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## Effects of whole seed priming on the *in vitro* germination of hybrid banana embryos (*Musa* spp.)

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### Summary

Trials were conducted to determine the effects of hydro- and hormonal priming on *in vitro* germination and regeneration of hybrid banana embryos. Fully-mature seeds of Pisang Jajee × *M. acuminata* ssp. *burmannicoides* were hydroprimed (for 1, 2, 3, 4 or 5 days) or hormone primed (gibberellic acid (GA<sub>3</sub>), 6-benzylaminopurine (BAP), indole-3-acetic acid (IAA)) at different concentrations (2, 5, 10, 15 and 20 ppm) for three days prior to *in vitro* culture. The embryos were extracted and initiated on basal MS medium devoid of growth regulators. Hydropriming for three days resulted in 40.8% germination compared with 20.8% germination of non-primed seeds. Priming in GA<sub>3</sub> at 10 ppm for three days doubled the germination (82.4%) and also enhanced growth parameters. Early shoot emergence was recorded with BAP priming (5 ppm), while priming with IAA (10 ppm) enhanced root length and root number.

### Introduction

Banana and plantains are important food crops. They provide nearly 25% of the daily calorie intake for around 70 million people across Africa (Tenkouano *et al.*, 2011). More than 65% of the total production (127.9 million tonnes) is grown as high value crops across Asia and Latin American countries.

Despite the dual status of banana, as both a subsistence and high value crop, its production and productivity is seriously threatened by a number of pests and diseases. Conventional breeding remains the preferred method to develop hybrids with resistance to biotic stresses. However, success depends on the number of fertile complete seeds produced (with a well-developed seed coat, endosperm and healthy viable embryos; Chin, 1996). In banana and plantain, the number of complete fertile seeds is severely limited by male and/or female sterility, genetic incompatibility, limited pollen vigour and/or failure of the pollen tube to reach the ovaries (Ortiz and Vuylsteke 1995; Ssebuliba *et al.*, 2006a)

Baskin and Baskin (2004) classified seed dormancy into physical (hard seed coat), physiological (inhibition by or imbalance of growth substances), morphological (absence or improper growth of seed coat, embryo and endosperm) and their combinations. McGahan (1961) described the failure of *Musa* seed germination as due to lack of water imbibition or exchange of gases. In *Musa* species, physical dormancy is caused by a

thick cuticle layer on the inner side of the seed integument (Graven *et al.*, 1996), but this varies with varieties (Chin, 1996). Morphological dormancy can be overcome by extracting the seeds at the right maturity (Uma *et al.*, 2011) while physical dormancy has been overcome by using stratification and scarification methods such as soaking in water and treatment with acid (Wattanachaiyingcharoen, 1990). Unfavourable conditions such as microorganisms that are present in the soil also affects the germination of *Musa* seeds (Stotzky *et al.*, 1962).

Due to low seed germination, many have opted for embryo rescue techniques especially for breeding materials (Ssebuliba *et al.*, 2006b). However, very little information is available on the dormancy and germination process in *Musa* seeds under natural and artificial conditions (*in vivo* and *in vitro*). These factors have necessitated *in vitro* embryo culture to increase the rate of germination and regeneration on appropriate growth medium (Santos *et al.*, 2001). The success of embryo culture has been reported to depend mainly on two factors, embryo maturity and culture medium. Mature hybrid embryos of *Musa* exhibited better germination in MS (Murashige and Skoog, 1962) basal medium compared with medium supplemented with plant growth regulators (Johri and Rao, 1984; Uma *et al.*, 2011). Other reports on *Musa* indicated that germination was enhanced by soaking seeds in water (Afele and De Langhe, 1991) and growth hormones (Chauhan *et al.*, 2009).

The present study was undertaken to determine the effects of hydro- and hormonal priming of seeds on *in vitro* germination and regeneration of hybrid banana embryos of Pisang Jajee (AA) × *Musa acuminata* ssp. *burmannicoides* (AA) (Calcutta 4).

## Materials and methods

### *Production of hybrid seeds*

Two non-parthenocarpic AA diploids, Pisang Jajee and *M. acuminata* ssp. *burmannicoides* maintained in the field gene-bank of NRC Banana, Trichy, Tamil Nadu, India, were crossed. Male flowers of *Musa* which were due to open the following day were forcibly opened and pollen from the anthers extracted using a dissection needle. Female flowers of Pisang Jajee were pollinated early in the morning when stigma receptivity was maximal. The crossed bunches were harvested at their full maturity and allowed to ripen at room temperature for 3-5 days. Seeds were extracted from fully ripened fruits and exposed to a water gravity test, the floats (partially developed seeds) were discarded and seeds that did not float (fully developed seeds) were used for the experiment.

