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Original Research Article

Effects of storage and priming on seed germination in soil and embryo culture of *Musa acuminata* Calcutta 4

ABSTRACT:

Aims:

Effects of 3 storage durations, 3 hydro priming protocols and 6 chemical priming protocols on germination in soil (*in vivo*) and embryo culture (*in vitro*) of *Musa acuminata* Calcutta 4 were investigated.

Study design:

Analysis of variance was used ($P=.05$) to test treatment effects in a Completely Randomised design. mean comparison was by LSD.

Place and Duration of Study: This study was carried out for a period of 10 months at the International Institute of Tropical Agriculture High Rainfall Station, Onne, in Rivers State, Nigeria.

Methodology:

Seed pre-sowing treatments consisted of 3 storage protocols, 3 hydro priming and 6 chemical treatment protocols. After which treated seeds were divided into two sets. One set was sown directly in soil and the other set subjected to embryo culture technique.

Results:

Seeds sown in soil immediately they were extracted had significantly higher germination than stored seeds. Germination declined by 20% and 23% after 2weeks and 4weeks of storage respectively. For embryo culture, seeds stored for 2weeks had significantly higher germination (40%) than seeds that were not stored or seeds stored for 4weeks (38%). Germination *in vivo* was significantly higher for seeds that were not hydro primed than for seeds hydro primed for 4days or 8days. Germination declined by 33% and 38% in seeds hydro primed for 4 and 8days respectively. Hydro priming for embryo culture for 4days increased germination significantly by 60% compared to those without hydro priming. All the chemicals reduced germination in both soil and *in vitro* procedures except that of Copper oxychloride in embryo culture which increased germination by 18%, compared to the control achieving 47% germination.

Conclusion:

Higher germination was recorded with *in vitro* than *in vivo* procedures irrespective of the treatments applied. Perhaps inherent factors in the seed coat and possible interactions in soil may account for the poor germination exhibited *in vivo* and will require further investigation.

Keywords: [*Musa acuminata*, Hydro-priming, chemical-priming, *in vivo*, *in vitro*]

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1. INTRODUCTION

Seed production is required in plantain and banana (*Musa* spp.) mainly for breeding purposes. At maturity, *Musa* seeds are black or dark brown stony bodies. The seed has a rough seed coat [1]. It contains an embryo, which is embedded in a copious endosperm and chalazal mass [2]. Seeds vary in size (about 4-6 mm), colour (brown or black) and shape (angular or globose). Seed shape varies due to compression between neighbouring seeds

15 [1]. The structure of the *Musa* seed is complex, hence making germination very difficult [3,
16 4,1]. It was found that seed viability was also affected by moisture content, oxygen and
17 temperature [5]. The presence of a semi-permeable inner membranous seed coat restricts
18 the movement of moisture and oxygen into the embryo. In addition, while a hard seed coat
19 provides effective protection during maturation, dispersal and dormancy, it hampers
20 germination because the embryo requires extra energy to rupture the seed coat.

21 Seed set in *Musa* spp. varies greatly among seed-fertile cultivars. This limitation in
22 variable seed set is further compounded by an extremely low rate, slow and non-uniform
23 germination in soil thus making creation of new cultivars and other breeding activities of
24 plantains and bananas difficult [1, 6]. In fact seed germination especially of hybrid seeds in
25 soil is reported to be less than 1% [7]. While seeds of *Musa balbisiana* (with the B genome)
26 readily germinate in culture and soil [8], seeds of *M. acuminata* (with the A genome) and
27 most interspecific hybrids have poor germination and are not viable especially if the fingers
28 are left to over ripen (blackened or rotten) before extraction [8]. However, a major source of
29 pollen in plantain & banana breeding is the wild diploid accession, *Musa acuminata* Calcutta
30 4, which though agronomically poor, produces abundant and viable pollen [9]. It is resistant
31 to black Sigatoka disease, but produces non-parthenocarpic fruits due to the presence of
32 two complementary recessive genes for parthenocarpy [10]. It is important in germplasm
33 enhancement because it serves as a source of plantain alleles and resistance to black
34 Sigatoka disease [11].

35 The parental differences between seeds from *M. balbisiana* and other accessions
36 could be histological, physiological or genetic in nature. Another study have identified single
37 sequence repeats (SSRs) that could help in understanding the divergence between *M.*
38 *acuminata* and *M. balbisiana* [12]. For example, in *Vicia* spp., germination ability has been
39 linked to permeability of the seed coat, a condition that was found to be controlled by a two-
40 gene system [13]. Similarly seed coat permeability in cotton *Gossypium hirsutum* L. was
41 found to be controlled by a single gene [14].

