Original Research Article

Effects of ~~s~~Storage and ~~p~~Priming on ~~s~~Seed ~~g~~Germination in ~~s~~Soil and ~~e~~Embryo ~~c~~Culture of *Musa acumunata* Calcutta 4

ABSTRACT:

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

Study ~~d~~Design: The experimental design was a completely randomized with three replicates~~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~~three storage protocols, three~~3~~ hydro priming and ~~6~~six 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 two~~2weeks~~ and 4four weeks of storage respectively. For embryo culture, seeds stored for ~~2~~two weeks had significantly higher germination (40%) than seeds that were not stored or seeds stored for 4four weeks (38%). Germination *in vivo* was significantly higher for seeds that were not hydro primed than for seeds hydro primed for four~~4days~~ or eight~~8~~ days. Germination declined by 33~~%~~ and 38% in seeds hydro primed for four4 and ~~8~~eight days respectively. Hydro priming for embryo culture for 4four days 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 ~~C~~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 acuminate~~,~~ ~~H~~hydro-priming~~,~~ chemical-priming~~,~~ in vivo~~,~~ in vitro~~.]~~

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 [1]. The structure of the *Musa* seed is complex, hence making germination very difficult [1,3, 4~~,1~~]. It was found that seed viability was also affected by moisture content, oxygen and temperature [5]. The presence of a semi-permeable inner membranous seed coat restricts

the movement of moisture and oxygen into the embryo. In addition, while a hard seed coat provides effective protection during maturation, dispersal and dormancy, it hampers germination because the embryo requires extra energy to rupture the seed coat.

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

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

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

In order to increase germination, seeds are scarified by physical or chemical means to permit imbibition and improve the rate of germination or shorten the time required for germination [15]. Unfortunately, some pre-sowing treatments such as chipping of testa, scorching and the application of temperature shocks are usually deleterious and often lethal to *Musa* seeds [16]. Other methods used to overcome dormancy in *Musa* seeds include

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treatment with different concentrations of potassium hydroxide, sulphuric acid and carbon dioxide [17]. The use of potassium hydroxide has also been found to improve germination and emergence of several other crop species [18]. This was demonstrated in oat (*Avena fatua* L.) as dormancy was broken and germination was significantly enhanced. Other studies have pointed out that seed germination and seedling vigour can be improved as a result of various hydro priming protocols [19-, 20, 21, 22, 23, 24].

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

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

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

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 Experimental Site and Planting Material

This study was carried out at the International Institute of Tropical Agriculture (IITA) High Rainfall Station, Onne (4°51'N, 7° 03'E, 10 m above sea level), in Rivers State, south-eastern Nigeria. The rainfall pattern is monomodal, distributed over a 10 month period from February through December, with an annual average of 2400 mm. Relative humidity remains high all year round with mean values of 78% in February, increasing to 89% in the months of July

and September. The mean annual minimum and maximum temperatures are 25^{0}~~C~~ and 27^{0}C, respectively, while solar radiation ~~/~~ sunshine lasts an average of 4four hours daily [27].

Seeds of the wild banana *M. acumunata,* Calcutta 4 (diploid AA) which is resistant to black Sigatoka disease were used to evaluate the effect of different seed treatments on direct seed germination in soil (*in vivo*) and on embryo culture (*in vitro*). Bunches were harvested when the fruits of the proximal nodal cluster (first hand) had reached physiological maturity. Harvested bunches were ripened with ethylene for four days, after which the seeds were extracted mechanically, washed and air-dried.

2.2 Treatments and Experimental Details

~~-~~Seed pre-sowing treatments consisted of three storage protocols, three hydro priming and six chemical treatment protocols~~Seed pre-sowing treatments consisted of the under listed protocols~~, after which treated seeds were divided into two sets. One set was sown directly in soil (*in vivo*) and the other set subjected to embryo culture technique (*in vitro*) after embryo rescue. Each set of treatments was replicated 3 times, in a completely randomized.

~~**Treatment protocols**~~

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

2.3 Crop Conduction

2.3.1 Planting in ~~s~~Soil – (*in vivo*)

On completion of treatment protocols, seeds were immediately washed with tap water and sown at a rate of 11 seeds per pot, in perforated plastic pots (16 cm x 13 cm x 4.9 cm), three-quarters filled with soil (soil, dried palm fibre and dried poultry manure in a 7:3:1 ratio).

