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Enhancing seedling growth of *Jatropha curcas*—A potential oil seed crop for biodiesel

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Abstract

The effects of aerosol smoke (AS), smoke-water (SW), potassium nitrate (KNO_3), naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA) on germination and seedling growth of *Jatropha curcas* were investigated. Seed coat removal accelerated water imbibition and germination occurred within 48 h. Seeds subjected to AS failed to germinate over a 90 day period. There were no significant differences in germination percentage between the treatments and untreated control (intact- and shelled-seed). However, shelled-seeds had the shortest mean germination time (MGT). Seedlings developed from treated seeds were planted in trays under shade house conditions and growth traits measured after 3 months. Soaking intact-seeds in SW, KNO_3 and NAA (24 h) produced significantly heavier and longer seedlings, which resulted in higher vigour indices (VI) compared to the control treatments. These results provide empirical evidence of the stimulatory effect of SW, KNO_3 and NAA on *J. curcas* seedling growth and vigour and the continuation of the effect over time. The approach of treating intact-seeds of *J. curcas* with plant growth substances prior to planting will help in producing healthy seedlings and possibly improve crop productivity.

Keywords: *Jatropha curcas*; Nitrogen salt; Smoke-water; Seedling vigour

1. Introduction

Jatropha curcas L. (physic nut) is a drought resistant shrub or tree belonging to the family Euphorbiaceae that originated from Central America and is cultivated in South America, Asia and Africa (Schmook and Serralta-Peraza, 1997). It offers potential as a biodiesel source (Martínez-Herrera et al., 2006). The fatty acid methyl ester of its seed oil is suitable for use as biodiesel, which meets the specification of international biodiesel standards (Azam et al., 2005). The co-products from *Jatropha* oil extraction are suitable for cellulosic ethanol production, meeting the alcohol demands of the biodiesel transesterification process (Visser et al., 2011). However, there are still many biological constraints that limit its yield and agronomic potential. Some of the inherent problems associated with *J. curcas* seeds are low germination and loss of vigour if not carefully stored due to its high oil content, which results in rancidity and weak seedling growth (Paramathma and Srimathi, 2006; Swarup, 2006). Effective propagation of healthy plants is a prerequisite for both breeders and growers. Seedling vigour is an important factor for the establishment of a plant (Van Staden et al., 2006). Numerous studies have shown that application of bio-stimulants and plant growth regulators (PGRs) increases germination ability of seeds and plays an important role in early developmental stages of seedlings in a wide range of plants (Afzal et al., 2005; Crunkilton et al., 1994; Russo and Berlyn, 1990; Swaminathaan and Srinivasan, 1996; Van Staden et al., 2006). Smoke technology has the potential to be used in the

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horticultural and agricultural industry for the production of healthier and more vigorous crops (Light and Van Staden, 2004).

Kureel (2006) reported that the seeds of *J. curcas* when soaked in water and GA_3 solutions (10 and 20 mg l^{-1}) for 24 h, germination occurs within 12, 8 and 5 days, respectively. Soaking *J. curcas* seeds in di-sodium hydrogen orthophosphate (Na_2HPO_4) for 6 h enhanced emergence by 25% and improved the quality of seedlings (Srimathi and Paramathma, 2006). Although these are few reported studies, there is not enough scientific information documented on optimization of seed germination and seedling growth of *J. curcas*. In particular, no studies have been reported on the effects of smoke so far.

In this study, the viability, moisture content and imbibition of *J. curcas* seeds were determined and the effect of aerosol smoke (AS), smoke-water (SW) and different plant growth substances on seed germination and seedling growth were investigated.

2. Materials and methods

2.1. Seed source

Fruits were collected from a 2-year-old monoculture of *J. curcas* at the University of KwaZulu-Natal Research Station Ukulinga, Pietermaritzburg, South Africa (latitude 30°41′ E; longitude 29°67′ S; altitude 781 m above sea level). The crop was established from seeds obtained from plantations at the Owen Sithole College of Agriculture, South Africa. The original seeds were imported from Malawi. Fruits were dehulled by hand, kept in paper bags and stored under laboratory conditions at 20 °C for 6 months before being used.

