree of genetic change and diversity of these populations is by and large unknown. Decades of regeneration could have contributed to these changes but likewise uncertain.

Understanding genetic diversity, provenance variations and structure of the tree species, particularly at juvenile age, serves as an important reference for tree breeding programs and conservation strategy (Wee *et al*., 2011). Several studies documented the genetic and provenance variations of *F.moluccana*; however, most of them used stand of existing and field trial plantations and none at the juvenile stage.

For instance, Kurinobu *et al.* (2013) explored provenances of tree plantation of *F.moluccana* and found variations in height growth and response to different spacing. Yuskianti and Shiraishi (2017) identified genetic diversity between populations of *F.moluccana* from two provenances in Indonesia. Meanwhile, genetic diversity study on gull rust diseases resistance of *F.moluccana* plantation using growth traits revealed that index of incidence of the diseases and severity varied significantly among families and provenance (Baskorowati *et al*., 2012). Still about the disease, a study was conducted exploring the molecular mechanisms of the *F.moluccana* against gall-rust disease through transcriptome assembly producing dataset from disease infected and non-infected trees (Shabrina *et al*., 2019).

Provenance and genetic variations in trees could be detected as early as seed germination and seedling growth and development. Azad *et al.* (2014) stressed that seedling growth is a precondition for conservation of genetic resources which depends upon understanding of breeding system, genetic traits, and evolutionary forces in forest tree improvement. Ivetic *et al.* (2016) argued that while genetic diversity in tree breeding is an important consideration to shorten rotation period and enhanced wood quality and quantity, tree geneticists emphasized that every step in production of forest genetic resources materials from collection to nursery, juvenile materials production has an effect on genetic diversity. A growing number of empirical investigations documented genetic variations and diversity among families and provenance of various tree species at juvenile stage.

For instance, Navegantes *et al.* (2018) reported a significant genetic variance for growth traits of tree progenies of *Euterpe oleracea* (Arecaceae) at the juvenile stage. The study of Azad *et al.* (2014) found out significant differences in seasonal variation in seedling growth among species population of *Tamarindus indica* (Fabaceae). Sudrajat (2015) observed significant genetic variations among 11 populations of *Anthocephalus cadamba* in fruits, seeds, and seedling traits. Watanabe *et al.* (2017) reported a significant effect of habitat degradation to genetic structure in juveniles of *Machilus thunbergii* (Lauraceae). Wee *et al.* (2011) documented the populations of *Gmelina arborea* (Lamiaceae) from four countries with a good level of genetic diversity, and molecular variance of the species among population. Carles *et al.* (2009) discovered a statistically significant variation among families of white spruce in terms of seed characteristics and germination variables. Meanwhile, growth and development of tree species are also affected by various forms of growth stressors.

Trees have to adapt in their environment with a multitude of biotic and abiotic stresses. Resilience and resistance mechanisms to these stresses are of special importance for long-lived tree species (Polle and Rennenberg, 2019). Tree species tolerance to these stresses, however, is dependent on their genetics, environmental situation and the combination of these two factors (Pessarakli *et al.*, 2015).

Examples of these abiotic growth stressors are soil nutrient and water stresses. There is evidence that major genera of the Fabaceae, where *F.moluccana* belongs, are already negatively affected by water stress such as drought and associated biotic and abiotic stressors (Muller and Gailing, 2015). Other common abiotic growth stressor is soil nutrient elements depression (Pessarakli *et al.*, 2015).

Evidently, tree species behave differently in ecological responses to growth stressors and exhibit a degree of adaptation when exposed to these stressors. For example, potential adaptation to drought stress was evident in *Pinus massoniana* (Pinaceae) by way of naturally removing carbon storage by the plant itself (Lin *et al.*, 2018). Response of *Pinus ponderosa* (Pinaceae) and *Populus tremuloides* (Salicaceae) to water stress revealed that ponderosa pine showed little elevational variation in drought related traits but avoided drought stress at low elevations by limiting transpiration through stomatal closure (Anderegg and HilleRisLambers, 2016). A study on simulated heat as growth stressor on the response and phenotypic plasticity of juvenile progeny of seven forest tree species revealed a significant population effect (Alfas *et al.*, 2018). Warwell and Shaw (2019) provided an insights on how tree populations of ponderosa pine may evolve in response to drought, and where maternal families of the species were differentially adapted to drought patterns associated with the climate of their origin. Kupers *et al.* (2019) documented that soil water potential (SWP) indirectly shape local species distributions of tropical forest seedlings. Fotelli *et al.* (2019) illustrated how Aleppo species had been negatively affected during summer drought by increasing potential evapotranspiration and rate of vapor pressure deficit. Meanwhile, soil nutrients growth stressors are often expressed in terms of soil pH (acidic and alkaline). Soil pH is considered as the “master soil variable” that influences myriads of soil biological, chemical, and physical properties and processes that affect plant growth and biomass yield; and can be applied in nutrient recycling, plant nutrition and soil remediation (Neina, 2019). As observed by Pessarikil *et al.* (2015), plant absorption ability is influenced by soil physical and chemical characteristics such as structure, texture, pH, fertility level and nutrient content.

Ngulube (1989) opined that while genetic variations can be traced at juvenile stage, a nursery evaluation phase in provenance elimination trials of tree species is imperative. A research and development (R&D) on tree breeding was launched by the Philippine government in 2015 to increase the production and improve the quality of *F.moluccana* wood. More than two hundred genetic materials of the species had been collected for genetic characterization and genetic diversity analysis of the tree breeding program, which need prioritization to expedite the genetic study.