### *Seed morphology and structure*

Fifty seeds were weighed, cut longitudinally to separate the individual components (embryo, endosperm and seed coat) and individual components weighed separately. The contribution of each component tissue as a proportion of the total weight of the seed was calculated (Orozco *et al.*, 2007).

### *Water uptake studies*

Freshly harvested seeds were washed thoroughly, sterilised using 5% NaOCl followed by

0.1% HgCl<sub>2</sub> for 15 minutes and rinsed in sterile water. A set of ten seeds were maintained in five different flasks with double-distilled water (DDW). Initial seed weight and seed weight after 1-5 days of soaking were recorded. Excess water was removed using filter paper before weighing. Percent weight gained by the seeds was calculated as a proportion of initial weight. Initial weight and final weight of seeds were recorded before and after priming treatments respectively.

#### *Hydro- and hormone priming*

For hydropriming, after pre-sterilisation, seeds were soaked in sterile distilled water on an orbital shaker at 80 rpm at 26°C for 1, 2, 3, 4 or 5 days. This was followed by excision of the embryo in a laminar air flow chamber for embryo culture. For hormone priming, after pre-sterilisation, seeds were treated for three days in 2, 5, 10, 15 or 20 ppm giberellic acid (GA3), 6-benzylaminopurine (BAP) or indole-3-acetic acid (IAA), followed by *in vitro* culture of the embryos.

#### *Culture conditions*

The seeds were cut open under a stereo microscope using a scalpel blade and the mushroom-shaped embryos transferred to a sterile 90 mm-diameter petri dish. The embryos were then inoculated onto culture medium slants with the haustorium embedded in the medium and the meristematic region exposed (Asif *et al.*, 2001). The culture medium was basal MS (Murashige and Skoog, 1962) supplemented with 4% sucrose and 0.2% Phytigel (pH 5.8) and autoclaved at 121°C for 20 minutes. The cultures were initially incubated in complete darkness for 15 days in a culture room maintained at 28 ± 2°C and 80% relative humidity. Later, the cultures were shifted to culture racks with a 16/8 hours light/dark cycle with light intensity of 1600 lux. Germination, days taken for germination (i.e days taken for the colour change of embryo from white to yellow) and days for shoot and root emergence were recorded. Shoot and root length and root number were recorded after 15 days of dark incubation.

#### *Experimental design and data analysis*

The experiments were laid out in a completely randomised design with five replicates per treatment and 15 seeds per replication. Percentage germination data were arc-sine transformed and ANOVA carried out using the statistical software AGRES. Means were compared using LSD.

## **Results**

#### *Seed morphology and structure*

Hybrid seeds of Pisang Jajee and *M. acuminata* ssp. *burmannicoides* were 3-5 mm in diameter, regular or irregularly globose in shape with a flat bottom. Seeds were blackish-brown with a warty and hard seed coat (figure 1a). The embryo was dull-white with a prominent haustorium and stalk (figure 1b). Average seed weight was 49.0 mg and individual seed weight ranged from 46.8 to 51.1 mg. Among the three components, the

seed coat constituted 74.3% of total seed weight, the whitish powdery endosperm 24.1% and the embryo 1.6%. The stalk increased in length during soaking to fill the gap between the embryo and the chalazal opening along the nucellar pad (figure 1c-f) within three days of hydropriming.