2.2. Viability test

Seed viability was determined for three replicates of 30 seeds each using 2,3,5-triphenyl tetrazolium chloride (TTC) solution. The seeds were shelled and imbibed for 24 h in water at 25 ± 0.5 °C. After cutting longitudinally to expose the embryo, seeds were soaked in 1% TTC for 24 h at 25 ± 0.5 °C in the dark. A seed with a red-stained embryo was scored as being viable (ISTA, 1999). Viability percentage was calculated as the number of the stained embryos to the total number of embryos used.

2.3. Moisture content and imbibition curve

2.3.1. Moisture content

The moisture content of the seeds was determined by drying seeds at 110 °C from three replicates of 30 seeds each. The seeds were weighed repeatedly (once-a-day) until a constant weight was reached. The moisture content was determined as a percentage based on original fresh weight (Kulkarni et al., 2007).

2.3.2. Imbibition curve

Four replicates of 15 seeds each were placed in 9 cm Petri dishes on two layers of filter paper (Whatman No. 1) moistened with 15 ml distilled water and allowed to imbibe at room temperature (25 °C). The increase in seed weight was determined after 2, 4, 8, 12, 24, 36, 48, 72 and 96 h. Seeds were blotted dry before weighing and returned to the wet filter paper.

2.4. Germination

2.4.1. Aerosol smoke

Out of two batches of seeds [intact and shelled (seed coat was removed) seeds] four replicates of 15 seeds each were placed separately in sieves and exposed to cool aerosol smoke for 30, 60 and 90 min (Sparg et al., 2006). This was achieved by placing the sieves inside a chimney, 150 cm above slow burning semi-dry and dry grass, leaves and branches collected from the University of KwaZulu-Natal Botanical Garden. After exposure to smoke, seeds were washed with distilled water and placed in paper towelling moistened with distilled water inside plastic bags and incubated at 25 °C under 16-h photoperiod provided by Osram® 75 W cool white florescence tubes with irradiance of 16 μmol m^{-2} s^{-1} at shelf level.

2.4.2. Smoke and PGRs solutions

The smoke dilutions were prepared from an aqueous smoke-extract that was produced from burnt grass material (*Themeda triandra*) as outlined by Baxter et al. (1994). Seeds were decontaminated by soaking in 0.2% mercuric chloride for 2 min and then rinsed with distilled water (Sparg et al., 2005). Three replicates of 30 seeds each were soaked for 24 h in distilled water using intact- or shelled-seeds representing the controls (C1 and C2, respectively). Three dilutions of smoke-water (SW) (1:500, 1:1000 and 1:1500), potassium nitrate (KNO_3) (10^{-5}, 10^{-6} and 10^{-7} M), indole-3-butyric acid (IBA) (10^{-5}, 10^{-6} and 10^{-7} M) and two concentrations of naphthalene acetic acid (NAA) (10^{-5} and 10^{-6} M) were tested by soaking intact- and shelled-seeds in these solutions for 24 h. The treated seeds were then placed on paper towelling and wetted with distilled water inside plastic bags. Germination was recorded daily and was considered complete once the radicle was ≥2 mm in length. The experiment continued for 5 days when all treatments had reached full germination. However, for the aerosol smoke (AS) treatment the observation continued for 21 days. The germination data were arcsine transformed for statistical analysis (Dezfuli et al., 2008). Mean germination time (MGT) was calculated using the equation $MGT = \Sigma n \times d / N$, where n is the number of seeds germinated between observation intervals, d the incubation period in days after time of observation and N the total number of seeds in the sample that germinated in the treatment (Balestri and Bertini, 2003).

2.5. Seedling growth

Treated and non-treated seedlings from germination trials were planted in plastic trays (20 × 10 × 5 cm) in a shade house of

the botanical garden of the University of KwaZulu-Natal, Pietermaritzburg, with an average midday light intensity of 331 μmol m^{-2} s^{-1}. The soil mixture in each plastic tray was made up of compost, bark (chipped and decomposed Pine), LAN (limestone ammonium nitrate) and 2:3:2 NPK in ratio 4:1:0.1:0.1. Trays were placed randomly in the shade house and watered twice weekly. Each tray had ten seedlings with three replications. After 3 months, seedling growth traits such as mass, number of leaves, stem width, stem length, root length and leaf area (LA) were measured and vigour index (VI) was calculated as percentage germination × (stem length + root length) (Dhindwal et al., 1991). The number of roots was not considered for measurement because *J. curcas* plant has a constant number of roots (one taproot plus four lateral roots) (Heller, 1996).