42 Due to their triploid nature, plantains and bananas are almost completely female sterile,
43 resulting in low seed set upon pollination and poor seed quality. If seeds from Tetraploid (4x)
44 and Diploid (2x) crosses can be made to germinate at relatively high frequency when planted
45 in soil, they can be grown in environments other than those under which they were produced
46 provided that an efficient method for seed germination is available. Perhaps seeds obtained
47 from the 4x - 2x crosses, if treated with some chemicals and sown in the soil could have
48 relatively high germination.

49 In order to increase germination, seeds are scarified by physical or chemical means to
50 permit imbibition and improve the rate of germination or shorten the time required for
51 germination [15]. Unfortunately, some pre-sowing treatments such as chipping of testa,
52 scorching and the application of temperature shocks are usually deleterious and often lethal
53 to *Musa* seeds [16]. Other methods used to overcome dormancy in *Musa* seeds include
54 treatment with different concentrations of potassium hydroxide, sulphuric acid and carbon
55 dioxide [17]. The use of potassium hydroxide has also been found to improve germination
56 and emergence of several other crop species [18]. This was demonstrated in oat (*Avena*
57 *fatua* L.) as dormancy was broken and germination was significantly enhanced. Other
58 studies have pointed out that seed germination and seedling vigour can be improved as a
59 result of various hydro priming protocols [19, 20, 21, 22, 23, 24].

60 Since germination of plantain and banana seeds in soil is abysmally low, hybrid seed
61 propagation in *Musa* is usually difficult [4, 7]. Therefore, the regeneration of hybrid seedlings
62 has relied more on *in vitro* culture of excised embryos [25], a technical and relatively more
63 expensive procedure than planting in soil. Improving *Musa* seed germination in the soil being
64 the natural medium of plant growth (designated as *in vivo*) could accelerate hybrid
65 development, selection, and evaluation of several cultivars in *Musa* breeding efforts that
66 meet the production and consumption requirements of target populations as advocated by
67 [26]. In addition enhanced seed germination would encourage seed storage in gene banks
68 [4] for germplasm preservation. Moreover, it would facilitate the production of large number
69 of segregating planting materials and decentralize hybrid distribution for research and
70 production under various agro-ecologies at a relatively low cost.

71 The main objective of this study was to investigate how to enhance germination of *Musa*
72 *acuminata* Calcutta 4 (AA genome) seeds when planted in soil (*in vivo*) and by embryo
73 culture technique (*in vitro*). Specifically:

- 74 I. whether varying storage durations will affect germination of seeds planted in soil (in
75 *vivo*) and embryo culture (*in vitro*) differently
- 76 II. determine how hydro priming protocols will affect germination of seeds planted in soil
77 (*in vivo*) and embryo culture (*in vitro*); and
- 78 III. find out how chemical priming with various chemicals will affect germination of seeds
79 planted in soil (*in vivo*) and embryo culture (*in vitro*);

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81 **2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY**

82 This study was carried out at the International Institute of Tropical Agriculture (IITA) High
83 Rainfall Station, Onne (4°51'N, 7° 03'E, 10m above sea level), in Rivers State, south-eastern
84 Nigeria. The rainfall pattern is monomodal, distributed over a 10month period from February

85 through December, with an annual average of 2400mm. Relative humidity remains high all
86 year round with mean values of 78% in February, increasing to 89% in the months of July
87 and September. The mean annual minimum and maximum temperatures are 25°C and 27°C,
88 respectively, while solar radiation / sunshine lasts an average of 4hours daily [27].

89 Seeds of the wild banana *M. acumunata*, Calcutta 4 (diploid AA) which is resistant to
90 black Sigatoka disease were used to evaluate the effect of different seed treatments on direct
91 seed germination in soil (*in vivo*) and on embryo culture (*in vitro*). Bunches were harvested
92 when the fruits of the proximal nodal cluster (first hand) had reached physiological maturity.
93 Harvested bunches were ripened with ethylene for four days, after which the seeds were
94 extracted mechanically, washed and air-dried. Seed pre-sowing treatments consisted of the
95 under listed protocols after which treated seeds were divided into two sets. One set was
96 sown directly in soil (*in vivo*) and the other set subjected to embryo culture technique (*in vitro*)
97 after embryo rescue. Each set of treatments was replicated 3 times.

