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Research Article

Factors Affecting Seedling Emergence and Dry Matter Characteristics in *Musa balbisiana* Colla

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The effects of storage duration (0, 2, 4, 6, 8, and 10 days), sterilization with sodium hypochlorite (0, 5, 10, 15, and 20%), and weaning media on seedling characteristics and dry matter content in *Musa balbisiana* seedlings were studied. The experiment was factorial in a completely randomized design with five replicates. The result indicates that increase in NaOCl concentration and number of days in storage significantly ($P = 0.5$) increased the period of seedling emergence. Also, soaking in NaOCl for 20 min had significant effect on average seedling emergence at 15 and 20% concentrations, compared to 10 min soaking at the same concentrations. The combined effects of storage duration and sterilization resulted in a decrease in the duration of seedling emergence. Seeds previously sterilized with either water or NaOCl had no significant effect on seedling growth, leaf and corm dry weight, but affected almost all the dry matter traits. A mixture of poultry manure, top soil, and river sand as weaning media gave better seedling growth and increased dry matter characteristics. We conclude that *M. balbisiana* seeds require after-ripening treatment to enhance germination, sterilizing seeds with 5% NaOCl for 10 min and air-drying under ambient condition for 2–6 days were found most appropriate, and a mixture of poultry manure, top soil, and river sand is recommended as weaning medium for growth and dry matter composition in *M. balbisiana* seeds.

1. Introduction

Bananas and plantains (*Musa* spp.) are giant perennial herbs that grow in the humid agroecological zones of the tropics where they are important staples and contribute significantly to the incomes of the rural dwellers that grow them in compound or home gardens [1]. Bananas and plantains are derived from intra- and interspecific crosses between the diploid wild species *Musa acuminata* Colla (genome A) and *M. balbisiana* Colla (genome B) [2]. Most cultivated *Musa* are triploids ($2n = 3x = 33$) with the following genomic constitutions: dessert bananas predominantly AAA, plantains AAB, and cooking bananas ABB [2]. The wild progenitors (*M. acuminata* and *M. balbisiana*) of the edible bananas produce seeds, while most of the edible clones are seedless with a few exception such as "Pisang awak" subgroup ABB [3].

A major constraint to *Musa* breeding is the scarcity of healthy planting materials, due to low seed-set and viability [4]. Intractable fertilization barriers such as moderate to high levels of female sterility and triploidy make genetic improvements of parthenocarpic *Musa* clones slow and technically difficult [5]. Hybrid plant production in the most common triploid clones is further complicated by low seed-set and germination caused by endosperm failure [4]. Seeds are excellent dispersal units which have emerged in the course of plant evolution and are important genetic delivery systems essential for sustainable agriculture. *Musa* seeds are orthodox seeds; that is, the seeds can be dried to very low level moisture content (below 7%), stored at subzero temperatures, and are employed only for propagation in breeding programs. The possibility of conserving bananas in the form of seed is envisaged for a long-term preservation of *Musa* sp. Under favorable environmental conditions, at least 3–6 weeks is required for the initiation of germination in soil, and germination may occur either in a flush or intermittently over a period of 3–15 weeks. Germination percentages differ

Table 1: Relative proportion (%) of weaning substrates.

Media	TP	PM	SD	RS
1	25	25	25	25
2	33.3	33.3	0	33.3
3	33.3	50	33.3	0
4	50	50	0	0
5	0	50	50	0
6	50	33.3	16.7	0

Spent sawdust (SD), river sand (RS), topsoil (TP), and Poultry manure (PM).

Table 2: Effects of sterilization (NaOCl) and storage duration on seedling emergence in *M. balbisiana*.

Conc. (%) NaOCl	Days of storage/duration of seedling emergence (days)					
	0	2	4	6	8	10
0 (control)	16.7	17.6	18.8	20.7	21.6	22.2
5	16.0	17.1	18.8	20.2	21.3	21.7
10	18.3	19.8	21.6	22.3	25.1	27.3
15	19.6	23.4	36.2	27.0	27.7	26.5
20	20.2	22.9	24.2	27.2	28.8	28.7
$LSD_{(0.05)}$	1.72	1.59	9.06	1.69	1.95	1.72

markedly between harvest lots [6–8], depending on the maturity of the fruit at time of seed harvest, postharvest age of the seed, and method of storage [6, 7].