Subjecting all these genetic materials to genetic laboratory analysis is not only expensive but also time consuming and to some extent redundant. Meanwhile, the previous related studies on *F.moluccana* cited above used stand of existing field trial plantations and none on the juvenile stage at nursery setting. Moreover, previous juvenile stage studies to understand genetic variation of tree species as exposed to growth stressors were explored for tree species other than *F.mollucana*. Nonetheless, those above cited empirical studies provide us a scientific bases to explore the varying effects of soil and water growth stressors to the forest genetic resources (FGR) among families of *F.moluccana* species at juvenile stage. Hence, this nursery experiment was conducted to fast-track genetic characterization of the provenance and families of forest genetic materials of *F.moluccana* in the secondary diffusion pathways. Specifically, the study aimed to identify variations, if any, among provenance and families of the selected FGR materials of *F.moluccana* at seedling stage as influenced by growth stressors (i.e. soil pH and water regimes) and prioritize materials that will be genetically characterized and analyzed in the laboratory.

Materials and methods

Study site, methods and protocols

The study site, methods and protocols employed were the same as with the experiment study of the *F.moluccana* in the primary diffusion pathway (Phase 1 of the study) (Casas *et al.*, 2020). The study was conducted in February 2016 to January 2018. The nursery site was established in the Central Mindanao University (CMU), Maramag, Bukidnon, Southern Philippines (7.8649°N, 125.0509°E). The nursery was laid-out towards north-south direction so as to control the effect of sunlight to the experimental plots. It had a dimension of 9m x 22m consisting of 20 beds. The dimension of the beds was about 1.3m x 5m. The height of the roof from the bed's flooring was 1.3m. In this study, five provenances were used from the secondary diffusion pathways (Fig. 1) as the species had been widely spread all over the country after it was first lodged in Bukidnon and Surigao del Sur from Papua New Guinea and Solomon Island, respectively (Siladan, 2010).These families were randomly chosen from the lists of the families from the five provenances with two families purposively sampled from each provenance.

Standardization of the treatments

The soil pH and water regime treatments were first standardized. The soil pH was first determined to compute the lime requirement. The soil had a pH of 4.2 which was considered as the acidic soil treatment. The lime had a 96% relative neutralizing power (RNP). Based on the computed lime requirement of the soil, 1kg of soil was mixed with 4.82g of lime to produce a pH of 7.2 to 7.5 for the alkaline soil treatment. The soil medium was then sterilized. The potted soil was subjected to regular watering to determine the day when the basic pH of 7.0 to 7.5 was fully achieved. Soil samples were taken and analyzed every week until the pH of 7.2 was achieved which took four weeks. This was the time when seed sowing commenced with acidic and basic soil treatments. In establishing a water regime protocol, a total of 25 three-month old seedlings of *F.moluccana* in the existing nursery of the university were subjected to different water regimes for one month using 200ml water in a bed with plastic roofing. About five seedlings were subjected for each of the following water treatments: daily; every other day; every two days; every three days; and every four days. Results showed that seedlings exhibited wilting in the second treatment and seedlings died in the third, fourth and fifth water treatments. Based on these results, the experiment used every other day watering of 200ml for each seedling for dry condition while every day watering of the 200ml for wet condition. The pre-germination treatment was conducted in which seeds were placed inside sock/cloth. The water was boiled and once boiling, seeds were dipped for 3 to 5 seconds. The seeds were transferred into a container filled with tap water and were soaked for 24 hours. After which, seeds were spread in wet cotton cloth and were covered with another cotton cloth. Seeds were sown as soon as radicles emerged.

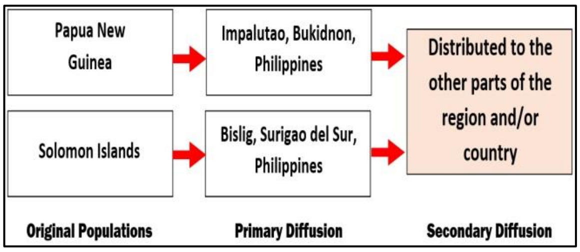

Fig. 1. Diffusion pathway of *Falcataria moluccana* from Papua New Guinea and Solomon Islands to the Philippines (Adapted from Siladan, 2010)

Field lay-out and experiment

The experiment was laid out following a Completely Randomized Design (CRD) in the nursery. Two soil treatments (acidic and alkaline) and two water regimes (dry and wet) were simulated in the nursery with the FGR materials of the 10 families. Ten seedlings in five replications were used for each of the four treatment combinations for a total of 2,000 seedlings. The treatments were as follows: Treatment 1-acidic and dry soil; Treatment 2-acidic and wet soil;

Treatment 3-alkaline and dry soil; and Treatment 4-alkaline and wet soil. The soil was first sterilized before it was mixed with sterilized fine sand with a ratio of 1:4 (sand-soil). A 1kg of soil was mixed with 4.82g of lime to produce the soil pH of 7.2. During the whole observation period, sample of the soil medium was analyzed every month to determine if the pH had not been affected with the water application and through time. A 5" x 8" polyethylene bag was used for potting. The polyethylene bags were spaced 15cm apart. Seed sowing was done after the 4th week from the time the potted soils had been placed in the experimental field area. This was the time when the effect of lime in the soil, achieving the pH of 7.2 had been achieved. Sowing was completely done in one day so as to minimize error/bias. A roof net was installed to prevent the seedlings from wilting. When the seedlings were ready for water treatment (dry and wet), a plastic roofing was installed and the net was placed over the plastic to prevent it from tearing off. Originally as proposed, after three months old, the seedlings would have been exposed to water regime treatment. However, after three months of growing in the nursery, the average height of the seedlings was only 4.16cm. This was way below the projected target average height of the seedlings of around 10cm. The stunted growth was attributed to poor aeration and drainage of the potting medium and possibly due to the strongly acidic and basic media used in this experiment. Consequently, water regime treatment was moved to another five months or at eight month-old seedlings with an average height of more than 10cm.