98 **Treatment protocols**

- 99 1. Three storage protocols of seed in transparent air-tight plastic jars at ambient
100 temperature for 0 {sowing immediately on extraction), 2weeks and 4weeks after
101 extraction of seeds;
- 102 2. Three hydro priming protocols, i.e., soaking of seeds in water for 0 (no soaking in water
103 before sowing), 4days and 8days before sowing. Seeds were soaked in water with 2
104 drops of Tween 80 (Sorbitan), agitated initially and allowed to stand for 24 hrs, with
105 changes of solution (at 24 hr. intervals) for the different soaking durations.
- 106 3. Six chemical treatment protocols, with copper-oxychloride (0.052M), 25% sulphuric acid
107 (0.23M), silver nitrate (0.06M) plus streptomycin sulphate (0.0002M), hydrogen peroxide
108 (0.1M), potassium nitrate (0.01M) and water (control). Seeds were soaked in chemical
109 solution with 2 drops of Tween 80 (Sorbitan), agitated initially and allowed to stand for 24
110 hrs.

111 **Planting in soil – (*in vivo*)**

112 On completion of treatment protocols, seeds were immediately washed with tap water and
113 sown at a rate of 11 seeds per pot, in perforated plastic pots (16 cm x 13 cm x 4.9 cm), three-
114 quarters filled with soil (soil, dried palm fibre and dried poultry manure in a 7:3:1 ratio).
115 Watering of sown seeds was carried out as required. Germination was considered to have
116 occurred when the plumule emerged about 1cm above the soil level.

117 **Embryo culture – (*in vitro*)**

118 Treated seeds were subjected to *in vitro* culture [28]. Seeds were surface sterilized (with 70
119 % methylated spirit for 2minutes) and transferred to a 1% solution of silver nitrate plus Tween
120 80 for 20minutes and rinsed in sterilized distilled water. Embryos were excised from seeds

121 using forceps and a scalpel under a stereoscopic microscope in a laminar flow cabinet. The
122 excised embryos were inoculated in culture tubes, each containing 20ml of modified MS
123 (Murashige and Skoog) medium [29]. The medium was half the standard concentration of
124 MS, supplemented with 3% sucrose, 2mg 1-1 glycerine, 0.5mg 1-1 nicotinic acid, 0.5mg 1-1
125 pyridoxine, 0.4mg 1-1 thiamine and 20mg 1-1 ascorbic acid. Gelrite (Sigma, USA) was used
126 to solidify the medium. Cultures were incubated under continuous light at a temperature of
127 10⁰C and examined daily. Germination was recorded when shoots emerged to about 1cm
128 above the medium.

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130 **Data Collection and Statistical Analyses**

131 The number of germinated seeds in both *in vivo* and *in vitro* procedures was recorded weekly
132 until no further germination occurred. The experimental design was a completely randomised
133 design with treatments replicated three times. Analysis of variance was used to test
134 treatment effects. All data were analysed using the GLM procedure of Statistical Analyses
135 Software and any effects found to be significant have been tested at a significance level of
136 5% while means were compared using the LSD test at $P \geq 0.05$. Graphs of means with
137 associated standard errors were drawn to show treatment effects.

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139 **3. RESULTS AND DISCUSSION**

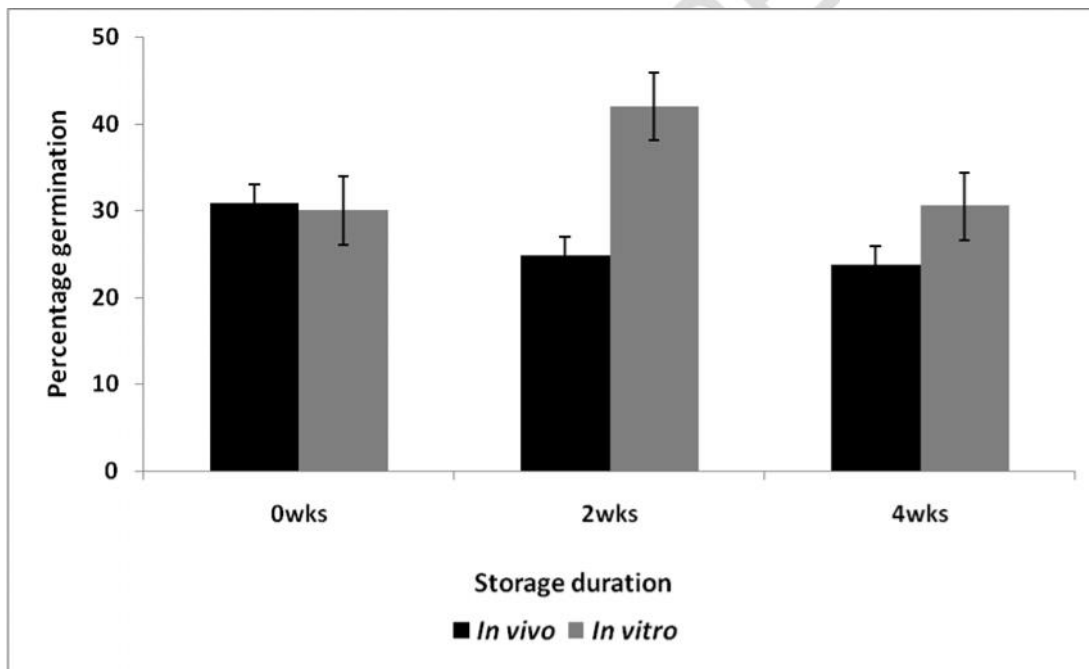
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141 **3.1. Seed Storage Duration**