Little is known about the factors that affect seed germination in *Musa*, except that germination is extremely variable and relatively difficult to obtain under natural conditions [5]. The use of *Musa* seedlings as research tools and the increased emphasis on banana breeding programs require improved germination rates. Understanding the germination process will help in the conservation strategies and breeding programs [9]. Seed germination is considered as a critical phase in the reproductive cycle and is of great importance for species fitness, and variation in germination percentage has been interpreted as an adaptation to ecological conditions [10, 11]. Several reports have indicated the limited and variable seed germination exhibited by *Musa* [6, 12]. The work in [5] used an optimized *in vitro* culture of zygotic embryos to identify factors affecting germination and seedling growth in *Musa acuminata* subspecies *malaccensis*. The work in [13] employed *in vitro* embryo culture technique to produce hybrid germplasm in *Musa balbisiana*. Therefore, the objective of this study is to evaluate the effects of seed treatment chemicals, storage conditions, and weaning media on seedling emergence, growth, and dry matter content in *Musa balbisiana*.

2. Materials and Method

2.1. Experimental Site and Collection of Samples. This work was carried out at the Rivers State University of Science and Technology, Port Harcourt, Nigeria (Department of Applied and Environmental Biology), and formerly at the International Institute of Tropical Agriculture (IITA), Onne station (lat. 4° 43 N, long 7° 01 E, and 10 m altitude above sea level). *Musa balbisiana* seeds were extracted from matured fruits obtained from the field genebank of IITA Onne. *M. balbisiana* Colla, a diploid ancestor of commercial parthenocarpic varieties, was used because of the availability of large quantities of seeds. The weaning substrates sawdust, river sand, topsoil, and poultry manure were obtained from Port Harcourt and Onne towns. JIK (3.5% a.i) a Nigerian commercial product of sodium hypochlorite (NaOCl) used for sterilization was obtained from the market. A total of 1000 freshly harvested and viable *Musa balbisiana* seeds were selected using floatation method. Moisture Content (MC) of the seeds was also determined [14]. The seeds were air-dried, and only properly filled ones were selected for use.

Table 3: Effects of soaking time and NaOCl concentrations on days to seedling emergence in *M. balbisiana*.

Soaking time (min)	NaOCl concentration/seedling emergence time (days)				
	0	5	10	15	20
10	17.6	19.3	20.4	22.6	23.6
20	17.6	20.0	22.4	24.2	26.2
30	19.3	21.1	23.8	23.8	24.9
$LSD_{(0.05)}$	1.33	1.23	1.42	1.31	1.51

2.2. Experimental Layout and Treatments. The experiment was factorial in a completely randomised design. The factors considered were number of days in storage after extraction (0, 2, 4, 6, 8, and 10 days) and sterilization with sodium hypochlorite (NaOCl, 3.5% active ingredient), with the following concentrations 5, 10, 15, and 20% and 0% as control (seeds soaked in tap water). After sterilization, seeds were washed under running tap water and air-dried under ambient room condition before planting. However, seeds with no-storage treatment (0) were planted immediately, while the remaining seeds were and stored in unsealed thin cellophane from where subsequent seeds were taken and planted accordingly.

There were five replicates and 10 seeds per replicate were sown in black nursery bags measuring 21 × 11 cm filled with sawdust. Medium sterilization was as described by [15] and watered with deionized water. Data on the effects of NaOCl concentration on days to seedling emergence after extraction was recorded. A seed was considered to have germinated when the radical had pierced through the testa up to 2 mm in height.