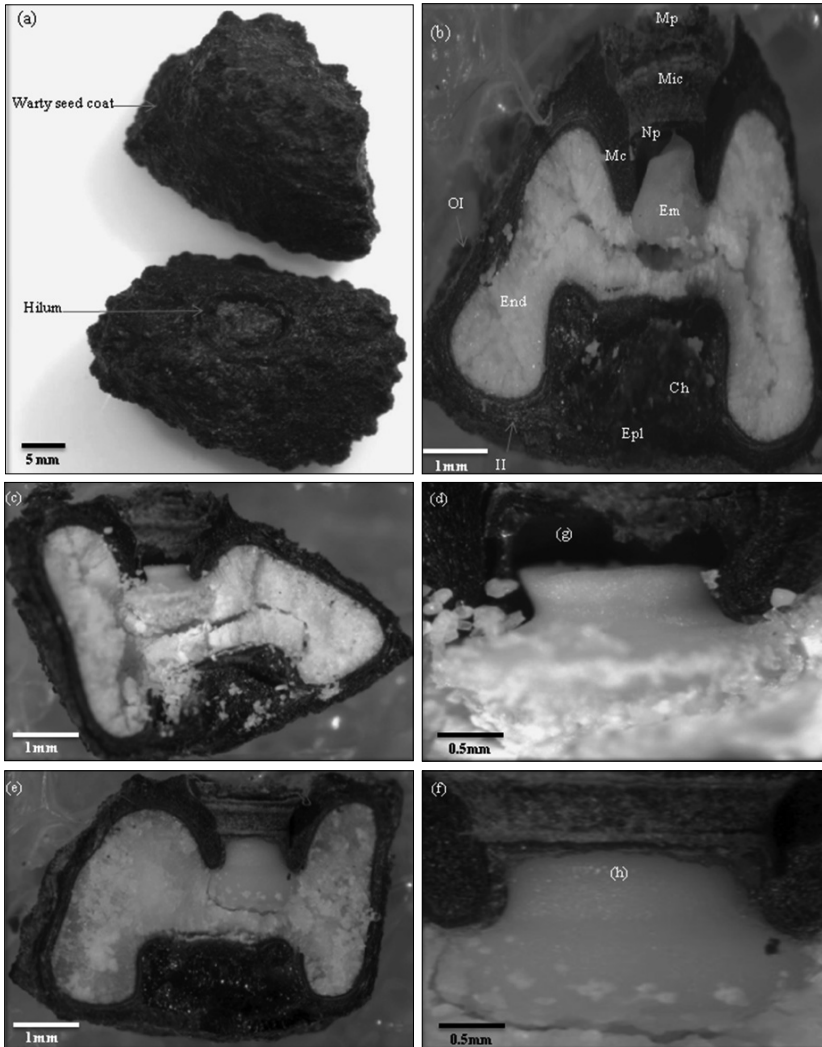


Figure 1. Structure of Pisang Jajee  $\times$  *M. acuminata* ssp. *burmannicoides* seeds: (a) globose-shaped seeds; (b) longitudinal section of the seed. Longitudinal section of the seed and their dimensions before and after hydropriming; (c) and (d) physical state of seed prior to hydropriming; (e) and (f) physical state of seed after hydropriming; (g) visible gap between shrunken embryo and micropylar cap before hydropriming; (h) swollen embryo filling the gap during the imbibition process (day-3). Ch = chalazal mass; Em = embryo; End = endosperm; Epi = persistent epidermal fragment; II = inner integument; Mc = micropylar collar; Mp = micropylar plug; Np = nucellar pad; OI = outer integument; S = stalk region; and H = haustorium.

*Water uptake by seeds*

There was a vigorous uptake of water during the first day of hydropriming, followed by further uptake until day-3 after which it appeared to gradually decline (figure 2). Measurement of the bisected seed before and after hydropriming showed that there was a 20% increase in diameter (948 to 1142  $\mu\text{m}$ ) during priming. The stalk increased in length from 407 to 492  $\mu\text{m}$  and the haustorium increased in diameter from 960 to 1246  $\mu\text{m}$ .

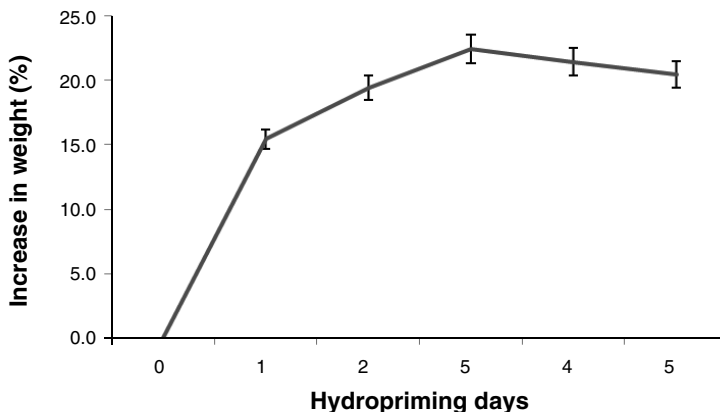
*Effect of hydropriming*

Figure 2. Effect of hydropriming on fresh weight gain of seeds of Pisang Jajee  $\times$  *M. acuminata* ssp. *burmannicoides*. Bars indicate standard deviation.