142 Best germination of 31% (*in vivo*) was achieved when seeds were not stored. Seeds sown in
143 soil (*in vivo*) that were not stored had significantly higher germination compared to seeds
144 that were stored for 2weeks or 4weeks (Fig. 1). Germination declined by 20% and 23% after
145 2weeks and 4weeks of storage respectively. However, seeds stored for 2weeks did not
146 significantly differ in germination from those stored for 4weeks. For embryo culture (*in vitro*),
147 seeds stored for 2weeks had 42% germination; significantly higher germination than seeds
148 that were not stored or seeds that were stored for 4weeks (Fig. 1). Germination increased by
149 40% at 2weeks of storage but declined by 38% beyond 2weeks at 4weeks of storage. There
150 was no significant difference in germination between seeds that were not stored and those
151 that were stored for 4weeks. On the average germination in embryo culture (*in vitro*) was
152 29% higher than germination in soil (*in vivo*) and was significantly better. Planting
153 immediately after seed extraction was best for soil (*in vivo*) and planting at 2weeks of
154 storage was best for embryo culture (*in vitro*). This was significantly better and 36% higher
155 than the best soil (*in vivo*) germination.

156 Germination in soil (*in vivo*) was significantly higher in seeds that were not stored
157 than for seeds stored for 2 or 4weeks (Fig. 1). Storage of seeds of pearl millet (*Pennisetum*

158 *glaucum*, Slapf & Habbnd) for 10 days reduced soil germination, and declined further after
159 14 days of storage [30]. A major limitation of stored seeds may result from the seed coat.
160 The seed coat contains ferulic acid and polyphenolic compounds that affect soil germination
161 by restricting the embryo development [1]. Germination in soil (*in vivo*) was reported to be as
162 erratic as <5% - 23% over 20days [31] In this study, *in vivo* germination ranged from 24% -
163 31% for storage duration. For embryo culture (*in vitro*) germination was significantly higher in
164 seeds stored for 2weeks, and declined thereafter. Perhaps lower moisture content could
165 have played a role in the higher level of germination observed after 2weeks of storage and
166 the decline beyond 2weeks to 4weeks [31]. While storage for 2weeks increased *in vitro*
167 germination, it reduced the rate of *in vivo* germination, although the data suggests that the
168 seeds in both instances retained viability for as long as 4weeks. For best results, seeds to be
169 planted in soil (*in vivo*) should be planted as soon as they are excised from the fruits while
170 seeds for embryo culture (*in vitro*) should be stored for 2weeks before use.
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172 Fig. 1. Effect of storage duration on *in vivo* and *in vitro* germination of *Musa*
173 *acuminata* Calcutta 4
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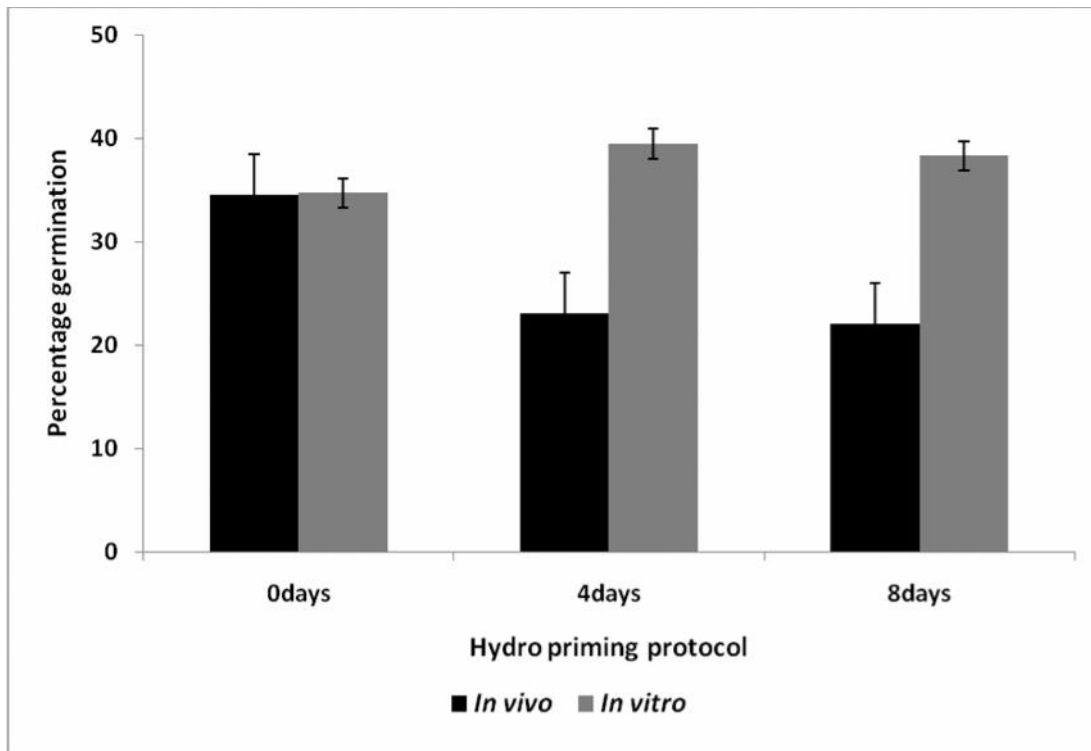
176 **3.2. Hydro Priming Protocol**

