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Research Article

Pre-Sowing Treatment, Formation and Transitional Changes of Leaves from Juvenile to Adult in *Vitex doniana* (Linn)

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Abstract

The study accesses the responses of pre-sowing treatment on germination and also takes a critical look at the seedling characteristics and developmental pattern of formation and transition of juvenile to adult leaves in V. doniana. A total of 1800 fruits from 15 trees were collected from parts of North Central Nigeria (FCT and Niger State). Randomized Complete Block Design (RCBD) was adopted for the study and there were six sub treatments from two main treatments; namely chemical and hot water treatments. The seeds were subjected in to chemicals and hot water treatments at various degrees in order to speedy up the germination and break the dormancy of the fruit. The germination was recorded daily from the date of sowing and continued till the germination ceased. Chi-square (X2) test showed no significant difference between the treatments and the locations. The Least Significant Difference (LSD) showed no statistical significant of area of first pairs of leaf sizes across the three locations while LSD showed significant of petiole lengths between the three location. It was recorded that generally compound leaf formation ranges from two leaflets (bifoliolate) to five leaflets (pentafoliolate), the result shows that 50% of the plants selected for the study transited from simple to pentafoliolate compound leaf. Similarly, transition stage was between 7th and 8th leaf stage with majority (65%) of transition at 8th leaf stage. However, the study revealed that after interruption of already formed compound leaves i.e. cutting off the main apexes, majority (57%) of the seedlings reformed to simple leaves; hence, different leaf reformations were recorded.

ABBREVIATIONS

RCBD: Randomized Complete Block Design MCI: Mild Cognitive Impairment; AD: Alzheimer's Disease; SSRIs: Selective Serotonin Reuptake Inhibitors

INTRODUCTION

Seeds of many plant species cannot germinate despite favorable environmental conditions required for germination. Main reasons for this problem, is termed as seed dormancy, which are hard and impermeable seed coat and presence of immature or dormant embryo [1]. The basis of dormancy varies in different plant species but basically it is classified as physiological, morphological, (morpho-physiological), combination of physical

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- Vitex doniana

and physiological factors [2,3]. Depending on the dormancy, various scarifications based on the use of physical or chemical agents may be used to break seed dormancy. Seed dormancy of some plants is prone to chemical agents such as plant growth regulators [4], potassium nitrate [5], hydrogen peroxide [6] and sulphuric acid [7]. Other plants may require physical agents to break their seed dormancy such as hot water [8], light and temperature [9].

Studies on the transition from juvenile to adult leaf forms in seed plants have focused on various issues including leaf sizes, development of heteroblasty in adult leaves, character of leaf margins, presence or absence of petioles phyllotaxy and transition from simple (juvenile) leaves to compound (adult) leaf forms.

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Variability in leaf forms is, perhaps, most profound among the legumes (Papilionaceae) and elicits simple leaves and varieties of compound leaves [10]. Olorode reported that transition from juvenile leaf forms to adult leaf forms generally exhibit specific patterns in each of the sub-families of *Papilionaceae (Mimosoideae, Caesalpinoideae* and *Papilionoideae)*. Beyond the sub-family specificities, there are also genus-specific patterns and distinct patterns among species of the same genus.

V. doniana called by different names in Nigeria. English (Black plume), Hausa (Dinya), Nupe (Edin), Yoruba (Orinla) and Igbo (Uchakoro) [11]. Is a medium sized deciduous tree, about 8-15m high? Leaves are glabrous, about 14-34cm long, usually containing 5 leaflets on stalks, 6-14cm in length. Leaflets are distinctly stalked. The leaf is dark green at the surface and pale grayish-green below or under [12]. It produces fruits which are plum – like, sweet and edible [13]. The fruit is green when mature and changes to dark brown when fully ripe, with the pulp surrounding a hard stone containing 1 - 4 seeds. It is a savanna species and can therefore be found in northern, eastern and western Nigeria [11].