Upon culture of the embryo after hydropriming, the embryo changed from white to yellow, the first visible sign of germination (figure 3b), which began on day-5 of incubation in darkness. Germination was followed by swelling of embryos and development of roots and shoots (figure 3c-g). Compared with control, hydropriming had a highly significant effect ( $P < 0.05$ ) on percentage germination and days taken for germination, shoot emergence, root initiation, number of roots and root length (table 1).

Germination increased with priming duration upto day-3 when it reached a maximum (40.8%). The lowest germination (20.8%) was recorded for unsoaked seeds (control). The days taken for germination varied with the priming duration, earliest germination was observed following three days hydropriming (5 days) followed by one and two days (5.4 and 5.6 days, respectively). Control seeds recorded the maximum time for germination (8.3 days). Earliest shoot (7 days) and root emergence (6 days) were observed following three days of hydropriming followed by two days hydropriming (7.2 and 7.1 days respectively), whereas it was delayed for control seeds. The longest shoot (4.5 mm) and root lengths (4.8 mm) were produced following three days hydropriming and they were shortest for the control (3.2 mm and 2.1 mm respectively). The maximum numbers of roots were also produced from seeds hydroprimed for three days (4.8), while minimum of 2.8 roots were produced from control seeds.

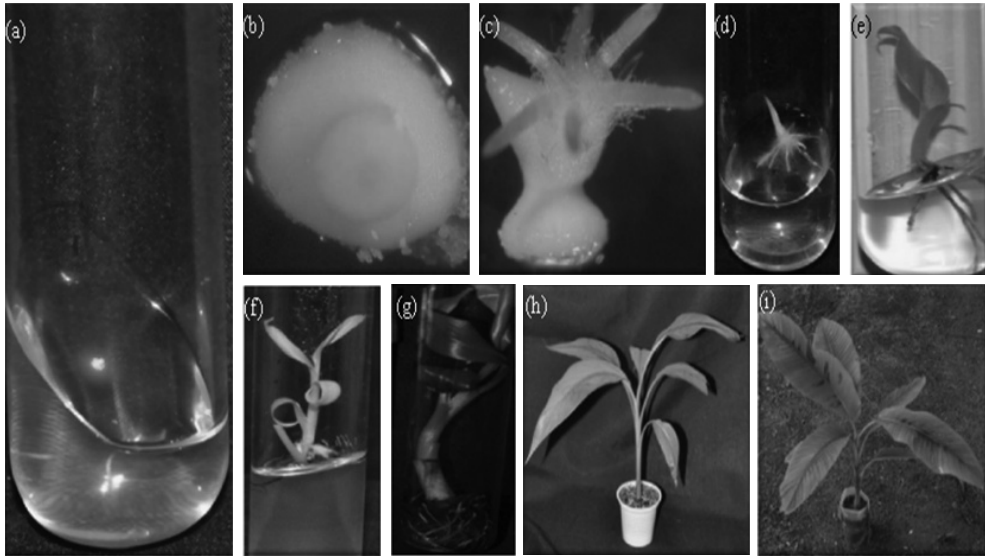


Figure 3. Stages of embryo development: (a) haustorium embedded in the medium with stalk region exposed; (b) initial response; (c) emergence of shoot and root; (d) after fifteen days of culture; (e) after 45 days of culture; (f) after 75 days of culture; (g) plant in rooting medium; (h) primary hardened plant; (i) secondary hardened plant ready for field planting.

Table 1. Effect of hydropriming on growth and development of embryos of Pisang Jajee  $\times$  *M. acuminata* ssp. *burmannicoides*.

Hydropriming period (days)	Germination (%)	Days taken for germination	Days taken for shoot emergence	Shoot length (mm)	Days taken for root emergence	Root length (mm)	No. of roots
1	36.4 <sup>c</sup>	5.4 <sup>a</sup>	7.4 <sup>a</sup>	4.1 <sup>a</sup>	8.2 <sup>c</sup>	3.8 <sup>bc</sup>	4.4 <sup>a</sup>
2	38.6 <sup>b</sup>	5.6 <sup>ab</sup>	7.2 <sup>a</sup>	4.3 <sup>a</sup>	7.1 <sup>b</sup>	4.4 <sup>ab</sup>	4.6 <sup>a</sup>
3	40.8 <sup>a</sup>	5.0 <sup>a</sup>	7.0 <sup>a</sup>	4.5 <sup>a</sup>	6.0 <sup>a</sup>	4.8 <sup>a</sup>	4.8 <sup>a</sup>
4	35.5 <sup>c</sup>	6.2 <sup>b</sup>	8.4 <sup>b</sup>	4.2 <sup>a</sup>	8.3 <sup>c</sup>	3.1 <sup>c</sup>	4.6 <sup>a</sup>
5	33.5 <sup>d</sup>	6.1 <sup>b</sup>	8.6 <sup>b</sup>	4.2 <sup>a</sup>	8.2 <sup>c</sup>	4.4 <sup>ab</sup>	4.5 <sup>a</sup>
Control	20.8 <sup>e</sup>	8.3 <sup>c</sup>	10.8 <sup>c</sup>	3.2 <sup>b</sup>	9.8 <sup>d</sup>	2.1 <sup>d</sup>	2.8 <sup>b</sup>
SE	0.50	0.39	0.36	0.53	0.45	0.55	0.46
CD ( $P = 0.05$ )	1.01	0.79	0.72	1.07	0.90	1.11	0.93
Level of significance	**	**	**	*	**	**	**