177 When seeds were sown in soil (*in vivo*), germination was significantly higher for seeds that
178 were not hydro primed than for seeds that were hydro primed for 4days or 8days (Fig. 2).
179 Germination declined by 33% and 38% in seeds hydro primed for 4 and 8days respectively.
180 Germination of seeds that were hydro primed for 4days did not significantly differ from those
181 that were hydro primed for 8days. In embryo culture (*in vitro*) germination of those not hydro

182 primed was significantly lower than those hydro primed for 4 and 8days (Fig. 2). Hydro
183 priming for 4days increased germination significantly by 60% compared to those without
184 hydro priming but reduced germination by 3% when hydro primed for 8days compared to
185 that hydro primed for 4days. There was no significant difference in germination between
186 seeds hydro primed for 4days and that hydro primed for 8days (Fig. 2). Comparing both
187 procedures, again on the average, germination in embryo culture (*in vitro*) was 29% higher
188 than in soil (*in vivo*) and was significantly better. Planting without hydro priming was best for
189 soil (*in vivo*). Planting after 4days hydro priming was best for embryo culture (*in vitro*)
190 resulting in a 15% higher germination than the best soil (*in vivo*) germination.

191 Seeds that were not hydro primed gave significantly higher *in vivo* germination
192 compared to seeds hydro primed for 4days and 8days (Fig. 2). Sowing of hydro primed
193 seeds in the soil led to decline in the rate of germination. It is likely that hydro priming
194 reduced the protection level provided by the seed coat under normal circumstances. Hence,
195 the seeds became exposed to microbial attack and other detrimental soil factors [31] or the
196 moisture content of the seeds could have exceeded the optimum required for germination
197 over the 4-8day period [31].

198 In embryo culture (*in vitro*) seeds hydro primed for 4 days gave significantly higher
199 germination than seeds hydro primed for 8days. Hydro priming for 5days was reported to
200 have increased *in vitro* germination in *M. balbisiana* more than hydro priming for 3 and 9days
201 [32]. They found germination after hydro priming for 5days was 94% (*in vitro*) within 7days
202 compared to 50% after 54days for greenhouse-sown seeds (*in vivo*). Similarly, in this study,
203 hydro priming only increased *in vitro* but not *in vivo* germination. Hydro priming is also
204 thought to increase free-radical scavenging enzyme activity, counteracting the effects of lipid
205 peroxidation and reducing leakage of metabolites [33, 34, 35]. It is likely that by the fourth
206 day of soaking, the embryos could have become metabolically active for rapid germination
207 under aseptic conditions. The reasons for the difficulty in achieving high germination of *Musa*
208 under natural conditions (*in vivo*) need to be further investigated.



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Fig. 2: Effect of hydro-priming on *in vivo* and *in vitro* germination of *Musa acuminata* Calcutta 4

