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Effect of Smoke-water on Seed Germination and Seedling Growth of Papaya (*Carica papaya* cv. Tainung No. 2)

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***Abstract*. Smoke-water is a chemical extract used to stimulate the germination of many plant species under cultivation. This study evaluated the efficacy of smoke-water on the seed germination and seedling growth of papaya (*Carica papaya* cv. Tainung No. 2). Smoke-water, prepared from dry rice straw (*Oryza sativa*) by burning and bubbling the smoke through water, was used for germination experiments, growth experiments, and anatomical structure changes of seeds. In the germination experiments, papaya seeds were soaked with different concentrations of smoke-water (0.1%, 0.2%, 1%, 2%, 3%, 4%, 5%, 7%, or 10%, v/v) for 24 h before planting. Low concentrations of smoke-water (0.1% or 0.2%, v/v) not only promoted the maximum rate of germination, but also shortened the germination time. Analysis of longitudinal sections of seeds treated with smoke-water concentrations of 0.1% or 0.2% v/v suggested that smoke-water could overcome water impermeability barriers, because it stimulated the seedcoat to rupture and allowed the radical to elongate and emerge faster. In the growth experiments, papaya seedlings were transplanted into peatmoss-filled pots that were saturated with different concentrations of smoke-water (0%, 1%, 2%, 3%, 4%, 5%, 7%, or 10%, v/v). Results showed that all growth parameters increased significantly compared with the control. In addition, smoke-water treatments consistently and significantly increased the percentage of nitrogen in roots and shoots and significantly increased the percentage of magnesium in shoots. In these experiments, smoke-water showed potent germination promotion at low concentrations and promoted multiple growth attributes such as chlorophyll content and seedling vigor index at all concentrations in papaya seedling production.**

Farmers throughout the world have traditionally used fire and smoke in grain drying practices. It is thought that these methods improve germination and seedling vigor (Paasonen et al., 2003). Recently, the germination response to smoke has been studied through the use of smoke-water, which is derived from burning plant material and bubbling the smoke through water. This has been shown to promote germination in wild plant species from many ecosystems (Light and Van Staden, 2004) and also to improve the growth and yield of agricultural and horticultural species (Brown et al., 2003), although it is necessary to dilute the concentration of the smoke-water before application to many species (Lloyd et al., 2000).

The main active germination compound of smoke-water derived from burned plant materials (Van Staden et al., 2004) and cellulose (Flematti et al., 2004) has been identified as butenolide [3-methyl-2*H*-furo(2,3-*c*)pyran-2-one], which is effective at very low concentrations (1 ppb). The compound has recently been referred to as "karrikinolide" (Commander et al., 2008). The action of smoke in promoting seed germination in many species is attributed to the presence of this compound (Soos et al., 2009). Dixon and Roche (1995) reported that the promotive effect of smoke is independent of seed size, seed shape, seed type, phylogenetic position, geography, and plant life form, i.e., whether annual, perennial, herbaceous, fire-sensitive seeder, or fire-tolerant resprouter. Smoke from a wide variety of biotic sources, including wood, straw, mixtures of dry and fresh plant material, and charred wood, can stimulate germination (Brown and Van Staden, 1997).

Papaya (*Carica papaya*) seed germination is affected by many environmental factors such as temperature, light, pH, oxygen, and soil moisture (Lange, 1961). Under normal conditions, germination can occur as soon as in 16 to 20 d. (Bhattacharya and Khuspe, 2001). Lange (1961) also found that the percent germination may vary from 3% to 71% depending on the cultivar and temperature. In unfavorable conditions, germination has been reported to be slow, erratic, and incomplete (Lange, 1961).

In Taiwan, the rice production area is more than 200,000 ha, creating a problematic volume of dry straw residue (Council of Agriculture Statistics Year Book, 2010) that would better serve agriculture if it could be repurposed. Papaya in Taiwan is grown on 3,287 ha and fruits yield 129,322 tons. The papaya industry is limited by seed germination rates. Therefore, the objective of this study was to evaluate the efficacy of smoke-water prepared from dry rice straw on papaya seed germination and the growth of the 'Tainung No. 2' papaya seedling.