SE, standard error; CD, critical difference; \*\* highly significant ( $P < 0.01$ ); \* significant ( $P < 0.05$ ); NS, non-significant ( $P > 0.05$ ). Germination percentage values were arc-sine transformed prior to ANOVA.

*Effect of hormone priming*

Compared with hydropriming, priming with GA<sub>3</sub> had a highly significant effect on all parameters (table 2). The germination percentage was maximum for seeds treated with 10 ppm GA<sub>3</sub> (82.4%), followed by 15 and 5 ppm (81.6 and 80.6%, respectively). The minimum germination of 40.8% was observed for control seeds (hydroprimed for three days). Early germination was recorded in seeds treated with 10 ppm GA<sub>3</sub> (3 days), followed by 2 and 5 ppm (3.4 and 3.5 days, respectively) but with no significant difference between them. Earliest shoot emergence and root emergence were both observed for seeds treated with 10 ppm GA<sub>3</sub> (5 days). Seeds treated with 10 ppm GA<sub>3</sub> resulted in the longest roots (7.9 mm). Maximum number of roots were produced in 10 ppm GA<sub>3</sub>-treated seeds (6.8) followed by 15 ppm (6.6) (not significantly different). Minimum number of roots was recorded for seeds treated with 2 ppm GA<sub>3</sub> (4.2).

Table 2. Effect of GA<sub>3</sub> priming on growth and development of embryos of Pisang Jajee × *M. acuminata* ssp. *burmannicoides*.

GA <sub>3</sub> concentration (ppm)	Germination (%)	Days taken for germination	Days taken for shoot emergence	Shoot length (mm)	Days taken for root emergence	Root length (mm)	No. of roots
2	78.4 <sup>e</sup>	3.4 <sup>ab</sup>	5.1 <sup>a</sup>	5.2 <sup>c</sup>	5.4 <sup>abc</sup>	4.8 <sup>c</sup>	4.2 <sup>c</sup>
5	80.6 <sup>c</sup>	3.5 <sup>ab</sup>	5.2 <sup>a</sup>	7.1 <sup>ab</sup>	5.3 <sup>ab</sup>	6.4 <sup>b</sup>	5.1 <sup>b</sup>
10	82.4 <sup>a</sup>	3.0 <sup>a</sup>	5.0 <sup>a</sup>	7.8 <sup>a</sup>	5.0 <sup>a</sup>	7.9 <sup>a</sup>	6.8 <sup>a</sup>
15	81.6 <sup>b</sup>	3.8 <sup>b</sup>	5.4 <sup>a</sup>	6.8 <sup>b</sup>	5.8 <sup>bc</sup>	5.3 <sup>c</sup>	6.6 <sup>a</sup>
20	79.4 <sup>d</sup>	3.9 <sup>b</sup>	5.6 <sup>a</sup>	6.6 <sup>b</sup>	5.9 <sup>bc</sup>	5.4 <sup>c</sup>	5.4 <sup>b</sup>
Control	40.8 <sup>f</sup>	5.0 <sup>c</sup>	7.0 <sup>b</sup>	4.5 <sup>c</sup>	6.0 <sup>c</sup>	4.8 <sup>c</sup>	4.8 <sup>bc</sup>
SE	0.29	0.47	0.41	0.48	0.43	0.40	0.56
CD ( $P = 0.05$ )	0.58	0.95	0.83	0.97	0.88	0.81	1-12
Level of significance	**	**	**	**	*	**	**

SE, standard error; CD, critical difference; \*\* highly significant ( $P < 0.01$ ); \* significant ( $P < 0.05$ ); NS, non-significant ( $P > 0.05$ ). Germination percentage values were arc-sine-transformed prior to ANOVA.