2.6. Data analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS® (SPSS Inc., Chicago, USA) release 15 statistical software. Tukey's test at 5% level of significance was used to separate differences between means of treatments.

3. Results

3.1. Seed viability, moisture content and imbibition

The mean viability and moisture content percentages of the fresh seeds used in the experiments were 90% and 11.7%, respectively. The imbibition curve for the shelled-seeds was steep at the beginning. However, within 24 h, the net water uptake plateaued and germination occurred within 48 h (Fig. 1). The imbibition curve for the intact-seeds remained steep up to 96 h (Fig. 1).

3.2. Germination

Seeds exposed to AS failed to germinate over the whole period of the experiment (21 days in incubator and 90 days in shade house). Soaking shelled-seeds in different concentrations of SW and PGRs for 24 h was detrimental with poor germination (data not shown). There were no significant differences in germination between the control treatments C1 and C2 (intact- and shelled-seeds, respectively) soaked in distilled water and the other treatments, where the intact-seeds were soaked for 24 h in SW, KNO_3, IBA or NAA and germinated (Table 1). However, there were variations only at C2 which had the shortest MGT compared to C1 and the other treatments (Table 1).

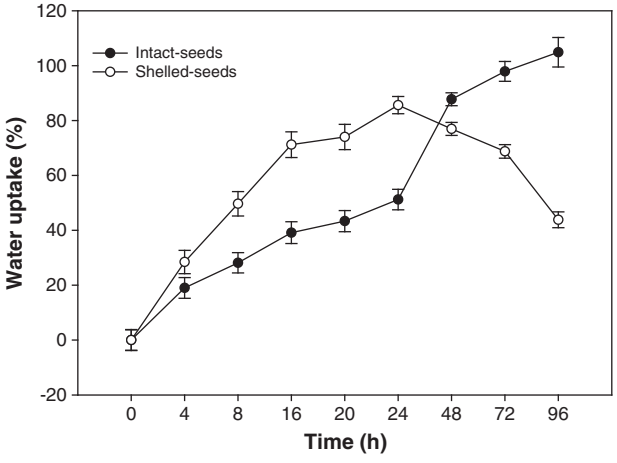

Fig. 1. Imbibition curve for *Jatropha curcas* seeds at 25 °C. Symbols are with standard error bars (±S.E.).

3.3. Seedling growth and vigour

Potassium nitrate at concentration of 10^{-5} M produced seedlings with a significantly greater weight, longer stem width and root length and higher vigour index (VI) (10^{-5} M) in comparison to controls [Figs. 2 and 3(A, D, E and G)]. There were no significant differences between the three concentrations of KNO_3 with the exception of LA. Naphthalene acetic acid at 10^{-5} and 10^{-6} M also achieved significantly greater seedling weight, stem length, stem width, root length (10^{-6} M) and higher VI compared to the control treatments [Figs. 2 and 4(A, C, D, E and G)]. Smoke-water at a dilution of 1:500 showed a significant increase in seedling weight, leaf number, stem width, stem and root length and VI compared to the controls [Figs. 2 and 5(A–E and G)]. Indole-3-butyric acid at all concentrations produced significantly larger stem widths than the control treatments [Fig. 6(D)]. Concentration of 10^{-7} M of IBA increased root length significantly when compared to the shelled-seeds (C2) [Fig. 6(E)].

4. Discussion

Our results indicate that seed coat removal accelerated water uptake with germination occurring within 48 h. The imbibition curve for the shelled-seeds was initially steep but within 24 h, the net water uptake plateaued (Fig. 1). However, the imbibition

Table 1
Effects of smoke-water (SW), potassium nitrate (KNO_3), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA) on seed germination of *Jatropha curcas*. MGT = mean germination time.

Treatment	Germination (%)	MGT (days)
C1 (intact-seeds)	93.7±0.48 a	5
C2 (shelled-seeds)	95.0±0.48 a	2
SW 1:500	95.0±0.57 a	3
SW 1:1000	98.3±0.25 a	3
SW 1:1500	100±0 a	3
KNO_3 10^{-5} M	98.3±0.25 a	3
KNO_3 10^{-6} M	100±0 a	3
KNO_3 10^{-7} M	100±0 a	3
IBA 10^{-5} M	96.7±0.50 a	3
IBA 10^{-6} M	98.3±0.25 a	3
IBA 10^{-7} M	98.3±0.25 a	3
NAA 10^{-5} M	96.7±0.50 a	3
NAA 10^{-6} M	100±0 a	3

Mean (±S.E.) germination percentage values followed by the same letter are not significantly different according to Tukey's test ($P<0.05$).