The effect of weaning media on growth parameters of *M. balbisiana* seeds was studied with six different weaning media, compounded in different ratios, namely, spent sawdust (SD), river sand (RS), topsoil (TP), and decomposed poultry manure (PM) (Table 1). Thereafter, 5-week-old seedlings (10 seedlings/weaning substrate) were planted in black nursery bags of 21 × 11 cm. Substrate sterilization and watering were as previously described, and data were taken at 4 and 8 weeks after transplanting on number of photosynthetically active leaves, height (cm) of seedling from

Table 4: Interaction between days after extraction (DAE) and varying concentrations of NaOCl on seedling emergence in *M. balbisiana*.

	Days to percentage seedling emergence								
	10%	20%	30%	40%	50%	60%	70%	80%	90%
DAE									
2	18.4	21.2	23.5	25.6	27.8	30.8	31.7	26.7	24.2
4	22.3	24.0	25.3	27.5	27.4	29.1	31.4	34.2	24.2
6	18.6	20.4	21.6	23.2	25.0	27.1	26.4	27.4	25.0
8	19.0	20.9	22.4	22.9	24.4	24.9	24.9	29.9	23.6
10	22.8	25.1	26.0	26.4	26.2	24.2	25.8	17.9	24.2
NaOCl Conc. (%)									
0 (control)	17.4	19.4	21.3	21.9	23.0	23.8	24.6	24.9	25.1
5	18.3	20.6	23.1	23.9	26.9	25.0	27.7	23.4	24.2
10	20.8	23.0	23.8	25.6	25.0	28.0	27.3	34.6	24.2
15	21.0	22.5	23.1	25.4	26.5	27.1	29.5	23.9	14.2
20	23.7	26.0	27.4	28.7	29.4	32.1	31.1	29.4	33.5
$LSD_{(0.05)}$	1.25	1.38	1.32	1.28	1.41	1.52	1.45	1.48	NS

DAE: Days after extraction.

the base of the pseudostem ("soil level") to the level of the last foliage, excluding the cigar leaves; plant girth/circumference (cm) taken from the base of the pseudostem above ground level and leaf area [16]. Leaf area = $(L \times W)0.8$, where L = length of leaf and W = width of leaf.

Destructive sampling was done at the end of 8 weeks on five randomly selected seedlings from each weaning medium, and data on the number of roots, length of longest roots (cm), and fresh weight (g) of corms, roots, leaves, and pseudostem, as well as their respective dry weights at 70°C, were recorded. Data were analyzed with GENSTAT Discovery Edition 1 [17] in a factorial completely randomized design. The least significant difference (LSD) at the 5% probability level was used to separate the means and detect the effects of storage, sterilization, weaning media, and interaction between storage and concentrations of sterilization medium on *M. balbisiana* seeds.

3. Results

Sterilization with sodium hypochlorite (NaOCl) at higher concentration significantly ($P = 0.05$) influenced the number of days to seedling emergence in *M. balbisiana*. The shortest duration in seedling emergence was observed in 5% concentration NaOCl which was not significantly different from the control (Table 2). There was no significant difference between seeds sterilized with 15 and 20% NaOCl, except in day 4 where seeds with 15% NaOCl sterilization took longer days (36.2 days) to emerge compared to 24.2 days in 20% NaOCl (Table 2). However, significant differences were observed between 20 and 10% concentration in all of the treatments. Thus, increase in both NaOCl concentration used in seed sterilization and number of days in storage resulted in an increase in the number of days to seedling emergence.

Soaking in NaOCl for 20 min had significant effect on average seedling emergence in *M. balbisiana* at 15 and 20% concentrations, compared to 10 min soaking at the same concentrations (Table 3). Also, soaking for 30 min at 20% NaOCl resulted in longer days to seedling emergence in *M.* balbisiana at various concentrations (Table 3).

There was a significant interaction effect between days after extraction and varying concentrations of NaOCl on seedling emergence, except at 90% emergence (Table 4). Seeds soaked in water (0%), which was the control, emerged earlier than other treatments, except in 90% germination, while seeds sterilized with 20% NaOCl took longer days to emerge. At two, 6, and 8 DAE had the shortest period to emergence at 10%, 20%, and 30%, respectively, while 8 DAE had the shortest seedling emergence period in 40–70%. At 10% emergence, 2 and 10 DAE had the shortest (18.4) and longest (22.8) days to emergence, while at 30% the maximum (26.0) and minimum (21.6) days to emergence were at 10 and 6 DAE. At 70% emergence, 8 and 2 DAE had the shortest (24.9) and longest (31.7) days to emergence. It was further observed that 8 and 6 DAE had the longest (25.0) days, while an equal number of days were noted for 2, 4, and 10 DAE to seedling emergence, which was the shortest. Thus, increase in days after extraction causes decrease in the number of days to emergence (Table 4).