Watering of sown seeds was carried out as required. Germination was considered to have occurred when the plumule emerged about 1cm above the soil level.

2.3.1 Embryo ~~c~~Culture – (*in vitro*)

Treated seeds were subjected to *in vitro* culture [28]. Seeds were surface sterilized (with 70 % methylated spirit for ~~2~~two minutes) and transferred to a 1% solution of silver nitrate plus Tween 80 for 20 minutes and rinsed in sterilized distilled water. Embryos were excised from seeds using forceps and a scalpel under a stereoscopic microscope in a laminar flow cabinet. The excised embryos were inoculated in culture tubes, each containing 20 ml of modified MS (Murashige and Skoog) medium [29]. The medium was half the standard concentration of MS, supplemented with 3% sucrose, 2 mg 1-1 glycerine, 0.5 mg 1-1 nicotinic acid, 0.5 mg 1-1 pyridoxine, 0.4 mg 1-1 thiamine and 20 mg 1-1 ascorbic acid. Gelrite (Sigma, USA) was used to solidify the medium. Cultures were incubated under continuous light at a temperature of 10^0C and examined daily. Germination was recorded when shoots emerged to about 1 cm above the medium.

Data Collection and Statistical Analyses

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

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3. RESULTS AND DISCUSSION

3.1. Seed Storage Duration

Best germination of 31% (*in vivo*) was achieved when seeds were not stored. Seeds sown in soil (*in vivo*) that were not stored had significantly higher germination compared to seeds that were stored for ~~2~~two~~weeks~~ or ~~4~~four weeks (Figure~~.~~ 1). Germination declined by 20~~%~~ and 23% after two~~2weeks~~ and ~~4~~four weeks of storage respectively. However, seeds stored for ~~2~~two weeks did not significantly differ in germination from those stored for ~~4~~four weeks. For embryo culture (*in vitro)*, seeds stored for ~~2~~two weeks had 42% germination; significantly higher germination than seeds that were not stored or seeds that were stored for ~~4~~four weeks (Figure~~.~~ 1). Germination increased by 40% at ~~2~~two weeks of storage but declined by 38% beyond ~~2~~two~~weeks~~ at ~~4~~four weeks of storage. There was no significant difference in

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germination between seeds that were not stored and those that were stored for 4four weeks. On the average germination in embryo culture *(in vitro)* was 29% higher than germination in soil (*in vivo)* and was significantly better. Planting immediately after seed extraction was best for soil *(in vivo*) and planting at 2two weeks of storage was best for embryo culture (*in vitro*). This was significantly better and 36% higher than the best soil (*in vivo*) germination.

Germination in soil (*in vivo*) was significantly higher in seeds that were not stored than for seeds stored for 2two or 4four weeks (Figure 1). Storage of seeds of pearl millet (*Pennisetum glaucum*, Slapf & Habbnd) for 10 days reduced soil germination, and declined further after 14 days of storage [30]. A major limitation of stored seeds may result from the seed coat. The seed coat contains ferulic acid and polyphenolic compounds that affect soil germination by restricting the embryo development [1]. Germination in soil (*in vivo*) was reported to be as erratic as <5% 23% over 20 days [31] In this study, *in vivo* germination ranged from 24% 31% for storage duration. For embryo culture (*in vitro*) germination was significantly higher in seeds stored for 2two weeks, and declined thereafter. Perhaps lower moisture content could have played a role in the higher level of germination observed after 2two weeks of storage and the decline beyond 2twoweeks to 4four weeks [31]. While storage for 2two weeks increased *in vitro* germination, it reduced the rate of *in vivo* germination, although the data suggests that the seeds in both instances retained viability for as long as 4four weeks. For best results, seeds to be planted in soil (*in vivo*) should be planted as soon as they are excised from the fruits while seeds for embryo culture (*in vitro*) should be stored for 2two weeks before use.