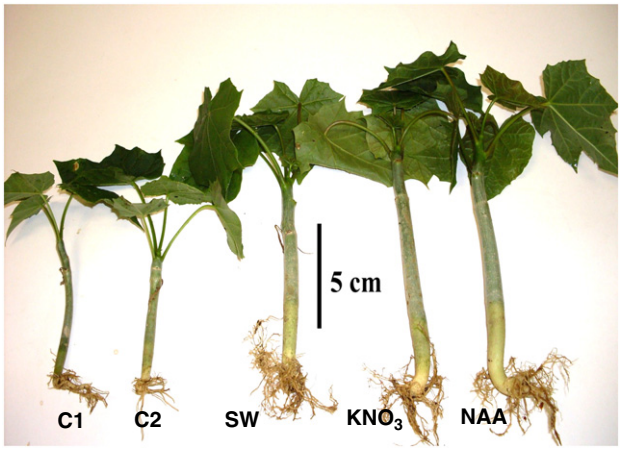

Fig. 2. Effect of different plant growth substances on *Jatropha curcas* seedlings under shade house conditions for 3 months. Controls (intact-seeds, C1; shelled-seeds, C2), smoke-water (SW), potassium nitrate (KNO_3) and naphthalene acetic acid (NAA).

curve for the intact-seeds was also initially steep but remained so up to 96 h (Fig. 1).

Seeds exposed to AS failed to germinate in the incubator (20 days) and shade house (90 days). Daws et al. (2007) showed the inhibitory effects of smoke on some seeds of arable weed species. This effect could be most likely attributed to inhibitory compounds that are present in the smoke (Light et al., 2010). Results of this study show no significant differences in germination percentage between all treatments. However, MGT was shorter for the shelled-seeds (2 days) and longer for the water-soaked intact-seeds (5 days) (Table 1).

Seed invigoration treatments such as hydropriming, osmo-priming, hardening, matripriming and PGRs have been successfully used for enhancing seed germination and seedling growth of many crops (Farooq et al., 2007). Our results indicate that KNO_3 at concentration of 10^{-5} M was beneficial in developing seedlings with heavier mass, wider stem width, longer roots and greater VI [Figs. 2 and 3(A, D, E and G)]. This