Measurement and analysis

The following parameters were measured: germination (%), seedling height (cm), root collar diameter (mm), biomass (g), and root shoot ratio. Percent germination was determined at 10 days after sowing, and height was measured for 245 days old seedlings or day before water regime was employed to determine the main effect of soil pH to seedling growth. At 370 days old seedlings (about 125 days after water treatments), another height measurement was done; and the root collar diameter, root-shoot ratio, and biomass were determined. Biomass measurement was done through destructive method of the 50% of the total seedlings sampled randomly. The samples were oven-dried at 65°C for three consecutive days. After oven drying, they were then placed in the styrobox to prevent them from absorbing moisture. This was followed by weighing the samples to determine the oven-dry weight. Statistical analysis using analysis of variance and pairwise mean comparison of the growth parameters was employed to determine the differential responses of the families FGRs to the soil pH and water regimes. The data were analyzed using Statistical Tool for Agricultural Research (STAR) software.

Results and discussion

Salient features of F.moluccana families

The sampled 10 *F.moluccana* families came from five provenances, namely: Agusan del Sur, Compostela Valley, Davao Oriental, Zamboanga del Norte, and Misamis Oriental, Philippines (Table 1).

Table 1. Provenance, diameter at breast height (DBH), total height (TH), elevation (Elev.), and terrain of the tree families.

Family Tree Code	Provenance	DBH (cm)	TH (m)	Elev. (masl)	Terrain
Fm047	Sn Francisco, Agusan del Sur	37	21.6	45	hilly
Fm059	Prosperidad, Agusan del Sur	54.9	29.1	50	hilly
Fm071	Mawab, Compostela Valley	24	18.5	91	hilly
Fm070	Mawab, Compostela Valley	36.5	21.1	92	hilly
Fm078	San Isidro Davao Oriental	37	20.6	483	hilly
Fm085	San Isidro Davao Oriental	48.6	26.3	489	hilly
Fm086	Mutia, Zamboanga del Norte	47.3	33.9	363	hilly
Fm099	Mutia, Zamboanga del Norte	82	37	157	hilly
Fm025	Gingoog, Misamis Oriental	25.5	17.9	79	hilly
Fm038	Gingoog, Misamis Oriental	37	19.6	69	hilly
	Average	42.98	24.56	191.8	

The family tree codes were consistent with the codes used in the field trials of the existing R&D tree breeding program. The average diameter at breast height (DBH) of the mother trees of the families was 42.98cm with the largest and smallest DBH of 54.9cm and 24cm, respectively.

The height ranged from 17.9m to 33.9m; and the average was 24.56m. Except for three families (Fm085, Fm086 and Fm078) where the elevation was more than 400 meters above sea level (masl), the rest of the families were located in an elevation of less than 400 masl which is most favorable to growth of *F.moluccana* (Lacandula *et al*., 2017).

Germination and seedling height at pre-water treatment

Families responded almost similarly to the two soil pH treatments in terms of germination except for Fm071 where percent seed germination was relatively lower than the rest of the families (Fig. 2a). This lower germination percentage of Fm071 could be attributed to the timing of seed collection since the seeds were collected at the latter part of the ideal collection season with the pods exhibiting possible over-maturity and insect damaged. Apparently, the two soil pH treatments had similar effect to percent seed germination of the families (Fig. 2b). Percent germination for both soil pH treatments was generally high (more than 92%). This finding is consistent with the results reported by Gentili *et al.* (2018) on germination rate of *Ambrosia artemisiifolia* which was higher at pH 5 to pH 7. Similarly, Sang *et al.* (2011) who studied the same species observed an optimum germination at soil pH between pH 5.0 and pH 8.0.

It shall be recalled that this study used approximately the same soil pH 4.2 for acidic and pH 7.2 for alkaline.Variations of seedling height among families were clearly shown in Fig. 3a. The seedlings of all families ostensibly grew favorably in acid soil, which difference of soil pH effect was illustrated in Fig. 3b. Seedling height growth of Fm078 was the tallest (18.95cm) while Fm047 the shortest (11.34cm) in each of the soil treatment.

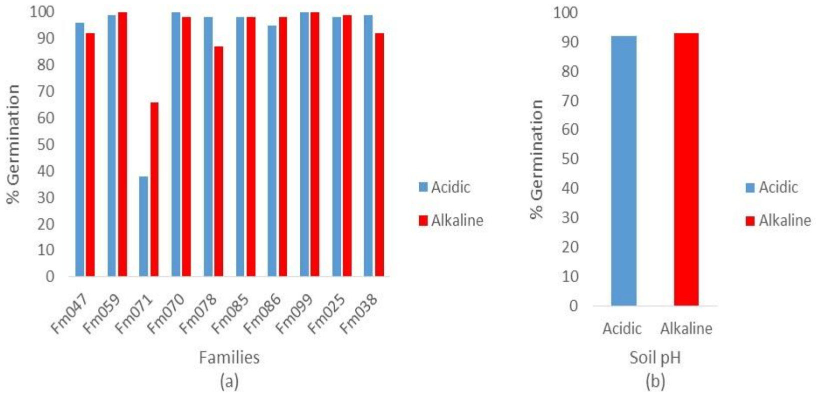

Fig. 2. Average percent germination of 10 days old *F.moluccana* seedlings by families (a) and by soil pH (b).