The stem bark extract of the tree is used for the control of hypertension and its anti - hepatotoxic effect and treatment of stomach ache, pains, disorders and indigestion [14]. In Ghana it is used for treatment of colds and cough in children and its bark in treatment of sterility [15]. Various parts of the plant are used by traditional medicine practitioners in Nigeria in the management and treatment of several disorders which include rheumatism, hypertension, cancer, and inflammatory diseases [16,17] assess the antibacterial effect of whole stem bark of Vitex doniana against some enterobactericeae, and supports the use of Vitex doniana by traditional medicine practitioners in the treatment of dysentary and gastroenteritis. He stressed further that, antimicrobial activity of the Vitex doniana extract could be attributed to the presence of phenolic compounds that have been liked with antimicrobial properties [18], stated that even though the bulk of their weight is water, leafy vegetables represent a veritablenatural pharmacy of mineral, vitamins and phytochemicals. He concluded that the potassium content of leafy vegetables is good in the control of diuretic and hypertensive complications, because its lower arterial blood pressure. Vegetables like V. doniana are important sources of protective foods, which are highly beneficial for the maintenance of good health and prevention of diseases [19,20]. Although, they can be raised comparatively at lower management cost and on poor marginal soil, they have remained under - utilized, due to lack of awareness of their nutritional values in favor of the exotic ones [21].

Despite its uses as source of income, food and medicine in this region, there has been little report concerning the breaking of physical seed dormancy and the study of seedling characteristics and development (morphological changes in seedling developments) of *V. doniana*. Therefore, the presence work focused on dormancy breakage, variation and similarities of seedling leaf sizes during the juvenile to adult leaf transition stage and also assessed the reformation pattern of leaf following the removal of main leaf apexes.

MATERIALS AND METHODS

Study area

The study was carried out at University of Abuja Biological Garden Mini Campus, Gwagwalada Federal Capital Abuja. The Federal Capital Territory (FCT) Abuja is located between latitudes 8°25 and 9°26 and longitudes 6°45 and 7°39. It has a total land mass of about 8,000 km², located geographically at the centre of the country (Nigeria).

The FCT has average weather conditions annually (warm, humid rainy and hot dry seasons) including brief harmattan occasioned by the North – East Trade wind with the main features of dust haze, intensified coldness and dryness. The rainy season begins from April and ends in October, when day time temperatures reach 28-30°C and night time lows over around 22-23°C. In the dry season, day time temperatures can be as high as 40°C and night time temperatures can be as high as 40°C and night time temperatures can dip to 12°C, resulting in chilly evenings (Nigerian Meteorological Agency - NIMET). Even the chilliest nights can be followed by day time temperatures well above 30°c. The high altitudes and undulating terrain of the FCT act as moderating influence on the weather of the territory. Rainfall in the FCT also reflects the territory's location on the wind ward side of the Jos Plateau and the zone of rising air masses. The annual total rainfall is in the range of 1,100mm to 1,600mm.

FCT falls within the Savanna Zone vegetation of the West African Sub-region with patches of rain-forest especially around the South Eastern parts of the territory (Figure 1).

Identification, collection and preparation of *Vitex doniana* fruits

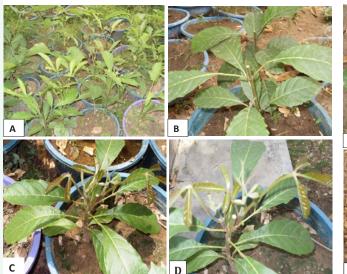
Vitex doniana is moderately abundant in north central Nigeria but it is the most widely distributed indigenous and wild species. The location, identification and collection of fruits cut across parts of FCT (Dutse and Gwagwalada) and Niger State (Izom, Minna, Kataeregi, Jima, Doko, Wuya, Kutigi, Labozhi, Kudu, Mokwa, Takuma, Jebba and Bokani). FCT and Niger State are parts of north central states of Nigeria which falls under guinea savanna. Vitex doniana trees were located and identified around major roads, linking from Abuja to Minna, Bida, Kutigi, Mokwa up to Jebba (North) areas in order to have a wider coverage and sample collection. Hence, the collection centres were grouped into three locations; namely Minna - Abuja (MIAB), Kutigi-Bida (KUBI) and Mokwa - Jebba (MOJE) locations. MIAB location comprises of Dutse, Gwagwalada, Izom, Minna, and Kataeregicentres, KUBI location comprise of Kutigi, Jima, Doko, Wuya, Labozhi while MOJE location comprise of Mokwa, Jebba, Kudu, Takuma, and Bokani).