Seeds previously sterilized with either water or NaOCl had no significant effect on number of leaves, seedling height, leaf and corm dry weight, and corm dry matter (Table 5). However, significant effects were observed on total dry matter, stem dry weight, and almost all the dry matter traits (Table 5). Although seeds soaked in water resulted in taller seedlings, seeds sterilized with sodium hypochlorite had higher percentage root and leaf dry matter.

Table 6 shows the effect of weaning media on seedling height, number of leaves, and dry matter yield and distribution in *M. balbisiana* seedlings. Seedlings grown in a mixture of poultry manure, top soil, and river sand (PM/TP/RS; weaning media 2) as well as those on poultry

Table 5: Effects of previous seed treatments on growth, dry matter yield, and distribution in *M. balbisiana* seedlings.

Previous seed treatment	Ht (cm)	NL	Dry weight (g)					Dry matter (%)			
			TDW	Root	Stem	Leaf	Corm	Root	Stem	Leaf	Corm
Water	48.6	7.7	22.1	2.04	9.43	9.22	1.44	10.3	37.8	45.6	6.39
NaOCl	47.8	7.5	16.1	2.12	4.31	8.62	1.03	14.6	26.7	52.4	6.37
$LSD_{(0.05)}$	NS	NS	4.40	2.93	2.93	NS	NS	2.91	4.7	4.6	NS

Ht: height of seedling, NL: number of leaves, and TDW: total dry weight.

Table 6: Effect of weaning media on growth, dry matter yield, and distribution in *M. balbisiana* seedlings.

Weaning media	Ht (cm)	NL	Dry weight (g)					Dry matter (%)			
			TDW	Root	Stem	Leaf	Corm	Root	Stem	Leaf	Corm
1	51.4	7.0	25.53	2.85	11.66	9.76	1.25	12.4	39.73	42.31	5.53
2	56.2	7.6	25.20	2.42	9.10	11.42	2.26	10.1	32.15	49.58	8.14
3	45.0	7.1	12.08	1.58	4.42	5.41	0.67	15.0	33.10	46.12	5.82
4	52.2	8.0	20.64	2.57	5.63	11.05	1.39	12.4	28.00	53.10	6.51
5	34.6	8.2	8.06	1.28	2.28	3.99	0.52	17.1	27.47	48.94	6.54
6	49.8	7.5	23.8	1.78	8.13	11.86	1.31	7.62	32.91	53.77	5.69
$LSD_{(0.05)}$	6.42	0.8	7.62	0.76	5.06	2.97	1.01	5.04	8.14	7.88	2.47

Weaning media: 1: PM/TP/SD/RS; 2: PM/TP/RS; 3: PM/TP/SD; 4: PM/TP; 5: PM/SD; 6: PM/TP/SD; PM: poultry manure; TP: top soil; SD: sawdust and RS: river sand; Ht: height of seedling; NL: number of leaves; TDW: total dry weight.