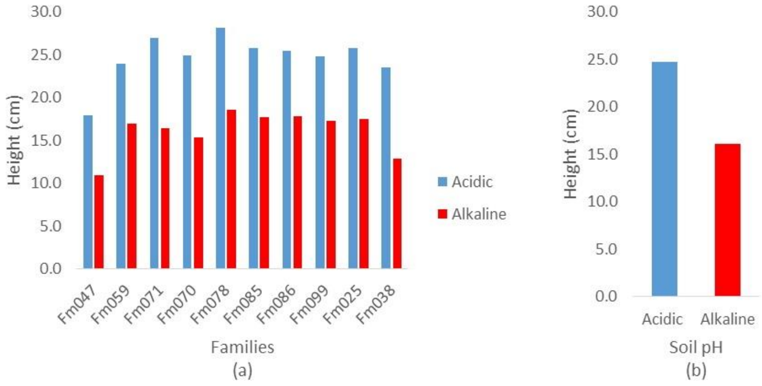

Fig. 3. Average height of 245 days old *F.moluccana* seedlings by families (a) and by soil pH (b) at pre-water treatments.

The difference on the effect between soil pH treatments to percent seed germination of the families was not significant (Table 2), implying that seed germination of the families has a wide soil pH amplitude. i.e. from acidic to alkaline.

This somewhat disagrees with the finding of Ghaderi-Far *et al.* (2010) where *Melilotus officinalis* species favors germination at pH 5-6 and decrease at acidic medium of pH 4 and alkaline pH 9. Similarly, the finding contradicts to the earlier study of Turner *et al.* (1988) on the germination of *Paulownia tomentosa* which showed that at soil pH 4.0 no seed germination occurred while seedling emergence was generally reduced at 4.5 pH. Meanwhile, significant differences on the effect of soil pH treaments to seedling height was observed, favoring acidic soil. Seedlings (at 245 days old) in acidic soils had average heights of 18.48cm which is significantly higher than the average height of 13.88cm in alkaline soils. Gentili *et al.* (2018) observed the same finding for *A. artemisiifolia* seedlings which performed better in acidic than alkaline/basic soil.

Soil pH favorable to tree seedling growth ranges from 5.5 to 6.5 (Turner *et al.*, 1988; Pierce *et al.,* 1999). Pessarikil *et al.* (2015) reported that in alkaline soils, phosphorous, iron and molybdenum deficiency are usually observed as plants also suffer from P deficiency, although a toxic amount of aluminum is found under this condition. Nonetheless, the authors observed that nutrient elements are naturally absorbed by the seedlings in optimum soil pH of 6-7.5, but under suboptimal conditions, higher or lower than this optimum pH, can affect nutrient availability status. Moreover, since *F.moluccana* is a nitrogen-fixing species, this enabled the species to fix atmospheric nitrogen and made available despite.

The acidic conditions which could contribute to better height growth. Calvo-Albarado *et al.* (2007) reported a higher yield and dominant stand height of the exotic tree species in acidic soil with well-defined dry season than the selected native tree species.

Table 2. Pair-wise comparison tests on the average germination (%) 10 days after sowing and height (cm) at 245 days *F.moluccana* seedlings with soil pH before water regime treatment.

Soil pH	Germination (%)	Height (cm)
Acidic	92.10^a	18.48^a
Alkaline	93.00^a	13.88^b

Means of the same letter are not signficantly different at $P < 0.05$.

Analysis of variance showed that soil pH treatments have no significant effects to seed germination but its effect to seedling height was highly significant (Table 3). These findings validate the above mentioned effects of the two soil pH treatments which had no significant effects to germination but differed significantly in seedling height.

Meanwhile the variation effect of family was highly significant to both seed germination and height growth. It was also observed that combination of soil pH and family had highly significant effect to seed germination, but none to seedling height.

The significant variation effect of family was clearly exemplified in the pairwise mean comparison of germination and height which differed among families. It is nonetheless observed that only one family (Fm071) had significantly lower seed germination compared to the nine families whose differences were not statistically significant and with very high seed germination, ranging from 92% to 100%. Soil pH as growth stressor had been clearly demonstrated in the seedling heights of the families.

Exhibiting the tallest height was family Fm078 (18.95cm) which difference was statistically significant with the shortest Fm047 (11.34cm) and Fm038 (14.20cm). Seedling height difference of the eight families was not significant; the seedling height of the shortest family was significantly different with those eight families. Variation of height growth response to this growth stressor among families may be due to the genetic traits of the families (Pessarakli *et al.*, 2015).

Table 3. Analysis of variance and pairwise mean comparison on seed germination (%) 10 days after sowing and height (cm) of families of 245 days old *F.moluccana* seedlings with soil pH at pre-water treatment.