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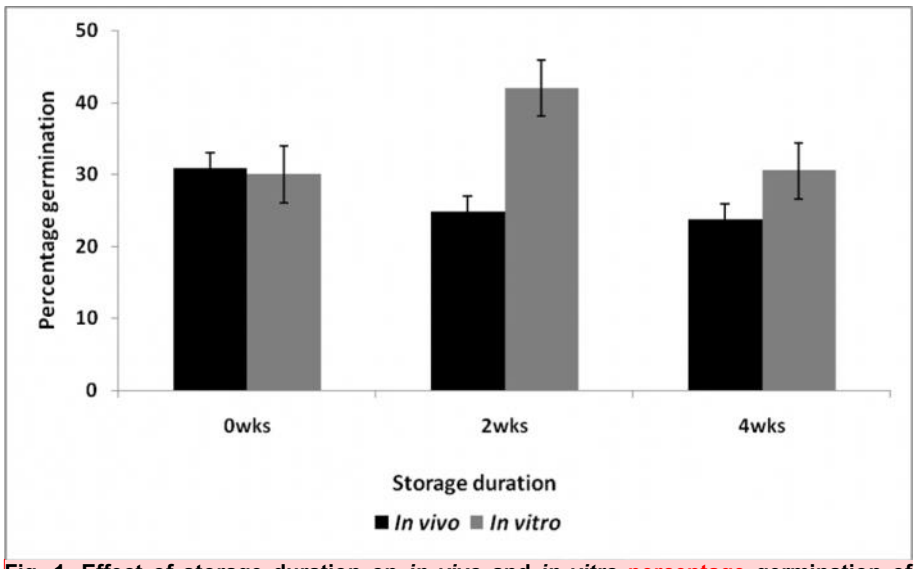

Fig. 1. Effect of storage duration on *in vivo* and *in vitro* percentage germination of *Musa acuminata* Calcutta 4

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Storage duration (Weeks).

On axis 'Y': Percentage germination (%)

Put the averages with their respective letters on the vertical bars.

3.2. Hydro Priming Protocol

When seeds were sown in soil (*in vivo*), germination was significantly higher for seeds that were not hydro primed than for seeds that were hydro primed for 4~~days~~four or 8eight days (Figure~~.~~ 2). Germination declined by 33~~%~~ and 38% in seeds hydro primed for 4four and 8eight days respectively. Germination of seeds that were hydro primed for 4four days did not significantly differ from those that were hydro primed for 8eight days. In embryo culture (*in vitro*) germination of those not hydro primed was significantly lower than those hydro primed for 4four and 8eight days (Figure~~.~~ 2). Hydro priming for 4days increased germination significantly by 60% compared to those without hydro priming but reduced germination by 3% when hydro primed for 8eight days compared to that hydro primed for 4four days. There was no significant difference in germination between seeds hydro primed for 4four days and that hydro primed for 8eight days (Figure~~.~~ 2). Comparing both procedures, again on the average, germination in embryo culture *(in vitro)* was 29% higher than in soil (*in vivo)* and was significantly better. Planting without hydro priming was best for soil *(in vivo*). Planting after 4four days hydro priming was best for embryo culture (*in vitro*) resulting in a 15% higher germination than the best soil (*in vivo*) germination.

Seeds that were not hydro primed gave significantly higher *in vivo* germination compared to seeds hydro primed for 4four days and 8eight days (Figure~~.~~ 2). Sowing of hydro primed seeds in the soil led to decline in the rate of germination. It is likely that hydro priming

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reduced the protection level provided by the seed coat under normal circumstances. Hence, the seeds became exposed to microbial attack and other detrimental soil factors [31] or the moisture content of the seeds could have exceeded the optimum required for germination over the 4-8 days period [31].