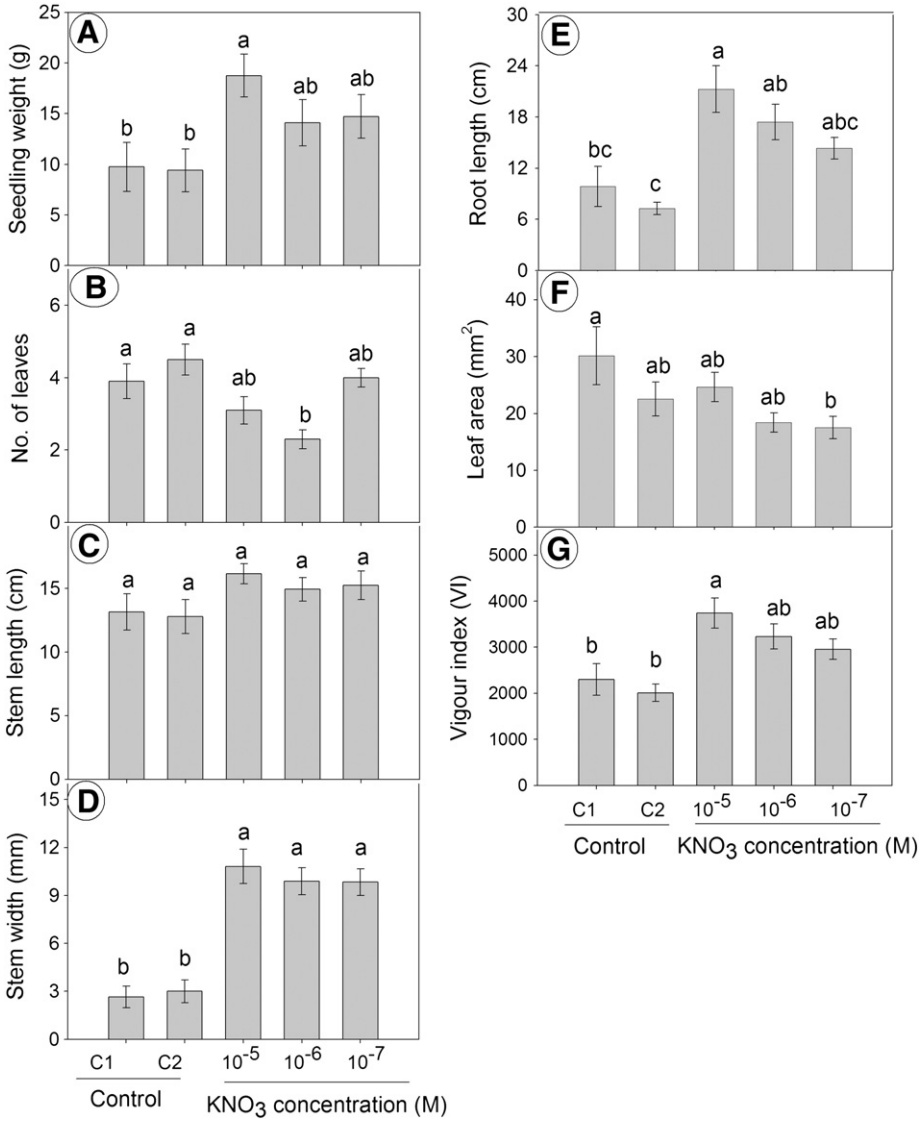

Fig. 3. Effect of different concentrations of potassium nitrate (KNO_3) on growth parameters of *Jatropha curcas* seedlings under shade house conditions for 3 months. Standard error (±) bars with different letter(s) are significantly different according to Tukey's test ($P<0.05$).