Five *Vitex doniana* trees were located and identified from each location and the trees were monitored through regular observation at potential collection sites (locations) to indicate when collection of fruits is likely to be worthwhile. This trend continued until the fruits become matured (ripe i.e. turn black) which signifies the best time for collection.

Methods of fruit collection

Shedded, *V. doniana* fruits were collected through hand picking from each of the trees crown. To have a very high viability

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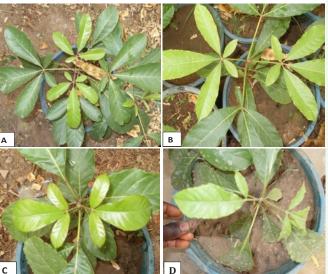


Plate (1): Stages of Emergence of Compound Leaves and Cutting Off of Compound Leaves Stages (A: Selected Plants for Compound leaf Studies- X0.2, B: Sample of Cut off of Main Apex- X0.2, C: Newly Reformed Penta-foliolate Compound Leaf-X0.2, D:Newly Reformed Tri-foliolate Compound Leaf- X0.2).

Plate (2): Response to Leaf Form following the Removal of Main Apex (A: Tetra-foliolate Compound Leaf but opposite is Penta-foliolate- X0.3, B: Pente-foliolate Compound Leaf - X0.2, C: Tri-foliolate Compound Leaf-0.2, D: Bi-foliolate Compound Leaf but Opposite is Tri-foliolate-0.3.).e Compound Leaf- X0.2).

Figure 1

of seed only physiologically matured and ripped fruits were picked. Collections were made from a total of fifteen trees across the three locations.

The spacing between sampled trees was 100m. Collections from the three location were also adopted in order to ensure collection from trees with slightly different environmental, climatic and soil factors.

The fruits were collected using sacks and perforated polyethene bags to provide enough aeration for the initial draining of moisture, reduce overheating and loss of viability. Collections from each tree were made in separate sacks and details of collections were recorded and labeled on each sack such as location and tree number. All collections were performed within 72h in August, 2013.

Chemical and hot water Pre-Sowing Seed Treatments (PSTs) for breaking dormancy

Fruits from each tree and locations were divided into six groups according to the number of treatments to be rendered to them. This was considered in order to compare effective rate of different PSTs, increase the percentage of dormancy breakage and also for the benefit of faster germination.

Chemical PSTs involve the use of two different concentrated chemicals namely; H_2SO_4 and HNO_3 . H_2SO_4 was 97.99% acidimetric with 89.07g/mol while, HNO3 was 65% with 63.01g/mol. The fruits were depulped and soaked in chemicals for 30 minutes after which they were removed and planted.

Hot Water PSTs were made up of two main categories; namely hot water PST's at 60°C and 100°C. Each category has two groups – depulped and undepulped. Using kerosene stove and cooking pots, water was heated to 60°C and 100°C for each - group depulped and undepulped respectively. Thermometer was used to measure water temperature. Depulped and undepulped fruits were submerged in to hot water of various degrees (60°C and 100°C) which was then allowed to cool gradually for 24hours to allow the seeds which are swollen after imbibing water. Hot water was also used because it can remove the cuticle and sometimes parts of the palisade layers of the seed coat its uniformity of being able to pre-treat the seeds without any special training.

Seed sowing/propagation and monitoring

All the treated seeds were directly planted into already prepared, arranged and well labeled seed pots. The date of planting (DOP) of each group of treated seeds were recorded and observations were made on daily basis. Twenty seeds per pot, per tree, five trees per location were planted. A grand total of 1800 seeds from 15 trees and from 3 locations were planted.

The monitoring of planted seeds started at 30^{th} Day after Planting (DAP) and continued until the germination ceased at 137^{th} DAP. The first noted germination was recorded on 73^{rd} DAP while the last noted germination was at 136^{th} DAP. The monitoring of seedlings continued until 50% of the seedlings attained transitions from simple to adult stage that is, at 420^{th} DAP.

Measurement of leaf sizes and petioles at 420th Day after Planting (DP)

Using a ruler, first pairs of leaves were measured following the length of their petioles. Twenty plants were randomly selected from each location for measurements. All the readings were taken in Centimetre (cm).