Material and Methods

Seed source. Commercial seeds of 'Tainung No. 2' papaya (*Carica papaya* L.) were purchased from Known-You seed company in Taiwan and stored at 9 °C.

Smoke-water preparation. A smoke-water solution was prepared by igniting 5 kg of dry rice straw (*Oryza sativa* cv. Japonica) material (gathered from a rice field in southern Taiwan) in a 20-L stainless steel barrel. Using compressed air, the smoke was continuously bubbled through a 500-mL graduated cylinder containing distilled water for 45 min. This smoke extract was filtered through Whatman No. 1 filter paper and used as the stock solution.

Germination experiments. Seeds were soaked in 10 different concentrations of smoke-water [0%, 0.1%, 0.2%, 1%, 2%, 3%, 4%, 5%, 7%, or 10% (v/v), respectively] for 24 h and then rinsed for 1 min in three changes of sterile water. Seeds were planted in Tref peatmoss (Australia; pH: 5.5, electrical conductivity: 2.5 $mS\cdot cm^{-1}$, organic matter content: 0.85%, total nitrogen: 160 $\mu g\cdot g^{-1}$, phosphorus: 78.66 $\mu g\cdot g^{-1}$, potassium: 166 $\mu g\cdot g^{-1}$, and magnesium: 80 $\mu g\cdot g^{-1}$) in pots (dimensions: 10 cm × 8.5 cm × 7 cm, 200 g of peatmoss per pot). Each pot was watered three times weekly. All treatments consisted of five replicates with 30 seeds in each and the entire experiment was repeated twice. Pots were placed in a growth chamber set to 8 h of light phase at 30 °C and 16 h of dark phase at 25 °C with 75% relative humidity. Irradiance was provided at 40 $\mu mol\cdot m^{-2}\cdot s^{-1}$ using daylight fluorescent lamps. Germination counts were made daily for 2 weeks. Germination was considered complete when the hypocotyls emerged. Average germination time (AGT) was calculated by: AGT = $\sum(t_i.n_i)/\sum n$, where t_i = time of seed germination in days, n_i = number of seeds germinated in time t_i (not the accumulated number but the number correspondent to the seeds germinated on that particular day), and $\sum n$ = maximum germination of a seed lot.

Longitudinal section of seed. Six concentrations of smoke-water [0%, 0.1%, 0.2%, 1%, 3%, or 10%, (v/v), respectively] were used to soak seeds for 24 h, after which they were rinsed with distilled water. Each treatment consisted of three replicates of 30 seeds each were placed in 90-mm petri dishes on three layers of folded paper towel and incubated at room temperature for 24 h. Seeds were embedded in a tissue-freezing medium and cut into 30-μm sections by a Cryostat microtome (Cryocut 1800, Germany). Staining was carried out in 0.1% (w/v) safranin and observed under a microscope.

Growth experiments. Seeds were soaked with distilled water for 24 h, sown in peatmoss, and grown in a greenhouse. Papaya seedlings at the two true leaf stage were transplanted (four plants/pot) into peatmoss-filled

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pots. Four pots were prepared for saturation with one of eight different concentrations of smoke-water [0%, 1%, 2%, 3%, 4%, 5%, 7% or 10% (v/v), respectively]. There were three replicates for each concentration. The pots were placed in a growth chamber and maintained for a growing period of 4 weeks. Each pot was irrigated three times weekly with water. At the end of the experiment, growth parameters were measured and mineral content was analyzed. The seedling vigor index (SVI) was calculated as SVI = [shoot length (mm) + root length (mm)] × percentage germination (Dhindwal et al., 1991).

Mineral nutrition analysis. Plant samples were collected and separated shoots and roots. The samples were washed under tap water, rinsed with 1% HCl, then thoroughly rinsed three times in distilled water. All samples were dried to constant weight at 70 °C in an oven before milling to a fine powder with an electric blender and subsequent storage until analysis. For the determination of mineral content, the dried sample powder (0.5 g) was placed in a muffle furnace at 200 °C for 2 h, 400 °C for 1 h, and then 550 °C for 2 h. The resulting ash was dissolved with 5 mL 2 N HCl and filtered (Whatman No.42) according to the AOAC official method (AOAC, Official Method 973.03, 1995). Determination of total nitrogen followed the micro-Kjeldahl method, determination of phosphorus followed the Molibidam Yellow method, and determination of potassium, magnesium, calcium, iron, manganese, zinc and copper was by atomic absorption spectrophotometer (Hitachi Model Z-2300).