manure/topsoil/sawdust/river sand (PM/TP/SD/RS; weaning media 1) and a mixture of poultry manure/top soil (PM/TP; weaning media 6) were significantly taller, compared to other treatments (Table 6). At least seven leaves were produced in each weaning medium, although significantly higher numbers were observed in weaning media 4 and 5. There were differences in dry weight traits; for instance, total dry weight was lowest in weaning media 5 (8.06 g) and weaning media 4 (20.64 g). The other weaning media had total dry weight (TDW) ranging from 23.8 g to 25.5 g. Root dry weight was higher in weaning media 1, 2, and 4 than in 3, 5, and 6. Stem dry weight was significantly different in weaning media 1, 2, and 6 with 11.66, 9.10, and 8.13 g, respectively. The least values for this trait were observed in weaning media 5 (2.28 g), 3 (4.42 g), and 4 (4.53 g). Higher leaf dry weight was obtained in weaning media 1, 2, 4, and 6, while the same for corm was highest in weaning medium 2 (2.26 g), followed by media 4, 6, and 1, that is, 1.39 g, 1.31 g, and 1.25 in that order. Percentage dry matter in root was higher in weaning media 5 and 3 and the least in 6 (7.62%). In stem, percentage of dry matter was highest in weaning medium 1 (39.73) and lowest in 5 (27.47). Significantly higher, percentage of leaf dry matter was produced in weaning medium 6 with 53.77, compared to weaning medium 1 which had 42.31. Similar observations were made in % corm dry matter.

4. Discussion

There are reports that storage duration [9, 18–20], soaking in water [6, 21, 22], and sterilization with sodium hypochlorite [20] affect seed germination characteristics in plants. The present work confirms that increase in NaOCl concentration and number of days in storage resulted in an increase in the number of days to seedling emergence in *Musa balbisiana*. Similar results were obtained by [9] on germination and seedling characteristics of *Periploca angustifolia* Labill after long duration in storage. This is because as seeds get older the membrane system gradually becomes permeable allowing many electrolytes to flow out of cells leading to loss of vigor in such seeds. Similar effects were also observed when seeds were soaked in higher concentrations of NaOCl for a long time. Though sterilization with NaOCl is reported to reduce competition with pathogens which could impede the rate of growth and development of seedlings, [20], also it prolongs seedling emergence. It could be that the seeds may have to be resterilized after 10 days to reduce the time for seedling emergence. Soaking in water had better seedling emergence than NaOCl. Our findings further show that delayed planting for 10 days after seed extraction reduced seedling emergence which could be attributed to loss of seed viability as a result of loss in moisture. It was evident from the study that soaking duration influenced earliness to emergence and the total number of seedlings that emerged, while sterilization significantly affected the quality of seedlings produced. Seedlings arising from previously sterilized seeds had less total dry weight accumulation than water soaked seeds. This could be as a result of long duration of soaking seeds in NaOCl concentration. According to [23], the percentage germination in *Musa balbisiana* is highly variable depending on factors such as fruit maturity at seed harvest, the postharvest age of the seed, and the method of storage. The work in [21] reported that embryo germination was achieved in *Musa balbisiana* seeds after soaking in water for five days, prior to embryo excision. Similarly, Simmonds [6] and [23] also reported that the soaking of *Musa* seeds before sowing was either deleterious or ineffective to germination.

A mixture of poultry manure, top soil, and river sand as a weaning medium resulted in better seedling growth

and dry matter characteristics, which is supposed to be a reflection of the chemical properties of the medium. It should be noted that 5 out of the 6 weaning media formulations had the same proportion of poultry manure as nutrient source. Lower bulk density enhances better root substrate relation [22], provided such media have the ability to hold the plant firmly in place and at the same time have adequate drainage and aeration [24]. A combination of poultry manure, top soil, sawdust, and river sand (PM/TP/SD/RS) with poultry manure, top soil, and river sand (PM/TP/RS) had higher bulk density and were more aggregated than sawdust and poultry manure (SD/PM), thus having relatively greater pore spaces for proper aeration. Media compaction and in situ media decomposition could cause an undesirable decrease in drainage and aeration leading to impediment of root growth. The decomposition of SD causes a depletion of available nitrogen within the potting mixture [25]. Thus, poor plant performance of SD/PM in the traits studied might be due to nutrient deficiency, especially nitrogen immobilization.

In conclusion, *M. balbisiana* seed needs after-ripening treatment to enhance seedling germination. Also, sterilizing *M. balbisiana* seeds with 5% dilution of sodium hypochlorite (NaOCl) at 10 min duration and air-drying under ambient tropical room condition for 2–6 days were found most appropriate. Finally, a mixture of poultry manure, top soil, and river sand is most appropriate as weaning media for growth and dry matter composition in *M. balbisiana*.

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