Observation, identification of compound leaf formation and removal of main apex at 480th Day after Planting (DAP)

At 420th DAP, a study of seedling (juvenile) to compound (adult) leaf stage was carried out. Twenty plants that attained compound leaf stages were randomly selected from each location and interrupted by cutting off the compound leaf i.e. removal of main apex using sharp and uncontaminated razor blade to see if subsequent leaf formation will be compound or simple leaf.

Statistical analysis

All the data collected were analyzed statistically using chisquare test, ANOVA and Least Significant Difference (LSD) to separate treatment means in germination of *V. doniana* at various treatment regimes.

RESULTS

Germination of fruits under different treatments from different locations

Table (1) shows that in Minna-Abuja (MIAB) Location, out of 600 seeds that were planted, only 81 seeds germinated (14 seeds for H_2SO_4 , 6 seeds for HNO_3 , 21 seeds for hot water at 60°C depulped, 18 seeds for hot water at 60°C undepulped, 7 seeds for hot water at 100°C depulped, 15 seeds for hot water at 100°C undepulped). Similarly in Kutigi-Bida (KUBI) Location, out of 600 seeds that were planted, 139 seeds germinated (34 seeds for H_2SO_4 , 15 seeds for HNO_3 , 33 seeds for hot water at 60°C depulped, 26 seeds for hot water at 60°C undepulped, 15 seeds for hot water at 100°C depulped, 16 seeds for hot water at 100°C undepulped). While in Mokwa-Jebba (MOJE) Location, out of 600 seeds that were planted, 105 seeds germinated (21 seeds for H_2SO_4 , 6 seeds for HNO₃, 25 seeds for hot water at 60°C depulped, 22 seeds for hot water at 60°C undepulped, 12 seeds for hot water at 100°C depulped, 19 seeds for hot water at 100°C undepulped) (Table 2).

However, the chi-square test between the two chemical treatments (H_2SO_4 and HNO_3), hot water at 60°C treatment for depulped and undepulped and hot water treatments at 100°C for depulped and undepulped seeds all showed no significant difference as p-values were greater than level of significance of 0.05 and conclusion drawn that germination of seeds in any location is independent of pre-sowing treatments rendered. Similarly, ANOVA test between the locations and treatments, first pairs of leaves and location show statistical differences at P > 0.05.

The area of first pair of leaves and petiole length

The mean for area of first pair of leaves in MIAB location was 27.93, in KUBI location 95.79 while in MOJE location 44.33. The Standard Error (SE) for MIAB location was 2.19, KUBI location 9.04 while MOJE location was 5.06. Generally, the first pair of leaves in KUBI location appears to be larger, followed by MOJE location and lastly MIAB location, however, the Least Significant Difference (LSD) showed no statistical significant across the three locations. That means leaf sizes were the same across the

| MAJOR TREAT- MENT | SUB TREAT- MENT | MIN | NA-A | BUJA (1 | MIAB) | LOCAT | ΓΙΟΝ | KU | TIGI-B | SIDA (F | (UBI) | LOCAT | ION | MOKWA-JEBBA (MOJE) LOCATION | | | | | |
|-------------------------|--------------------------------|-----------|-----------|-----------|-----------|-----------|------------|-----------|-----------|-----------|-----------|-----------|------------|-----------------------------|-------|-------|-------|-------|------------|
| | | TREE I | TREE 2 | TREE 3 | TREE 4 | TREE 5 | TO- TAL | TREE 1 | TREE 2 | TREE 3 | TREE 4 | TREE 5 | TO- TAL | TREE1 | TREE2 | TREE3 | TREE4 | TREE5 | TO- TAL |
| CHEMI- | H ₂ S0 ₄ | 3 | 2 | 4 | 4 | 1 | 14 | 6 | 4 | 10 | 9 | 5 | 34 | 4 | 3 | 4 | 5 | 5 | 21 |
| CAL | HNO ₃ | 2 | 2 | 0 | 2 | 0 | 06 | 0 | 3 | 4 | 5 | 3 | 15 | 1 | 0 | 2 | 0 | 3 | 06 |
| нот | DEPULPED | 6 | 3 | 4 | 3 | 5 | 21 | 7 | 6 | 11 | 5 | 4 | 33 | 3 | 5 | 6 | 5 | 6 | 25 |
| WATER (60°C) | UN- DEPULPED | 6 | 0 | 5 | 6 | 1 | 18 | 5 | 3 | 3 | 6 | 9 | 26 | 5 | 4 | 5 | 3 | 5 | 22 |
| нот | DEPULPED | 0 | 0 | 4 | 3 | 0 | 07 | 1 | 3 | 4 | 2 | 5 | 15 | 5 | 0 | 3 | 2 | 2 | 12 |
| WATER (100°C) | UN- DEPULPED | 1 | 0 | 4 | 6 | 4 | 15 | 3 | 4 | 2 | 3 | 4 | 16 | 2 | 3 | 5 | 3 | 6 | 19 |
| то | TAL | 18 | 7 | 21 | 24 | 11 | 81 | 22 | 23 | 34 | 30 | 30 | 139 | 20 | 15 | 25 | 18 | 27 | 105 |