Statistical analysis. Statistical analysis of experimental data were performed using SAS 9.2 (SAS Institute, 2002) and subjected to one-way analysis for variance by a completely randomized design statistical model. Mean values among treatments were compared by Duncan's multiple range tests at the 1% ($P \leq 0.01$) level of significance.

Results

Effect of smoke-water on seed germination. Smoke-water was successful in improving the germination of papaya seeds. Smoke-water at low concentration treatments (0.1% and 0.2%) resulted in significantly higher germination rates (87.3% and 86.7%, respectively) and reduced average germination time (8 d) in comparison with the control (0% smoke-water; 70.7% germination rate; and 9 d average germination time). However, high concentrations of smoke-water treatments (1% and above) showed no significant increase in germination rate and did not reduce the germination time (Table 1).

Longitudinal sectioning of papaya seed. The microstructure of a mature papaya seed contains a fully developed embryo with differentiated meristems, radicle, and cotyledons. In mature seeds, the seed capsule has three layers, consisting of mucilage, seedcoat, and a mesophyll layer of seedcoat. Inside the seedcoat is the single cell layer of micropylar endosperm, which covers the cap-like endosperm and the radicle tip. In this study, results of longitudinal sectioning of papaya seeds treated with smoke-water showed that low concentrations of smoke-water (0.1% and 0.2%) stimulated the endosperm to stretch and the seedcoat to rupture, allowing the radicle to elongate and emerge, thus completing seed germination (Fig. 1).

Effect of smoke-water on growth parameters and nutritional composition of seedling. Smoke-water enhanced the growth of papaya seedlings. Seedlings grown while saturated with 10% smoke-water showed significantly better growth parameters (with maximum elongation of shoots and roots of 16.7 and 21.9 mm, respectively) than the control seedlings (11.1 and 11.8 mm, respectively). The seedlings also showed a significant increase in shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight in comparison with the control. On the other hand, 10% smoke-water plants had significantly greater number of leaves, elevated chlorophyll content, and greater seedling vigor index (9.0, 34.5, and 38650, respectively) than the control seedlings (7.8, 29.7, and 22970, respectively; Table 2).

Smoke-water treatments did not affect the entire mineral content profile of papaya seedlings (Table 3), although nitrogen (N) levels were significantly altered. All concentrations of smoke-water treatments significantly increased levels of N in roots (2.3%) compared with the control (1.8%). In the shoot, significant increase in the level of N (4.1%) were found only with 10% smoke-water treatment (4.1% N vs. 3.2% N in control). Furthermore, treatment with 5% or greater smoke-water resulted in significantly increased levels of magnesium in shoots (1.33%) compared with the control (1.28%). The other elements were not significantly changed with smoke-water treatment.

Discussion

When used as an imbibition solution for papaya seeds, low concentrations of smoke-water (0.1% and 0.2%, v/v) significantly improved the germination rate and stimulated earlier radical emergence compared with the control. With high concentrations of smoke-water (1% to 10%, v/v), the results of germination rate were not significantly different from the control. However, Drewes et al. (1995) found that high concentrations of smoke-water could be inhibitory to germination but could be reduced to promoting levels through irrigation (De Lange and Boucher, 1993).

Light et al. (2009) reported that smoke from burning plant material stimulated seed germination of numerous species worldwide. Thus, smoke-water may be acting on the seedcoat in a way similar to scarification, by which the dormant embryo may more efficiently imbibe water and uptake oxygen (Egerton-Warburton, 1998). An important component of biomass smoke is ethylene, a chemical cue known to stimulate the germination of seed. The action of ethylene includes promoting radial cell expansion, increasing seed respiration, and promoting endosperm to rupture (Kucera et al., 2005).

Kulkarni et al. (2006) found that smoke-water had similar effects as plant growth regulators. Furthermore, Chiwocha et al. (2009) reported that Karrikins, a new family of plant

Table 1. Effect of smoke-water treatment on the percent germination of 'Tainung No. 2' payaya.