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

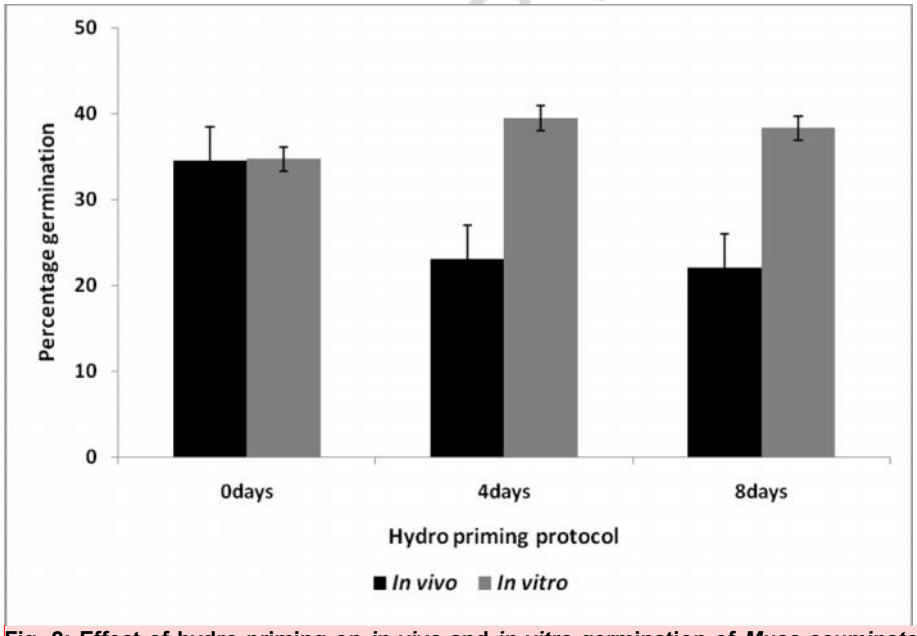

Fig. 2: Effect of hydro-priming on *in vivo* and *in vitro* germination of *Musa acuminata* Calcutta 4

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3.3. Chemical Priming Protocol

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 (Figure 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% (KNO_3), 8% (CuOCl), 11% ($AgNO_3$ + Streptomycin), 21% (H_2O_2) and significantly by as much as 63% (H_2SO_4) 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% ($AgNO_3$ + Streptomycin), 21% (KNO_3), 23% (H_2O_2) and significantly by 50% (H_2SO_4) 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).

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

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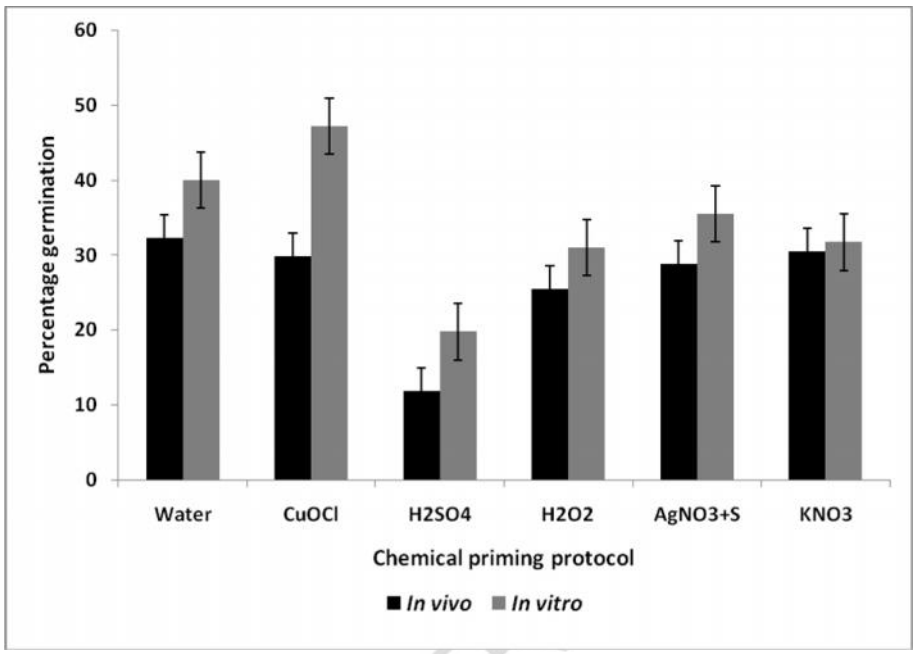

Fig. 3: Effect of chemical priming on *in vivo* and *in vitro* germination of *Musa acuminata* Calcutta 4

Comment [Acer4]: See the suggestions in Figure 1.

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

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

4. CONCLUSION

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Consistently 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. In this study, sowing seeds extracted immediately without hydro priming was best for (*in vivo*), while for embryo culture (*in vitro*), storage for ~~2~~two weeks and hydro priming for ~~4~~four days or priming with copper oxychloride gave the best results.

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