Table 2: A Comparison of Summary of Inter Trees, Treatments and Inter Locations Cumulative Frequency Germination.

| | TREATMENTS | | | | | | | | | | |
|--------------------------|--------------------------------|------------------|---------------------------------|--------------------------------|----------------------------------|---------------------------------|-------|--|--|--|--|
| LOCATION | H ₂ SO ₄ | HNO ₃ | HOT WATER (60°C) UNPULPED | HOT WATER (60°C) UNDEPULPED | HOT WATER (100°C) DEPULPED | HOT WATER (100°C) UNDEPULPED | TOTAL | | | | |
| MINNA ABUJA (MIAB) | 14 | 6 | 21 | 18 | 7 | 15 | 81 | | | | |
| KUTIGI-BIDA (KUBI) | 34 | 15 | 33 | 26 | 15 | 16 | 139 | | | | |
| MOKWA-JEBBA (MOJE) | 21 | 6 | 25 | 22 | 12 | 19 | 105 | | | | |

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three locations (Table 3). Similarly, the mean for petiole length of first pair of leaves in MIAB location was 1.01, in KUBI location 2.92 while in MOJE location 1.79. The Standard Error (SE) for MIAB location was 0.06, KUBI location 0.24 while MOJE location was 2.65. The petiole length showed Least Significant Difference (LSD) in Minna-Abuja and Kutigi-Bida locations, 0.032 and 0.014 respectively (Table 4). By implication, different locations may show differences in petiole lengths but the leaf sizes may likely be the same.

Seedling to Compound Leaf Formation and Leaf Reformation Following Removal of Main Apex

The result of seedling to compound leaf formation i.e. transition from juvenile to adult stage and the result of interruption (cutting) of compound leaf showed that out of sixty (60) plants selected for transition studies from three location, thirty (30) plants transit from simple to penta-foliolate, twenty-six (26) from simple to trifoliolate, three (3) from simple to bifoliate while one (1) from simple to tetra-foliolate compound leaf. Considering the transition stages, thirty nine (39) plants changed form at 8th leaf stage, nineteen (19) at 7th leaf stage while two (2) at 9th leaf stage compound (See Plates 1- 2, table 3 and 4).

The result of reformation of compound leaf after interruption (cutting) showed that out of sixty (60) plants studied from three locations, thirty-four (34) plants transformed to simple leaf, twenty (20) plants reformed to penta-foliolate compound leaf, five (5) plants reformed to tri-foliate while only one (1) reformed to bi-foliolate compound leaf. For the stages, thirty-four (34) plants changed form to simple leaf at 1st stage from the cut-off points and maintained the simple leaf all through, eleven (11) plants reformed compound leaf at 4th leaf stage, eight (8) plants reformed compound leaf at 3rd leaf stage while seven (7) plants reformed compound leaf at 5th leaf stage from the cut off points of compound leaf. This shows that majority of the compound leaf stage(s) that were interrupted transformed to simple leaf (34) while the plants that reformed compound leaf was between 3rd and 5th leaf stage from the cut-off points of compound leaf (Figures 2-5) (Table 5,6).