3.3. Chemical Priming Protocol

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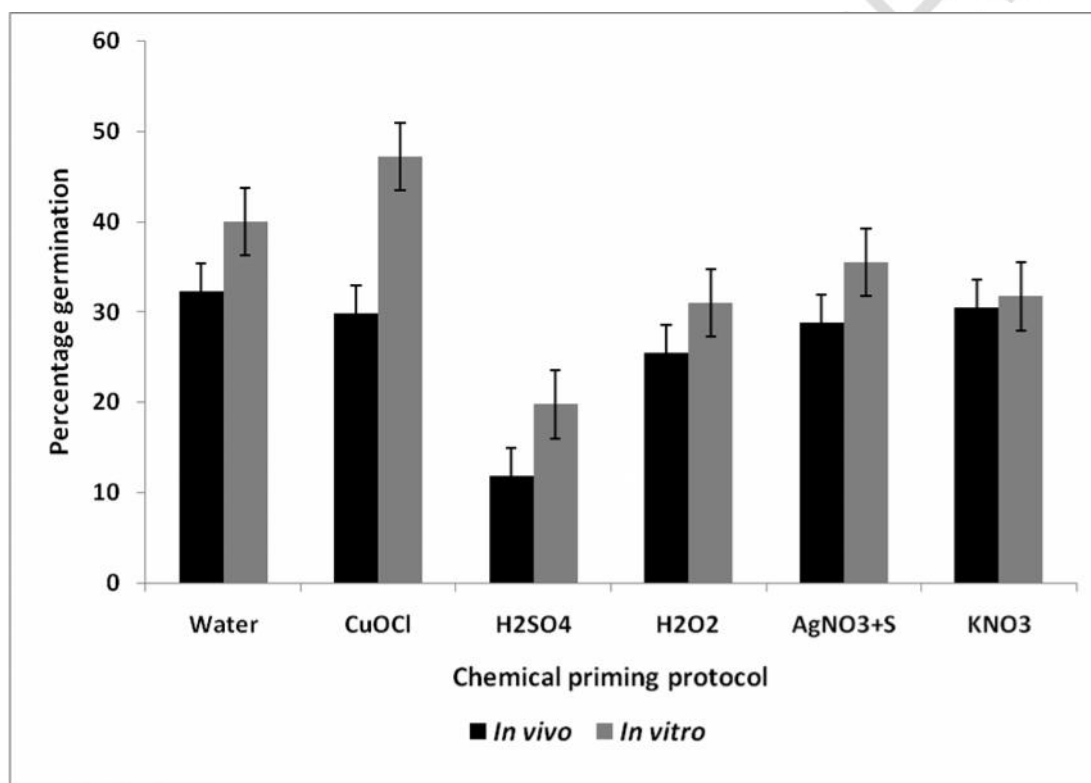
Germination of seeds primed with sulphuric acid was significantly lower than those of seeds primed with other chemicals or the control in both *in vivo* and *in vitro* procedures (Fig. 3). Other than this, for soil, (*in vivo*) there was no significant difference in germination of seeds primed with other chemicals and the control (water). In fact, all the chemicals reduced germination by 6% (KNO₃), 8% (CuOCl), 11% (AgNO₃ + Streptomycin), 21% (H₂O₂) and significantly by as much as 63% (H₂SO₄) compared to the control. However, in embryo culture (*in vitro*), copper oxychloride increased germination by 18% compared to the control achieving 47% germination. All other chemicals reduced germination by 11% (AgNO₃ + Streptomycin), 21% (KNO₃), 23% (H₂O₂) and significantly by 50% (H₂SO₄) compared to the control. Germination in all chemical priming treatments was lower for *in vivo* than for *in vitro* procedures. The best *in vitro* germination was with copper oxychloride priming which was significantly better and 46% higher than the best *in vivo* germination, the control -priming with water (32% germination).

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Chemical priming of seeds did not improve germination of *M. acuminata* Calcutta 4, although their efficacy has been reported in several other crop species [36]. This study has shown that sulphuric acid priming at the concentration used, significantly reduced both *in*

230 *vivo* and *in vitro* germinations (Fig. 3). Germination *in vivo* did not significantly differ between
231 chemically primed seeds and the control. This suggests that the chemical priming at the
232 concentrations used, did not improve *in vivo* germination. However, significantly higher
233 germination was reported with application of copper oxychloride in hybrid plantain seeds
234 when applied at low concentrations as a fungicide to soil [6] rather than as a seed primer.
235 This perhaps indicates that soil treatment rather than seed treatment could be an avenue for
236 further exploration. They also identified the average weather conditions such as air
237 temperature and sunshine at the time of seed treatment as a significant factor influencing the
238 germination of the seeds.

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241 **Fig. 3: Effect of chemical priming on *in vivo* and *in vitro* germination of *Musa***
242 ***acuminata* Calcutta 4**

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244 For *in vitro* germination, priming with copper oxychloride produced a significantly higher
245 germination than other chemicals implicating perhaps its anti-fungal properties relative to the
246 other chemicals in embryo culture.

247 Consistently higher germination was recorded with *in vitro* than *in vivo* procedures
248 irrespective of the treatments applied. Almost all studies conducted have reported the same
249 trend [32, 37, 38, 39].