Compared with hydroprimed seeds, treating seeds with BAP had a highly significant effect on percentage germination, days taken for shoot emergence, shoot length, root number and root length, while it had a non-significant effect on the days taken for germination and root emergence among the various treatments analysed (table 3). Seeds treated with 5 ppm BAP recorded the maximum germination (72.6%) and earliest shoot emergence (5 days) and root emergence (6 days). Shoot and root length and number of roots produced were also maximum (9.8 mm, 6.9 mm and 5.8 roots respectively) in this treatment.



Table 3. Effect of BAP priming on growth and development of embryos of Pisang Jajee  $\times$  *M. acuminata* ssp. *burmannicoides*.

BAP concentration (ppm)	Germination (%)	Days taken for germination	Days taken for shoot emergence	Shoot length (mm)	Days taken for root emergence	Root length (mm)	No. of roots
2	69.9 <sup>b</sup>	4.4	5.2 <sup>ab</sup>	7.3 <sup>c</sup>	6.3	4.8 <sup>c</sup>	4.6 <sup>b</sup>
5	72.6 <sup>a</sup>	4.0	5.0 <sup>a</sup>	9.8 <sup>a</sup>	6.0	6.9 <sup>a</sup>	5.8 <sup>a</sup>
10	69.4 <sup>bc</sup>	4.3	5.4 <sup>ab</sup>	8.8 <sup>ab</sup>	6.5	6.2 <sup>ab</sup>	5.4 <sup>ab</sup>
15	68.9 <sup>cd</sup>	4.6	5.5 <sup>ab</sup>	8.4 <sup>b</sup>	6.8	5.9 <sup>b</sup>	5.8 <sup>a</sup>
20	68.1 <sup>d</sup>	4.8	5.9 <sup>b</sup>	7.1 <sup>c</sup>	6.8	5.1 <sup>c</sup>	4.8 <sup>b</sup>
Control	40.8 <sup>e</sup>	5.0	7.0 <sup>c</sup>	4.5 <sup>d</sup>	6.0	4.8 <sup>c</sup>	4.8 <sup>b</sup>
SE	0.30	0.42	0.55	0.49	0.46	0.50	0.56
CD ( $P = 0.05$ )	0.60	0.84	1.1	0.99	0.92	1.0	1.12
Level of significance	**	NS	**	**	NS	**	**

SE, standard error; CD, critical difference; \*\* highly significant ( $P < 0.01$ ); \* significant ( $P < 0.05$ ); NS, non-significant ( $P > 0.05$ ). Germination percentage values were arc-sine-transformed prior to ANOVA.

Compared with control seeds, treating seeds with IAA resulted in significant differences in percentage germination, shoot length, days taken for root emergence, root length and root number (table 4). Seeds treated with 10 ppm IAA recorded maximum germination (63.2%) followed by 2 ppm (62.9%). Earliest shoot and root formation was observed in 10 ppm IAA-treated seeds (within 4 and 7 days, respectively) and it also recorded the maximum shoot length (6.8 mm), root length (9.9 mm) and root number (10.8).

Among the various priming treatments, hydropriming for three days enhanced germination up to 40.8% compared with 20.8% in unsoaked seeds. Among the three hormones tested, priming in GA<sub>3</sub> at 10 ppm for three days doubled the germination (82.4%) in a minimum time of 3 days and also enhanced the other growth characteristics. BAP priming at 5 ppm for three days was found optimum with regard to days taken for shoot emergence and shoot length while IAA at 10 ppm for three days was found optimum with respect to root length and root number.

## Discussion

Hybrid seeds of Pisang Jajee  $\times$  *M. acuminata* ssp. *burmannicoides* were globose in shape with a flat bottom, similar to the shape reported by Chin (1996). The seeds were complete with seed coat (outer and inner integuments), endosperm and embryo, chalazal mass,

Table 4. Effect of IAA priming on growth and development of embryos of Pisang Jajee × *M. acuminata* ssp. *burmannicoides*.