Family Tree Code	Provenance	Gemination (%)	Height (cm)
Fm047	Sn Francisco, Agusan del Sur	94.00^{a}	11.34^{c}
Fm059	Prosperidad, Agusan del Sur	99.50^{a}	16.64^{ab}
Fm071	Mawab, Compostela Valley	52.00^{b}	16.66^{ab}
Fm070	Mawab, Compostela Valley	99.00^{a}	15.49^{ab}
Fm078	San Isidro, Davao Oriental	92.50^{a}	18.95^{a}
Fm085	San Isidro, Davao Oriental	98.00^{a}	17.47^{ab}
Fm086	Mutia, Zamboanga del Norte	96.50^{a}	16.62^{ab}
Fm099	Mutia, Zamboanga del Norte	100.00^{a}	16.87^{ab}
Fm025	Gingoog, Misamis Oriental	98.50^{a}	17.52^{ab}
Fm038	Gingoog, Misamis Oriental	95.50^{a}	14.20^{bc}
Average		92.55	16.18
CV (%)		12.80	21.31
Sources of variations	Soil pH	df =1, F=0.29^{ns}	df =1, F= 89.00**
	Family	df=9, F=29.80**	df=9, F=7.50**
	Soil pH x Family	df = 9, F = 3.87**	df = 9, F =0.50^{ns}

Different letters indicate significant differences at $P < 0.05$

ns = not significant ** =highly significant at $P<0.01$ * =significant at $P<0.05$

Seedlings height, root collar diameter, root shot ratio, and biomass of the families of F.moluccana as influenced by soil pH and water treatments

The families performed well in acidic wet soil except for the root-shoot ratio (Fig. 4). This was followed by acidic dry, alkaline wet, and alkaline dry, with the later producing poor seedling growth. Families Fm78, Fm71, and Fm086, were dominant in height growth (Fig. 4a) in all treatments compared to the rest of the families. Dominant diameter growth rates across treatments were the following families Fm025, Fm071, Fm085, and Fm078 (Fig. 4b). Producing relatively high biomass across treatments were families Fm086, Fm071 and Fm078; while families FM047 and Fm025 dominated only in acidic wet soil (Fig. 4d). The root-shoot ratio of family Fm059 (Fig.4c) was extremely high in acidic wet soil, with other families performing uniformly in this growth parameter across treatments. Noticeably, two families performed well as clearly exemplified by their seedling growth, namely, Fm078 (Davao Oriental), and Fm071 of Compostela Valley.

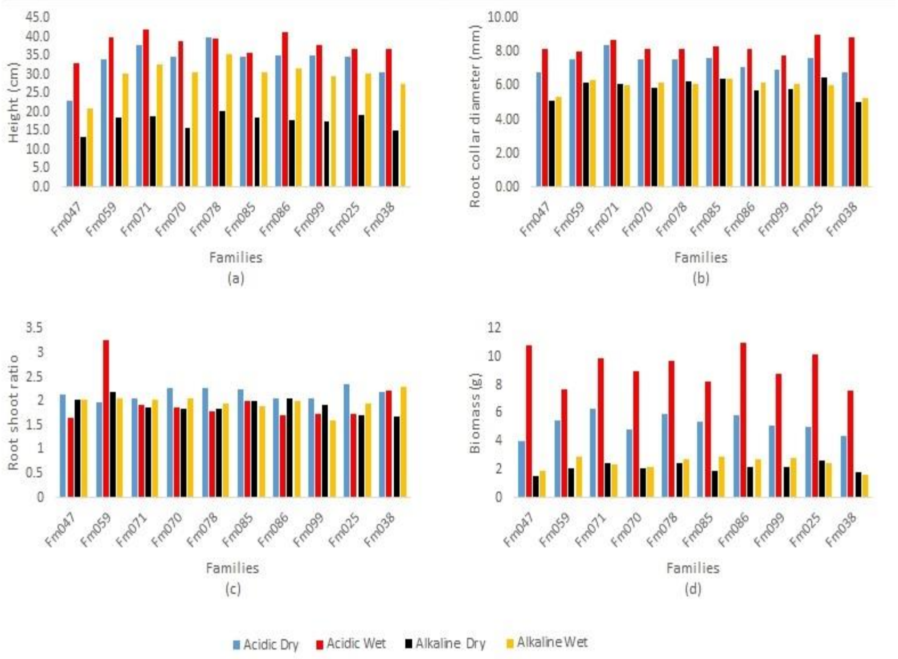

Fig. 4. Seedling height (a), root collar diameter (b), root-shoot ratio (c), and biomass (d) with soil pH and water treatments.

Analysis of variance showed that the growth stressors (soil pH and water) both had highly significant effects on seedling height, root collar diameter, and biomass; but their effect to root-shoot ratio was not significant (Table 4). Interaction between the two growth stressors yielded similar effects. Moreover, the family factor exhibited a highly significant variation effect on height and root collar diameter, but no significant effect on root-shoot ratio and biomass parameters. However, when family factor was combined with soil pH and water regime, the variation effect was not significant in any of the growth parameters. The data also indicates that none of the treatments had any significant effect on the root-shoot ratio of the seedlings.

Table 4. Analysis of variance of seedling height (cm), root collar diameter (mm), root-shoot ratio and biomass (g) of *F.moluccana* with soil pH and water regimes.