250

251 **4. CONCLUSION**

252 Consistently higher germination was recorded with *in vitro* than *in vivo* procedures
253 irrespective of the treatments applied. Perhaps inherent factors in the seed coat and
254 possible interactions in soil may account for the poor germination exhibited *in vivo* and will
255 require further investigation. In this study, sowing seeds extracted immediately without hydro
256 priming was best for (*in vivo*), while for embryo culture (*in vitro*), storage for 2weeks and
257 hydro priming for 4 days or priming with copper oxychloride gave the best results

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260 **REFERENCES**

- 261 1. Graven P, De Koster CG, Boon JJ. Bouman F. Structure and macromolecular
262 composition of seed coat of the *Musaceae*. *Annals of Botany*. 1996. 77:105-122.
263 2. Cronquist A. An integrated system of classification of flowering plants. Columbia
264 University Press, New York. 1981.
265 3. Kiew R. Notes on the natural history of the Johore banana, *Musa gracilis* Holtum.
266 *Malayan Nature Journal*. 1987. 41:239-248.
267 4. Chin, HF Germination and storage of bananas seeds. In: frison, ea., horry jp. de waele
268 d. (eds) new frontiers in resistance breeding for nematode, fusarium and Sigatoka.
269 INIBAP Proceedings of workshop held in Kuala Lumpur, Malaysia. 1995. 218-227.
270 5. Owen EB. The storage of seeds for maintenance of viability. Commonwealth
271 Agricultural Bureaux. *Field Crops Bulletin*. 1956. 43:81.
272 6. Dumpe BD, Wokoma ECW. Factors influencing germination of hybrid plantain seeds
273 in soil. *Plant Foods for Human Nutrition* 2003. 58: 1–11,
274 7. Ortiz R, Vuylsteke D. Factors influencing seed set in triploid *Musa* spp. L. and
275 production of euploid hybrids. *Annals of Botany*. 1995. 75:151-155.
276 8. Simmonds NW. The germination of banana seeds. *Tropical Agriculture Trinidad*. 1952.
277 29:2-16.
278 9. Swennen R, Vuylsteke D. Breeding black Sigatoka resistant plantains with a wild
279 banana. *Tropical Agriculture Trinidad*. 1993. 70 (1):74-77.
280 10. Simmonds NW. Segregations in some diploid bananas. *Journal of Genetics*. 1953. 51:
281 458-469.
282 11. Vuylsteke D, Ortiz R. Plantain-derived diploid hybrids (TMP2x) with black Sigatoka
283 resistance. *American Journal of Horticultural Science*. 1995. 30:147-149.
284 12. Ravishankar KV, Sampangi-Ramaiah M.H, Ajitha R, Khadke GN, Chellama V.
285 Insights into *Musa balbisiana* and *Musa acuminata* species divergence and
286 development of genic microsatellites by transcriptomics approach. *Plant Gene* 2015.
287 4:78-82
288 13. Donnelly ED, Watson JE. McGuire, JA. Inheritance of hard seed in *Vicia*. *Journal of*
289 *Hereditary*. 1972. 63:361-365.
290 14. Lee JA. Inheritance of hard seed in cotton. *Crop Science*. 1975. 15:149-152.
291 15. Lopez JH, Aviles RB. The pre-treatment of seeds of four Chilean prosopis to improve
292 their germination response. *Seed Science and Technology*. 1988. 16:239-246.
293 16. Simmonds NW. The evolution of the bananas. Longmans, London. 1962.
294 17. Simmonds NW. Experiments on the germination of banana seeds. *Tropical Agriculture,*
295 *Trinidad*. 1959. 36 (4):259-273.
296 18. Gao YP, Zheng GH, Gusta LV. Potassium hydroxide improves seed germination and
297 emergence in five native plant species. *American Journal of Horticultural Science*.
298 1998. 32 (2):274-276
299 19. McDonald MB. Seed priming. In: black m, bewley jd. (eds.). *Seed technology and its*
300 *biological basis*. Sheffield Academic Press, England, 2000. 287–325.