Smoke-water concn	Germination rate (%)	Avg germination time (days)
Control	70.67 b^z	9 a
0.1%	87.33 a	8 a
0.2%	86.67 a	8 a
1%	61.33 b	9 a
2%	72.67 b	9 a
3%	72.00 b	9 a
4%	64.00 b	9 a
5%	66.67 b	9 a
7%	62.67 b	9 a
10%	66.67 b	9 a

^{z}Values in each column with the same letter are not significantly different according to Duncan's significant difference test ($P \leq 0.01$).

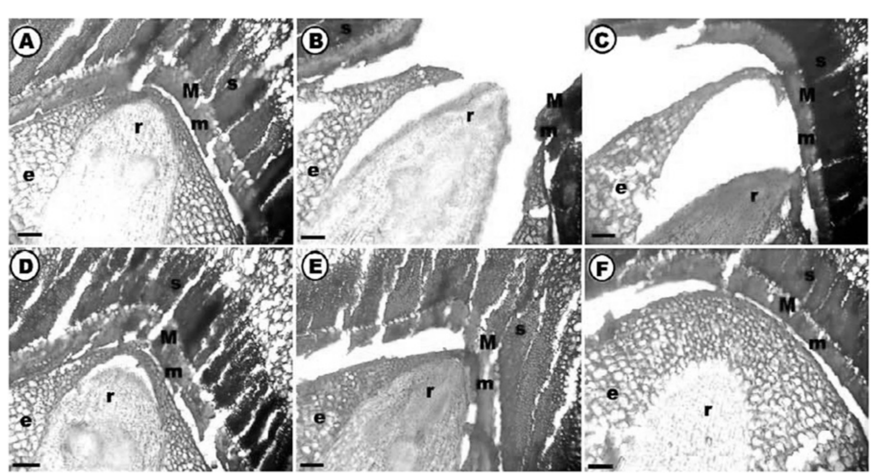

Fig. 1. Effect of smoke-water on longitudinal section of papaya seed; seeds treated with (**A**) control, and (**B–F**) different concentrations of smoke-water [0.1%, 0.2%, 1%, 3%, and 10% (v/v), respectively] for 24 h and incubated at room temperature for 2 h, then stained with 0.1% safranin. r = radicle; e = endosperm; M = mesophyll layer of seedcoat; m = micropylar endosperm; s = seedcoat. Scale bars: 10 μm.

Table 2. Effect of smoke-water on growth parameters of papaya seedlings after 4 weeks.

Smoke-water concn	Shoot length (cm)	Root length (cm)	Shoot fresh wt (g)	Shoot dry wt (g)	Root fresh wt (g)	Root dry wt (g)	No. of leaves	Chlorophyll content	Seeding vigor index
Control	11.14 e[z]	11.83 g	0.88 d	0.09 d	0.19 e	0.01 b	7.83 d	29.73 d	22970 e
1%	13.57 d	14.44 f	1.12 c	0.11 c	0.28 d	0.02 a	8.42 bcd	29.84 d	28010 d
2%	14.16 d	16.30 e	1.13 c	0.11 c	0.28 d	0.02 a	8.17 dc	30.00 d	30460 c
3%	14.51 dc	17.19 de	1.27 b	0.13 bc	0.31 cd	0.02 a	8.17 dc	29.98 d	31700 c
4%	14.17 d	18.32 dc	1.29 b	0.13 bc	0.32 cd	0.02 a	8.50 bcd	31.11 c	32490 c
5%	15.57 bc	19.32 bc	1.71 a	0.15 ab	0.48 a	0.02 a	9.42 a	32.80 b	34890 c
7%	15.78 ab	20.57 ab	1.72 a	0.16 a	0.38 bc	0.02 a	8.83 abc	33.27 b	36350 b
10%	16.71 a	21.94 a	1.73 a	0.17 a	0.40 b	0.02 a	9.00 ab	34.48 a	38650 a

[z]Values in each column with the same letter are not significantly different according to Duncan's difference test ($P \leq 0.01$).