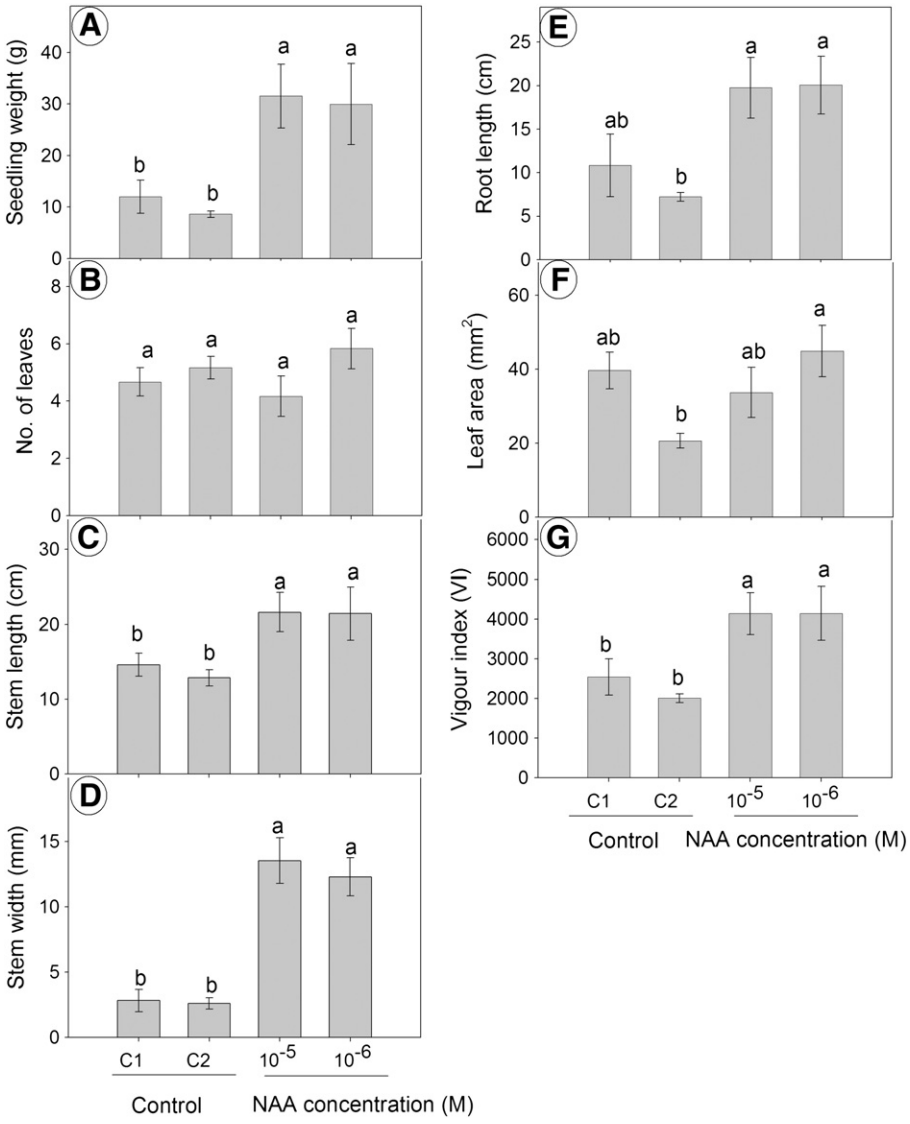

Fig. 4. Effect of different concentrations of naphthalene acetic acid (NAA) on growth parameters of *Jatropha curcas* seedlings under shade house conditions for 3 months. Standard error (±) bars with different letter(s) are significantly different according to Tukey's test ($P<0.05$).

result is in agreement with Kattimani et al. (1999), who showed that ashwagandha (*Withania somnifera* Daunal.) seeds soaked in 1% KNO_3 solution for 24 h produced vigorous seedlings with higher dry matter accumulation and longer roots compared to the water-soaked seeds. Studies have shown improved seedling growth and VI of soybean, canola and sunflower using KNO_3 (Mohammadi, 2009; Mohammadi and Amiri, 2010; Singh and Rao, 1993). Pre-sowing treatment of seeds of the threatened medicinal herb *Angelica glauca* with KNO_3 improved seedling vigour under nursery conditions (Butola and Badola, 2004).

Inclusion of PGRs during pre-soaking, priming or pre-sowing treatments has improved seed performance of several crops (Jeong et al., 1994; Korkmaz et al., 2004). However, the precise action on the plant growth depends on the concentration of the PGRs and the sensitivity of the concerned organ (Vamil et al., 2010). Plant growth regulators affect the balance between photosynthesis and photorespiration in plants. For instance, NAA is known to improve plant water relations and the rate of photosynthesis (Maske et al., 1997). This could be the reason that NAA-treated seeds of *J. curcas* produced heavier seedlings, longer and wider stems, longer roots and a higher VI compared to the control treatments [Figs. 4(A, C, D, E and G)]. All concentrations of IBA significantly produced seedlings with larger stem width than the untreated control [Fig. 6(D)]. For root length, a significant difference was observed by IBA at 10^{-7} M compared with the shelled-seed control [Fig. 6(E)]. These results are comparable with those of Olaiya (2010) and Olaiya et al. (2009) who reported that pre-sowing treatments of tomato seeds with NAA and IBA were effective in enhancing seedling growth. Vamil et al. (2010) reported that seeds of bamboo (*Bambusa arundinaceae*) treated with IBA and NAA (10 μM or 100 μM) enhanced seedling growth, LA and chlorophyll content. This enhancement in seedling growth was attributed to improvement of water absorption by seedlings and improved photosynthesis (Maske et al., 1997). Similar results were reported by Grzesik (2006) for *Zinnia elegans*, *Matthiola*