- 301 20. Halmer P. Methods to improve seed performance in the field. In: benech-arnold rl,
302 sanchez r. (eds.). Handbook of seed physiology. Food Product Press, New York.
303 2004. 125–156
- 304 21. Ghassemi-Golezani K, Sheikhzadeh-Mosaddegh P, Valizadeh M. Effects of hydro-
305 priming duration and limited irrigation on field performance of chickpea. Res. J. Seed
306 Sci. 2008. 1:34–40
- 307 22. Ghassemi-Golezani K, Chadordooz-Jeddi A, Nasrullahzadeh S, Moghaddam M.
308 Effects of hydro-priming duration on seedling vigor and grain yield of pinto bean
309 (*Phaseolus vulgaris* L.) cultivars. Not. Bot. Hor. Agro. ClujNap. 2010. 38:109–113.
- 310 23. Ghassemi-Golezani K., Hosseinzadeh-Mahootchy A, Zehtab-Salmasi S. Tourchi M.
311 Improving field performance of aged chickpea seeds by hydro-priming under water
312 stress. Int. J. Plant Animal Environ. Sci. 2012. 2:168–176.
- 313 24. Ghassemi-Golezani K, Hosseinzadeh-Mahootchy A. Influence of hydro-priming on
314 reserve utilization of differentially aged chickpea seeds. Seed Technology, 2013.
315 135: (1):117-124
- 316 25. Vuylsteke D, Swennen R, De Langhe E. Tissue culture technology for the improvement
317 of African plantains. In: fullerton ra, stover rh. (eds.). Sigatoka leaf spot diseases of
318 bananas INIBAP Proceedings, San José, Costa Rica. 1990. 316-337.
- 319 26. Coffman, WR. Smith ME. Role of public, industry and international research centre
320 breeding programs in developing germplasm for sustainable agriculture. In: sleeper,
321 da, barker, tc. bramel-cox, pj. ((eds) plant breeding and sustainable agriculture,
322 considerations for objectives and methods, CSSA Special Publication. 1991. 18:1-9.
- 323 27. Ortiz R, Austin PD, Vuylsteke D. IITA High Rainfall Station African humid forest.
324 American Journal of Horticultural Science. 1997. 32: 969-972.
- 325 28. Vuylsteke D, Swennen R, De Langhe E. Tissue culture technology for the improvement
326 of African plantains. In: fullerton ra, stover rh (eds.). Sigatoka leaf spot diseases of
327 bananas. INIBAP Proceedings, San José, Costa Rica. 1990. 16-337.
- 328 29. Vuylsteke D. Shoot-tip culture for the production, conservation and exchange of *Musa*
329 germplasm. Practical manuals for handling crop germplasm *in vitro* 2. International
330 Board for Plant Genetic Resources, Rome. 1989. 56p
- 331 30. Singh J, Govila OP, Agrawal PK. Preliminary results from a study of seed germinability
332 of pearl millet (*Pennisetum typhoides* L.) F₁ hybrids and their parents during accelerated
333 ageing test. Seed Science and Technology. 1988. 16:685-692
- 334 31. Vineesh PS, Skaria R, Mukunthakumar S, Padmesh P, Decruse SW. Seed germination
335 and cryostorage of *Musa acuminata* subsp. *Burmannica* from Western Ghats. South
336 African Journal of Botany. 2015. 100:158–163
- 337 32. Afele JC, De Langhe E. Increasing *in vitro* germination of *Musa balbisiana* seed. Plant
338 Cell, Tissue and Organ Culture. Kluwer Academic Publishers, Netherlands. 1991. 27:
339 33-36.
- 340 33. McDonald MB. Seed deterioration: physiology, repair and assessment. Seed Sci.
341 Technol. 1999. 27: 177–237
- 342 34. Hsu CC, Chen CL, Chen JJ, Sung JM. Accelerated aging-enhanced lipid peroxidation in
343 bitter melon seeds and effects of priming and hot water soaking treatments. Sci. Hortic.
344 2003. 98: 201–212.
- 345 35. Wang HY, Chen CL, Sung JM. Both warm water soaking and solid priming treatments
346 enhance anti-oxidation of bitter melon seeds germinated at sub-optimal temperature.
347 Seed Sci. Technol. 2003. 31 (1): 47–56. DOI: <https://doi.org/10.15258/sst.2003.31.1.06>
- 348 36. Copeland LO, McDonald MB. Principles of seed science and technology. 3rd edition.
349 Macmillan, New York. 1995. 409p.
- 350 37. Chin HF. Germination and storage of banana seeds. In: Frison AE, Horry JP, De Waele
351 D. (eds.). New Frontiers in Resistance Breeding for Nematodes, Fusarium and
352 Sigatoka. INIBAP, Montpellier, France. 1996. 218-227.

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354
355
356
357
38. Uma S, Lakshmi S, Saraswathi MS, Akbar A, Mustaffa MM. Embryo rescue and plant regeneration in banana (*Musa* spp.). *Plant Cell Tissue Organ Cult.* 2011. 105–111.
 39. Uma S, Lakshmi S, Saraswathi MS, Akbar A, Mustaffa MM. Plant regeneration through somatic embryogenesis from immature and mature zygotic embryos of *Musa acuminata* ssp. *burmannica*. *In Vitro Cell Dev. Biol. Plant.* 2012. 48, 539–545.

UNDER REER REVIEW