DISCUSSION

The major problem encountered in propagating of *V. doniana* in the study area which fall under guinea savannah is

| Table 3: Petiole Length of First Pair of Leaves (In Centimetre) across the Three Locations. | | | | | | | | | |
|--|----------------------|----------------------|----------------------|--|--|--|--|--|--|
| | MIAB Location | KUBI Location | MOJE Location | | | | | | |
| Total | 20.2 | 58.3 | 35.7 | | | | | | |
| Mean | 1.01 | 2.92 | 1.79 | | | | | | |
| SE | 0.06 | 0.24 | 2.65 | | | | | | |
| LSD P<0.05 | 0.032 | 0.014 | 0.001 | | | | | | |

 Table 4: Area of First Pair of Leaves (in Centimetre) across the Three Locations.

| | MIAB Location | KUBI Location | MOJE Location |
|------------|----------------------|----------------------|----------------------|
| Total | 558.61 | 1915.75 | 886.59 |
| Mean | 27.93 | 95.79 | 44.33 |
| SE | 2.19 | 9.04 | 5.06 |
| LSD P<0.05 | 0.26 | 0.87 | 0.61 |

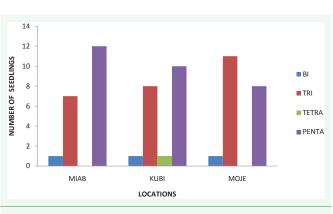
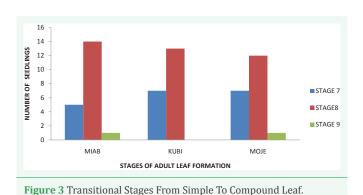
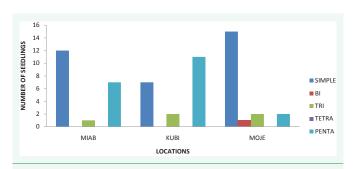
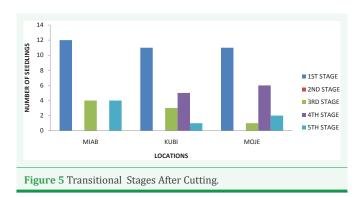


Figure 2 Leaf Formation (Foliolate) Before Cutting









poor seeds germination caused by the water impermeable seed coat which exerts physical dormancy. Seed dormancy hinders the completion of germination of an intact viable seed under favourable conditions [23,24].

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| Table 5: A Numerical Table for Frequency Distribution of Seedling to Compound Leaf Formation. | | | | | | | | | | | | |
|---|---------------------------|-----|--------------|---------------|--------|-----------------|-----------------|-----------------|------------------|--------|--|--|
| LOCATION | TRANSITION BEFORE CUTTING | | | | | | | | | | | |
| LOCATION | | L | EAF FORMATIC | ON (FOLIOLATI | STAGES | | | | | | | |
| | BI | TRI | TETRA | PENTA | DOUBLE | 7 TH | 8 th | 9 th | 10^{TH} | DOUBLE | | |
| MINNA-ABUJA | 1 | 7 | - | 12 | - | 5 | 14 | 1 | - | - | | |
| KUTIGI-BIDA | 1 | 8 | 1 | 10 | - | 7 | 13 | - | - | - | | |
| MOKWA-JEBBA | 1 | 11 | - | 8 | - | 7 | 12 | 1 | - | - | | |
| TOTAL | 3 | 26 | 1 | 30 | - | 19 | 39 | 2 | - | - | | |

Table 6: A Numerical Table for Frequency Distribution of Reformation of Compound Leaf following Removal of Main Apex.

| LOCATION | TRANSITION AFTER CUTTING | | | | | | | | | |
|-------------|--------------------------|--------|-----------|-------------|--------|-----------------|-----------------|----------|-------------------|-----------------|
| LUCATION | | LEAF I | FORMATION | (FOLIOLATE) | STAGES | | | | | |
| | SIMPLE | BI | TRI | TETRA | PENTA | 1 ST | 2 ND | 3^{RD} | 4^{TH} | 5^{TH} |
| MINNA-ABUJA | 12 | - | 1 | - | 7 | 12 | - | 4 | - | 4 |
| KUTIGI-BIDA | 7 | - | 2 | - | 11 | 11 | - | 3 | 5 | 1 |
| MOKWA-JEBBA | 15 | 1 | 2 | - | 2 | 11 | - | 1 | 6 | 2 |
| TOTAL | 34 | 1 | 5 | - | 20 | 34 | - | 8 | 11 | 7 |