Sources		Height	Root collar diameter	Root-shoot ratio	Biomass
	df	*F-value*	*F-value*	*F-value*	*F-value*
Soil pH	1	808.54^{**}	329.9^{**}	1.98^{ns}	444.1^{**}
Water regime	1	18.43^{**}	24.62^{**}	0.35^{ns}	85.69^{**}
Family	9	7.21^{**}	3.8^{**}	1.04^{ns}	1.52^{ns}
Soil pH x Water regime	1	8.48^{**}	14.99^{**}	1.96^{ns}	61.74^{**}
Soil pH x Family	9	1.45^{ns}	1.64^{ns}	0.45^{ns}	0.81^{ns}
Water regime x Family	9	0.56^{ns}	0.77^{ns}	1.03^{ns}	0.72^{ns}
Soil pH x Water regime x Family	9	1.05^{ns}	0.76^{ns}	1.26^{ns}	1.01^{ns}
CV (%)		*16.33*	*10.85*	*31.05*	*34.82*

** =highly significant at $P<0.01$ * =significant at $P<0.05$ ns = not significant.

Pairwise comparison test of average height, root collar diameter, and biomass showed significant differences (Table 5) with higher mean values for seedlings in acidic soil in either wet or dry conditions.

The observations on height growth of the seedlings was consistent with the observation above when the seedlings were still 8 month-old and before water treatments were employed. However, this was not observed in the root-shoot ratio of the seedlings where the effect of acidic wet soil was not significantly different from alkaline soil either in dry or wet conditions. Many authors like Maskova and Herben (2018), indicated that adjustments in root-shoot ratio is one of the plant's ability to compensate for limiting resources such as soil nutrient.

In acidic soil, phosphorus is limited since it is fixed by aluminum and iron (Ch'ng *et al.*, 2014). Bhattacharya (2019) stressed that phosphorus deprivation usually leads to a higher root-shoot ratio and to changes in root architecture; and as adaptation mechanism, an important contribution to scavenging the soil for phosphorus is root extension. Moreover, Saboya and Borghetti (2012) observed that seedlings of three native tree species of Cerado increased their root length by 243% greater than the shoot length to ensure access to water sources. The findings of these earlier studies however, were not evident in this study, because while the soil was acidic and wet, the soil aeration of the soil medium used in the experiment was very poor, thus, root growth was greatly restricted.

In fact, the seedlings in general exhibited stunted growth. For instance, the height of the 8 months old seedlings was way below the 4 months old *F.moluccana* seedlings in the nearby central nursery which were not subjected to any soil pH or water stress.

The stunted growth was attributed to poor aeration and drainage of the potting medium which was 1:4 (sand-soil). The prescribed potting medium which was used in growing the seedlings in the central nursery was 7:2:1 (soil: rice hull: vermicast), which in contrast had better drainage and aeration. Santosa (2014) documented that the suitable soil medium for *F.moluccana* seedlings was that with adequate porosity for root development that enhanced soil aeration and drainage; as he found out that the best soil medium for *F.moluccana* was a mixture of 50% mud soil, 40% rice husk and 10% compost.

Table 5. Pair-wise mean comparison tests on the height (cm), diameter (mm), root-shoot ratio, and biomass (g) of *F.moluccana* seedlings for soil pH at each level of water.

Soil pH	Height		Root collar diameter		Root-shoot ratio		Biomass	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Acidic	33.52^{a}	38.00^{a}	7.41^{a}	8.34^{a}	2.16^{a}	1.99^{a}	5.26^{a}	9.29^{a}
Alkaline	17.65^{b}	18.51^{b}	5.89^{b}	6.01^{b}	1.91^{a}	1.99^{a}	2.14^{b}	2.47^{b}

Different letters indicate significant differences at $P < 0.05$

The difference in the average height, root collar diameter, and biomass between acidic wet and acidic dry soil was significant (Table 6). In terms of root-shoot ratio, no significant difference was observed among treatments. Moreover, there was no significant difference in all growth parameters between alkaline wet and alkaline dry. While seedlings grew favorably in acidic soil, it was evident that *F.moluccana* seedlings grew faster in acidic wet soil than acidic dry soil conditions. For obvious reasons, plants grow well on adequate water supply because water is a key component in all physiological processes in plants. This result was observed by Kupers *et al.* (2019) on seedling of tree species distribution with a more positive growth response and were more abundant at higher soil water potential (SWP) and grew faster on the wet end than dry distributed species**.** Azad *et al.* (2014) reported that seedling growth of *Tamarindus indica* height and root collar diameter showed significant positive correlation with mean monthly rainfall. Similarly, Wang *et al.* (2012) documented a negative morphological effect of water deficit to the seedling of *Medicago falcata* resulting in reduced biomass production. Alfas *et al.* (2018) observed the presence of oxidative stress of the progeny of seven tree species due to negative effect of drought to plant physiological processes. However, the finding of this study on the no significant effect of water to root shoot ratio of the seedlings of *P.falcataria* contradicts with the findings of some authors. For example, Silva *et al.* (2012) reported that compared to well-watered plants, an intermittent water limitations applied to *Ligustrum japonicum* during the establishment resulted in new shoot dry mass reduction, and negatively affected root-shoot ratio, as they also observed patterns of shoot and root growth that varied considerably among plants. Sun *et al.* (2014) reported that water stress had negative significant effect to a one-year-old *T. ciliata var. pubescens* seedlings in terms of root growth, root mass ratio, root tip numbers and leaf mass ratio, although it had no effect on the growth of ground diameter. Similarly, apart from height, basal diameter, and biomass production, Wu *et al.* (2008) also observed root length of *Sophora davidii* seedlings was negatively affected by drought stress. The primary reason of this contradiction was probably due to poor aeration of the soil medium used in this study.