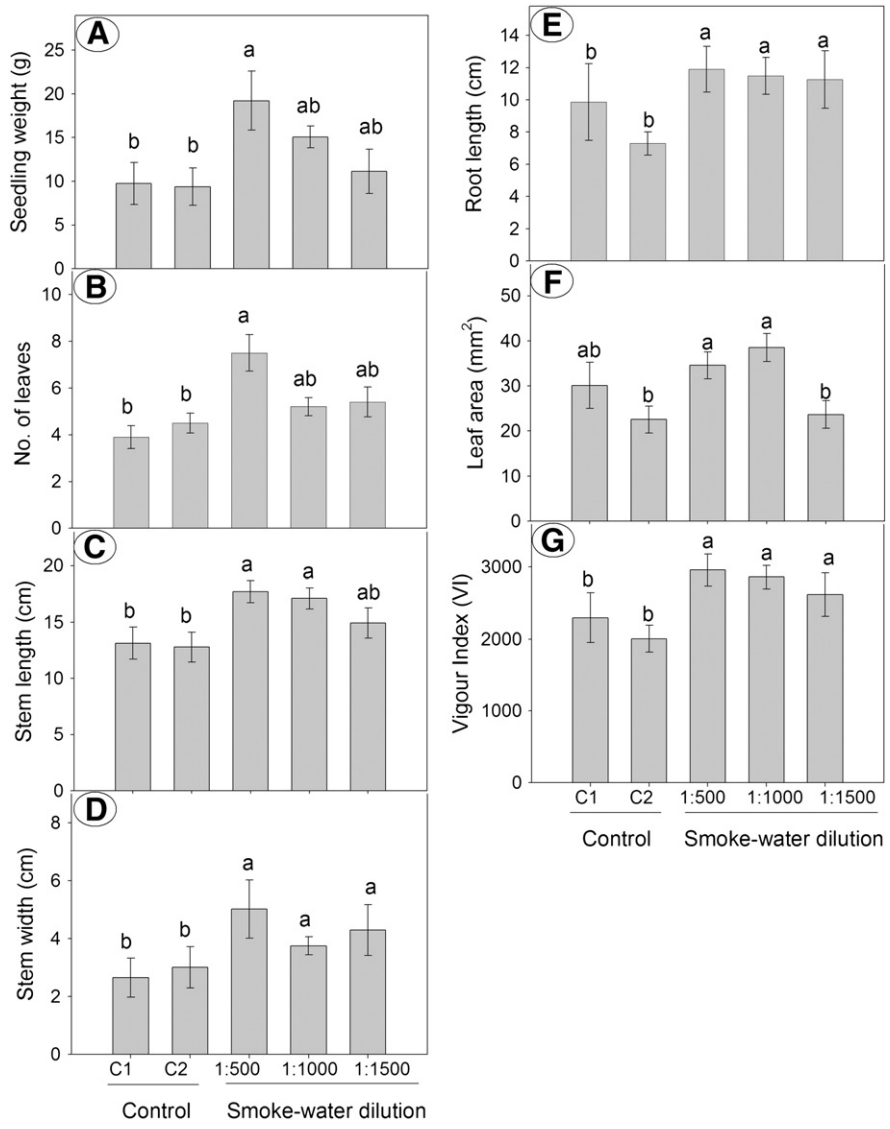

Fig. 5. Effect of different dilutions of smoke-water on growth parameters of *Jatropha curcas* seedlings under shade house conditions for 3 months. Standard error (±) bars with different letter(s) are significantly different according to Tukey's test ($P<0.05$).

incana and *Antirrhinum majus*, where NAA-treated seedlings were longer, had improved branching and were of better quality than the non-treated seedlings. In another study (Chauhan et al., 2009), *Macrotyloma uniflorum* (Lam.) seed treatment with NAA showed a remarkable improvement in seedling growth. *Phaseolus mungo* L. seed treatment with IBA showed increased root length and the best expansion of cotyledons (Chauhan et al., 2010). In this study, although IBA showed some improvement for stem width, it was not more effective than other treatments. In this study, the effect of PGRs was maintained for 3 months. These results are in agreement with our earlier studies on the application of PGRs on *J.curcas* plants for promoting branching (Abdelgadir et al., 2009), and subsequently studying the effect of these PGRs on flowering and fruiting after 1 year (Abdelgadir et al., 2010). The results of these studies showed that the effect of PGRs was sustained for a long period. The long-term effect of PGRs may differ from species to species and the levels of concentration applied.

The influence of SW on seedling characteristics and vigour of *J. curcas* was highly significant at a dilution of 1:500, which produced heavier seedlings with numerous leaves, improved stem thickness, longer stem and root length, and augmented VI compared to the control treatments [Figs. 2A and 5(A–E and G)]. This certainly provides evidence for the stimulatory effect of SW on seedling vigour. These results are in agreement with Van Staden et al. (2006) who reported that smoke-water at a dilution of 1:500 significantly increased seedling mass and VI of okra, tomato and maize compared to the untreated controls. Similar results were obtained for rice (Kulkarni et al., 2006) and the medicinal plant *Dioscorea dregeana* Kunth Dur. and Schinz (Kulkarni et al., 2007). Taylor and Van Staden (1996) demonstrated stimulated root formation in *Vigna radiata* L.