IAA concentration (ppm)	Germination (%)	Days taken for germination	Days taken for shoot emergence	Shoot length (mm)	Days taken for root emergence	Root length (mm)	No. of roots
2	62.9 <sup>a</sup>	4.8	6.2	4.8 <sup>c</sup>	5.4 <sup>ab</sup>	6.8 <sup>c</sup>	6.4 <sup>d</sup>
5	61.4 <sup>bc</sup>	4.6	6.4	5.8 <sup>b</sup>	5.5 <sup>ab</sup>	8.1 <sup>b</sup>	8.3 <sup>bc</sup>
10	63.2 <sup>a</sup>	4.0	6.0	6.8 <sup>a</sup>	5.0 <sup>a</sup>	9.9 <sup>a</sup>	10.8 <sup>a</sup>
15	62.3 <sup>ab</sup>	4.7	6.8	5.8 <sup>b</sup>	5.7 <sup>b</sup>	5.8 <sup>d</sup>	9.3 <sup>b</sup>
20	60.4 <sup>c</sup>	4.9	6.9	4.2 <sup>c</sup>	5.9 <sup>b</sup>	7.5 <sup>bc</sup>	7.4 <sup>c</sup>
Control	40.8 <sup>d</sup>	5.0	7.0	4.5 <sup>c</sup>	6.0 <sup>b</sup>	4.8 <sup>e</sup>	4.8 <sup>e</sup>
SE	0.31	0.56	0.57	0.47	0.39	0.45	0.50
CD ( $P = 0.05$ )	0.63	1.13	1.15	0.95	0.79	0.92	1.02
Level of significance	**	NS	NS	**	**	**	**

SE, standard error; CD, critical difference; \*\* highly significant ( $P < 0.01$ ); \* significant ( $P < 0.05$ ); NS, non-significant ( $P > 0.05$ ). Germination percentage values were arc-sine-transformed prior to ANOVA.

nucellar pad, micropyle and micropylar plug. The embryo was mushroom-shaped with a well-developed haustorium and stalk. This is in agreement with various descriptions of banana seeds (McGahan, 1961; Chin, 1996). In the present study, the seed weight ranged from 46.8 to 51.1 mg (mean 49.0 mg). Similar variation for seed weight has been reported by Chin (1996) and Puteh *et al.* (2011) for wild types of *M. acuminata*.

The floating seeds either lacked an embryo or had a partially developed embryo or endosperm. In some cases, seeds had thin seed coats. Such abnormalities have been reported by Shepherd (1954) and Vuylsteke *et al.* (1990) in plantain hybrids. The proportion of complete hybrid seeds was 68% in the present study. This can vary depending on parental combination (Anon, 2009 and 2010).

Some factors inhibit the rate and amount of water imbibition, including seed composition, permeability of the seed coat and availability of water in liquid or gaseous phase. In this study, water uptake increased rapidly over the first day and then more slowly up to day-3, after which it remained stable for a day and decreased beyond day-4. Slow absorption of water after three days of hydropriming could be due to saturation of the water channel along the testa and operculum (Puteh *et al.*, 2011) and initiation of cell division. A similar trend of rapid water uptake in the initial hours has also been reported for seeds of three wild types of banana from Malaysia (Puteh *et al.*, 2011). The third phase exhibits a declining trend for fresh weight gain. This is due to the passive nature of the imbibition process which depends on the matric potential of the seed and the absence of a water-impermeable layer of palisade cells and sclereids (Graven *et al.*, 1996). Similar

results were also reported by Afele and De Langhe (1991) in *M. balbisia* and by Pillay *et al.* (2011) in plantain diploids. However, swelling of the embryo subsequent to water imbibition occurred only in the presence of viable embryos. Although dormancy linked to the seed coat and especially the desiccation of the chalazal mass has been reported by Chin (1996) in *M. gracilis*, Puteh *et al.* (2011) have successfully demonstrated that banana seeds lack physical dormancy.

In the present study, hydropriming of seeds for three days was found to enhance germination from 20.8 to 40.8%. Similarly Pillay *et al.* (2011) have reported enhanced germination from 30 to 75% in *M. gracilis* with hydropriming. The reason could be hydropriming facilitated reactivation of the metabolic system, leading to the rehydration of proteins, enzymes and cellular organelles. Similarly Afele and De Langhe (1991) reported in wild diploid *M. balbisia* (BB), that embryos from open-pollinated unsoaked seeds showed 56% germination and embryos of soaked seeds recorded up to 94% germination under *in vitro* condition. Asif *et al.* (2001) reported that embryos from unsoaked open pollinated seeds of *M. acuminata* ssp. *malaccensis* recorded maximum germination (93%). The above mentioned variations in germination are in parallel with our experiments; however the slight deviations could be attributed to the hybrid nature of the seeds (Pisang Jajee  $\times$  *Musa acuminata* ssp. *burmannicoides*) and AA genome of the parents. Pancholi *et al.* (1995) had reported 73.3% germination of embryos on basal MS medium from unsoaked seeds of *M. velutina*. Santos *et al.* (2001) has also reported that variation in germination percentage of *Musa* hybrids embryos could be due to genotypic effects. From these results it can be suggested that germination of even healthy hybrid embryos depends on genotype and parental combinations besides seed priming.