Table 6. Pair-wise mean comparison tests on the height (cm), diameter (mm), root-shoot ratio, and biomass (g) of *F.moluccana* seedlings for water regime at each level of soil pH.

Water regime	Height		Root collar diameter		Root-shoot ratio		Biomass	
	Acidic	Alkaline	Acidic	Alkaline	Acidic	Alkaline	Acidic	Alkaline
Dry	33.52^{b}	17.65^{a}	7.41^{b}	5.89^{a}	2.16^{a}	1.91^{a}	5.26^{b}	2.14^{a}
Wet	38.00^{a}	18.51^{a}	8.34^{a}	6.01^{a}	1.99^{a}	1.99^{a}	9.29^{a}	2.47^{a}

Different letters indicate significant differences at $P < 0.05$

Difference on height, root collar diameter, root-shoot ratio, and biomass production of F.moluccana seedlings by families and provenance

Individual families responded differently to the two growth stressors (soil pH and water regime) as seen in the pairwise comparison of the mean height and diameter growth among families (Table 7). However, no significant difference was observed on root shoot ratio and biomass among families. In support to these findings, Wang *et al.* (2012) observed that seedlings among populations of *Medicago falcata* differed significantly on their physiological and morphological responses to soil water stress. Specifically, the authors observed significant difference on diameter among population of the species as exposed to water stress, but contrary to the finding of this study, the authors also found out a significant difference on the relative root length among population of the species. By growth parameter, Fm078 exhibited the tallest height (30.07cm) and the shortest was observed in Fm047 (20.46cm). Other families with seedling heights above the average of 26.92cm were: Fm071 (28.99cm), Fm059 (27.71cm), Fm099 (27.65cm), Fm085 (27.12cm), and Fm025 (27.10cm). Comparison of their means revealed that the tallest seedling height (Fm078) was significantly different with the shortest (Fm047) and the next shortest seedling height (FM038). Also, the seedlings of the Fm04 was significantly shorter than the rest of the seedling of the other families. Difference of the seedling growth among other families was not significant. Similar differences among families were observed in terms of seedling root collar diameter, where Fm025 (7.31mm) and Fm071 (7.30mm) had the largest growth, while the smallest diameter was exhibited again in Fm047 (6.36mm). Families whose seedling root collar diameter growth was above the average of 6.91mm were: Fm085 (7.19mm), Fm078 (7.04mm), and Fm070 (6.96mm). Seedlings diameter of Fm025 and Fm071were significantly larger than Fm047 and Fm038. Moreover, the smallest seedling diameter of Fm047 was only significantly different with Fm085.

The rest of the families did not differ significantly in root collar diameter. It is important to emphasize that while the values of the seedlings among families posted different root-shoot ratio and biomass production, their difference was not statistically significant, implying that on these growth parameter, the families were relatively even. The study by Navegantes *et al.* (2018) observed that genetic gains of progenies of *Euterpe oleracea* were more expressive for the traits plant height and girt circumference which is in agreement with this study.

Table 7. Pairwise mean comparison on seedling height (cm), root collar diameter (mm), root-shoot ratio, and biomass (g) of families of *F.moluccana* with soil pH and water treatments.

Family Tree Code	Provenance	Height (cm)	Diameter (mm)	Root-Shoot Ratio	Biomass (g)
Fm047	Sn Francisco, Agusan del Sur	20.46^{c}	6.36^{c}	1.97^{a}	4.58^{a}
Fm059	Prosperidad, Agusan del Sur	27.71^{ab}	7.02^{abc}	2.37^{a}	4.54^{a}
Fm071	Mawab, Compostela Valley	28.99^{ab}	7.30^{a}	1.97^{a}	5.24^{a}
Fm070	Mawab, Compostela Valley	26.62^{ab}	6.96^{abc}	2.01^{a}	4.52^{a}
Fm078	San Isidro Davao Oriental	30.07^{a}	7.04^{abc}	1.97^{a}	5.22^{a}
Fm085	San Isidro Davao Oriental	27.12^{ab}	7.19^{ab}	2.04^{a}	4.65^{a}
Fm086	Mutia, Zamboanga del Norte	28.36^{ab}	6.79^{abc}	1.96^{a}	5.44^{a}
Fm099	Mutia, Zamboanga del Norte	27.65^{ab}	6.67^{abc}	1.83^{a}	4.76^{a}
Fm025	GingoogMisamis Oriental	27.10^{ab}	7.31^{a}	1.94^{a}	5.06^{a}
Fm038	GingoogMisamis Oriental	25.12^{b}	6.50^{bc}	2.09^{a}	3.89^{a}
Average		*26.92*	*6.91*	*2.01*	*4.79*