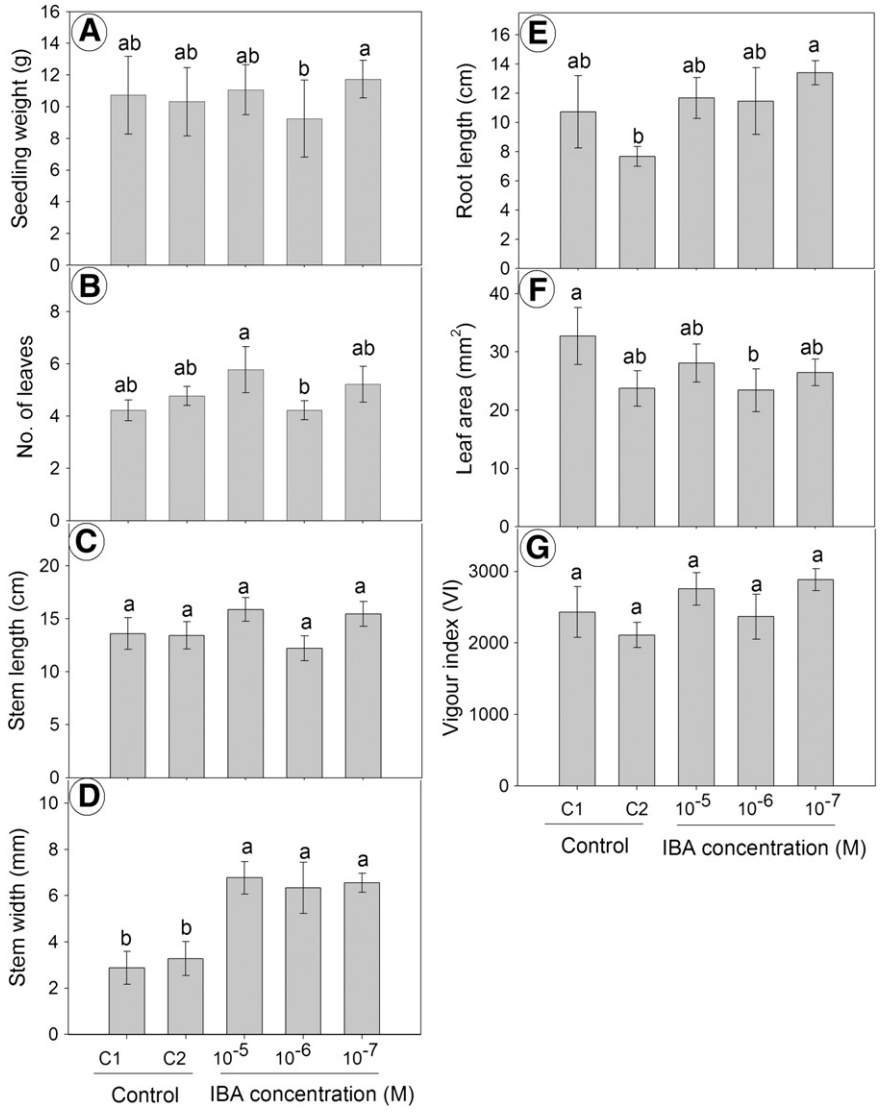

Fig. 6. Effect of different concentrations of indole-3-butyric acid (IBA) on growth parameters of *Jatropha curcas* seedlings under shade house conditions for 3 months. Standard error (±) bars with different letter(s) are significantly different according to Tukey's test ($P<0.05$).

Wilczek. by smoke extract, indicating that smoke constituents may play a significant role in promoting rooting.

5. Conclusions

Treatment of *J. curcas* intact-seeds with different concentrations of KNO_3, NAA, IBA and SW did not show a significant effect on germination but they proved to be very effective in improving subsequent seedling growth parameters. Although shelled-seeds showed shorter MGT, soaking of these seeds in treatment solutions was detrimental. Reducing the soaking period of shelled-seeds can help to overcome this problem. However, in practise, seed coat removal of *J. curcas* for large-scale plantation is not feasible and therefore use of intact-seeds is recommended. This study suggests that these plant growth substances can be useful in achieving vigorous seedlings from intact-seeds for successful establishment of a *J. curcas* crop.

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