Water imbibition leads to expansion of the embryo through the thin-walled endocarp followed by the rupture of outer layers and finally, the emergence of the radicle (Bewley, 1997). Hydropriming for three days resulted in the earliest shoot and root emergence (7 and 6 days, respectively) and maximum shoot and root lengths. The results are in line with the findings of Afele and De Langhe (1991) who have reported that pre-soaking of intact seeds leached out some of the inhibitors resulting in rapid germination. Beyond three days, germination decreased with a delay in germination which could be attributed to possible microbial contamination (Pillay *et al.*, 2011).

Phytohormones play a regulatory role in reserve mobilisation during germination. Bewley and Black (1994) also suggested that endogenous growth promoting and inhibiting compounds were directly involved in germination. Whenever endogenous hormonal levels are affected due to environmental or any other stress it can be regulated by external application of hormones (Kabur, 1987). However, the effect depends on endogenous supply and their relationship with metabolic activities such as reserve mobilisation, root and hypocotyl growth, cotyledon mass and chlorophyll synthesis in cotyledons (Richard, 1997). GA<sub>3</sub> resulted in the highest and fastest germination (82.4% in three days) compared with BAP (72.6% in four days) and IAA (63.2% in four days). GA<sub>3</sub> might have altered the membrane permeability and energy levels in the embryo / embryonic axis and further increased the level of poly-(A) RNA which is believed to contain the mRNA for  $\alpha$ -amylase synthesised by the aleurone cells and responsible for the hydrolysis of starch to maltose (Cardwell, 1989). As the GA<sub>3</sub> concentrations were increased from 2 to 10

ppm, there was an increase in germination after which, it declined and germination was also delayed. The exogenous application of GA<sub>3</sub> might have enhanced the availability of endogenous GA<sub>3</sub> influencing the germination and seedling growth as reported earlier in other crops (Jones and Stodart, 1977; [Pharis and King, 1985](#); [Karszen et al., 1989](#); [Chen et al., 2005](#); [Chauhan et al., 2009](#)). Similar results of enhanced germination with GA<sub>3</sub> (at 0.4 ppm) have been reported in *M. acuminata* ssp. *burmannica* seeds stored for nine months ([Uma et al., 2012](#)). GA<sub>3</sub> is also reported to weaken the tissues removing the mechanical restraint enforced by radicle covers ([Kucera et al., 2005](#)).

Among various cytokinins, BA (benzyl-adenine) was reported to be more active in breaking dormancy and promoting germination of celery and lettuce seeds ([Biddington and Thomas, 1976](#)). [Farrant et al. \(1993\)](#) reported that elevated cytokinin levels observed during the histodifferentiation process in recalcitrant seeds leads to embryo and endosperm formation. A similar response was also observed in banana seeds with 5 ppm BAP resulting in 72.6% germination compared with 40.8% in control seeds. It also reduced the time taken for shoot emergence and shoot length. The enhancement of germination by BAP is attributed to the release of ethylene ([Sinska and Gladon, 1984](#)). Exogenous applications of cytokinins have also been reported to be involved in the germination of *Pinus sylvestris* L. ([Richard, 1997](#)) and *Amaranthus hybridus* L. ([Tiryaki, 2006](#)). Although the days taken for shoot emergence was the same in both BAP and GA<sub>3</sub> primed seeds as reported by [Chuanren et al. \(2004\)](#), the shoot length was higher with BAP. Similar results have been reported in oat ([Wright, 1966](#)).

Use of auxins like IAA ([Pal et al., 1970](#)), IBA, IPA ([Singh, 1970](#)) and 2,4-D in seed treatment have been reported to increase seed germination, growth and yields in some horticultural crops ([Muddappa and Mathai, 1985](#)). In this present study, IAA at 10 ppm was found to enhance the germination by 20% compared with hydropriming. IAA also promoted the root growth in terms of root number and their length. The enhanced root growth might also be attributed to the active role of IAA in promoting root initiation and growth as reported in the plant tissue culture of several crop plants.

Hormonal priming for three days improved the percentage germination of hybrid embryos compared with hydropriming. Among the three growth regulators tested, GA<sub>3</sub> was found to be more effective and efficient.

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