The findings of the study revealed that H₂SO₄ Pre-Sowing Treatments (PSTs) for only 30 minutes recorded 23% germination which was higher than HNO₃ PSTs with only 9% germination. Hot water at 60°C depulped PSTs recorded 26% germination while 100°C depulped PSTs recorded 11% germination. This shows that warm water at 60°C depulped PSTs gave the highest rate of germination followed by H₂SO₄ PSTs. Consequently, V. doniana fruits have potentials to germinate irrespective of Pre-Sowing Treatments (PSTs) that could be given. However, differences in terms of Cumulative Frequency Germination (CFG) depend on environmental and genetic factors such as climatic and soil differences. Hence, a combination of two or more PSTs such as hot water at 60°C and chemical (H_2SO_4) as revealed from the result will definitely increase the percentage and chances of germination of V. doniana fruits. This finding agrees with the finding of other studies such as that of [24], in which was reported that in Coted'Ivoire, V. doniana seeds were dipped in to Sulphiric acid 95% for 60 minutes and subsequently in warm water for 72 hours (3 days) and germinated after 26 days but the germination rate was only 34% [25]. Who reported that in Albizialebbeck, 40 minutes was effective while both 20 and 60 minutes gave poorer germination while in Caesalpinaspinosa any duration of soaking within the tested pre-treatment time from 1 to 4 hours gave almost 100% germination. In Cassia sieberiana, 45 minutes gave 90-95% germination; In Delonixregia 3 to 6 hours gave 95% germination while in Senna bicaparis 60 minutes duration gave 95 to 100% germination.

The significantly higher percentage germination of hot water at 60°C depulped PSTs gave a contrary result when compared with [26] reported that hot water pretreatment is not a suitable technique in seeds of Adansonia digitata as well as Afzeliaafricana as subjecting the seeds to hot water could lead to the seed embryo been killed because of prolonged contact with boiled water. The result is similar to the view of [27], who offered that pre-treatment of Acacia Senegal seeds in hot water for 10 min, increased its growth potential.

On the other hand, HNO₂ PSTs recorded the lowest CFG across the three Locations followed by hot water at 100°C depulped. This denotes that a combination of PSTs such as HNO, with hot water at 100°C (boiling point) could be destructive, reduce the chances of germination or even impede the germination of V. doniana fruits. The HNO₂ appears to be destructive because of its high acidic nature while hot water treatment at 100°C may be too hot that it affected the viability of the fruit.

Studies have also shown that leaves of V. doniana are opposite, glabrous 14-34cm long, stalks 6-14cm long usually with 5 leaf lets on stalks [28]. Supported by [29] who reported that leaves are opposite, digitately compound with 3-7 leaflets, petide 5-20cm long the middle leaflets are usually the largest. The findings of this study can attest to the above summation, thou only the first pairs of leaf were measured which are generally smaller than the middle leaves. Generally, the first pair of leaves in KUBI location appears to be larger, followed by MOJE location and lastly MIAB location. The differences in leave sizes could be as a result of genetic or climatic (availability of water and shade) factors.

The result of this study confirms that the leaf formation ranges from two-leaflets (bi-foliolate) to five leaflets (penta-foliolate) and transition stage is between 7th and 9th leaf stage, majority of the leaves show transition from simple to penta-foliolate and majority changed form at 8th leaf stage. This study is in line with other studies such as that of [10] in which it was reported that juvenile leaves in the Mimosoideae are compound pinnate while adult leaves are compound bipinnate. Very similar to juvenile leaf to adult leaf transition in the papiolionoideae in which simple and opposite leaves precedes the emergence of adult leaves as exemplified by Vigna unguiculata and Cajanus cajan.

CONCLUSION

It was revealed that hot water at 60°C is the most effective; however, a combination of it with acid treatment at 98% concentration for 2 hours will yield more and better results. It is also inferred from this study that in V. doniana seedling development

and transition, leaf formation ranges from two-leaflets (bi-foliolate) to five leaflets (penta-foliolate), the transition stage was between 7th and 9th leaf stage, majority of the leaves show transition from simple to penta-foliolate, and changed form at 8th leaf stage. Interestingly, interruption of compound leaf i.e. removal of main apex can lead to reformation of different leaf formation either simple or compound leaf. Consequently, there is a great need to better understand the biology of indigenous tree species such as *V. doniana* so that their adaptive benefit in local conditions can be promoted and sustained.

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