Different letters indicate significant differences at $P < 0.05$

Based from the above findings, the top performing families which exhibited dominant growth were: Fm071of Compostela Valley, Fm078 and Fm085 of Davao Oriental, and Fm059 of Agusan del Sur. On the other hand, families which performed poorly included: Fm027 of Agusan del Sur, Fm038 of Misamis Oriental, and Fm099 of Zamboanga del Norte. Meanwhile, with the varied response of the families to growth stressors, it is evident that certain genetic traits unique to its family had influenced the growth traits of *F.moluccana* at juvenile stage. This finds support from Sudrajat (2015) where a genetic variation patterns on the fruits, seeds, and seedling morphological characteristics of the 11 natural populations of *Anthocephalus cadamba* were observed and the genotype of species explained most of the variance for these characteristics. Similarly, the findings conformed with the report of Warwell and Shaw (2019) that phenotypic selection of tree species supports the interpretation of clinal variation among populations within tree species as reflecting adaptive variation in response to past natural selection mediated by climate and water stress. By provenance (Table 8), Davao Oriental provenance exhibited the tallest height (28.59cm), and the largest root collar diameter was obtained by the Compostela Valley provenance (7.13mm). Agusan del Sur provenance yielded the highest root-shoot ratio (2.17), and Zamboanga del Norte provenance produced the highest biomass (5.10g). While obtaining the highest root-shoot ratio, Agusan del Sur posted the shortest height (24.08cm) and the smallest root collar diameter (6.69mm). The smallest root-shoot ratio was obtained by the Zamboanga del Norte provenance (1.89), while Misamis Oriental provenance posted the lowest biomass (4.48g). Based on the average growth parameters, the seedling growth of the provenances was relatively poor and stunted due to the soil media used in the experiment with problem on soil aeration. While mathematically the five provenances differed on the four growth parameters, statistical test showed that they are not significantly different from each other. There is a strong indication that the families on the five provenances are genetically related to each other. This was also observed by Yuskianti and Shiraishi (2017) in the families of *F.moluccana* from two provenances in Central and West Java and Mindanao Philippines which had a closer genetic relationship. The proximity of the locations of the provenances which are located in the Southern Philippines and island of Mindanao may largely explain that the provenances came from the same population. Ngulube (1989) reported the same finding on provenance of his study on genetic variations of *Gliricidia sepium* where none of the seedling traits assessed (height, diameter and dry-weight) was related with any of the seed-origin site characteristics.

Apparently, there is an indication of a relatively low genetic diversity of the available FGR materials of *F.moluccana* in the tree breeding program, although this needs to be validated by the appropriate genetic characterization studies using lab-based approaches. Hence, there is a need to diversify collection of FGR materials which should not focus only in Mindanao but other parts of the Philippines to better understand the genetic diversity of the species. Emphasizing the importance of species diversity, Zytynska *et al.* (2011) stressed that maintaining sufficient genetic diversity of the tree species will enhance diversity of other plants and animals.

Table 8. Pairwise mean comparison on seedling height (cm), root collar diameter (mm), root-shoot ratio, and biomass (g) by provenance of *F.moluccana* with soil pH and water treatments.

Provenance	Height (cm)	Diameter (mm)	Root-Shoot Ratio	Biomass (g)
Agusan del Sur	24.08^a	6.69^a	2.17^a	4.56^a
Compostela Valley	27.81^a	7.13^a	1.99^a	4.88^a
Davao Oriental	28.59^a	7.11^a	2.00^a	4.93^a
Zamboanga del Norte	28.01^a	6.73^a	1.89^a	5.10^a
Misamis Oriental	26.11^a	6.90^a	2.01^a	4.48^a
Average	26.92	*6.91*	*2.01*	*4.79*

Different letters indicate significant differences at $P < 0.05$

Conclusion

Understanding the genetic and provenance differences of tree species at juvenile stage makes it imperative for tree geneticist to expedite laboratory analysis and lessen the time, cost and resources in tree breeding program. This is clearly demonstrated in this study. Indeed the protocol used is highly relevant to expedite and cost effectively undertake tree genetic analysis. The variation in the seedling growth parameters shows that indeed families from the five provenances in the secondary pathways responded differently to growth stressors. This was exhibited in the differences of the germination, height and diameter of the seedlings of the families.

Although mathematically there was difference on the seedling root shoot ratio and biomass among families, statistically the study did not find significant differences in these growth parameters among families. Generally, seedling growth of the families was poor and stunted due to the very poor aeration of soil medium used in the experiment. Acidic soil medium facilitates and enhances height and diameter growth performance and biomass production of *F.moluccana* seedlings. Likewise, water is a significant factor, hence, an acidic wet soil medium favors growth of *F.moluccana* seedlings. Acidic dry soil provided the next best alternative soil medium provided that a certain level of amount of water is maintained to prevent the seedlings from wilting and desiccation. Growth of *F.moluccana* seedlings in alkaline or fertile dry or wet soil media is relatively poor as manifested by the poor growth parameters. Although requiring validation thru genetic analysis, there is a strong indication that families from the five provenances are related with each other due to their proximity, suggesting that genetic diversity of *F.moluccana* is low requiring a wider range of collection of FGR materials to include areas outside Mindanao Island. The top most performing families include: Fm071, Fm078, Fm059, and Fm085. Considering the time and budget elements in the genetic characterization and laboratory analysis, the order of priority (from highest to lowest) will be as follows: families Fm071, Fm078, Fm059, Fm085, Fm086, Fm025, Fm047, Fm038, Fm099, and Fm070. Considering phenotypic traits, the order of priority by provenance will be as follows: Davao Oriental, Compostela Valley, and Zamboanga del Norte. Agusan del Norte and Misamis Oriental provenances will be the least priority in the genetic laboratory analysis.

Acknowledgement

The study was funded by the Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development – Department of Science and Technology (PCAARRD-DOST), Los Baños, Philippines. The authors would like also to appreciate the administrative support of Central Mindanao University, University Town, Musuan, Bukidnon Philippines; and to Mr. Ian-Kent B. Famador, University Statistician, for the statistical analysis.

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