Table 3. Effect of smoke-water on mineral content of papaya seedlings (4 weeks old).

Smoke-water concn	Macroelement (%)						Microelement (ppm)		
	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Iron	Manganese	Zinc	Copper
	Root								
Control	1.81 b[z]	1.21 a	6.96 a	1.15 a	1.48 a	158.4 a	416.8 a	88.6 a	7.4 a
1%	2.41 a	1.27 a	6.79 a	1.31 a	1.54 a	161.4 a	420.5 a	88.6 a	7.5 a
2%	2.28 a	1.22 a	6.94 a	1.33 a	1.54 a	167.2 a	438.6 a	96.9 a	7.5 a
3%	2.37 a	1.19 a	6.76 a	1.35 a	1.55 a	165.2 a	399.0 a	88.4 a	7.4 a
4%	2.40 a	1.15 a	6.69 a	1.38 a	1.53 a	167.2 a	389.6 a	90.6 a	7.4 a
5%	2.36 a	1.20 a	7.01 a	1.15 a	1.56 a	166.5 a	387.1 a	91.9 a	7.4 a
7%	2.32 a	1.11 a	7.01 a	1.28 a	1.57 a	165.2 a	435.4 a	90.9 a	7.4 a
10%	2.49 a	1.14 a	7.00 a	1.38 a	1.57 a	162.9 a	394.5 a	90.2 a	7.5 a
	Shoot								
Control	3.24 c	1.16 a	5.76 a	1.93 a	1.28 b	88.5 a	123.8 a	47.1 a	3.3 a
1%	3.35 c	1.16 a	5.83 a	1.94 a	1.29 b	91.7 a	122.8 a	48.6 a	3.3 a
2%	3.61 bc	1.16 a	5.98 a	1.88 a	1.26 b	90.9 a	125.0 a	48.1 a	3.3 a
3%	3.59 bc	1.18 a	5.99 a	1.84 a	1.27 b	93.8 a	124.9 a	45.0 a	3.3 a
4%	3.55 bc	1.16 a	5.92 a	1.94 a	1.27 b	90.9 a	119.4 a	46.6 a	3.3 a
5%	3.80 ab	1.17 a	5.90 a	1.94 a	1.34 a	89.3 a	125.6 a	45.0 a	3.3 a
7%	3.92 ab	1.16 a	5.86 a	1.91 a	1.33 a	86.3 a	117.6 a	45.8 a	3.3 a
10%	4.14 a	1.15 a	5.79 a	1.86 a	1.33 a	89.5 a	124.5 a	44.9 a	3.3 a

[z]Values in each column with the same letter are not significantly different according to Duncan's significant difference test ($P \leq 0.01$).

growth regulators found in smoke, had a potential in breaking dormancy of seeds, triggering seed germination and controlling seedling growth in taxa. Karrikins act at concentrations below 1 ppb and are hypothesized to influence the production or metabolism of other phytohormones (Chiwocha et al., 2009; Flematti et al., 2004; Van Staden et al., 2004). Gibberellic acid (GA) plays a key role in dormancy release and promotion of seed germination, whereas abscisic acid (ABA) induces and prolongs seed dormancy (Kucera et al., 2005). Plant-derived smoke extracts can increase endogenous GA levels and decrease ABA level (Chiwocha et al., 2009). This indicates that the smoke-water contains major promotive compounds that stimulate germination of numerous smoke-responsive species and are active at very low concentrations.

Low concentrations of smoke-water (0.1% and 0.2%, v/v) applied to papaya seeds stimulated the endosperm stretch, caused the seedcoat to rupture, and allowed the radicle to elongate and emerge. Plant-derived smoke compounds have been reported to induce two major changes in seedcoat morphology. First, smoke treatment produced an intense chemical scarification at the seed surface with the external cuticle plasticized to form numerous small spheres on the seed surface. Second, smoke significantly altered the permeability of the internal cuticle directly associated with the breaking of seed dormancy (Egerton-Warburton, 1998).

The application of low concentrations of smoke-water to stimulate germination seems to be positive as a result of the amounts of ethylene, GA, and butenolide in the smoke-water. However, high concentrations of smoke-water may have these same components of levels inhibitory to germination. These findings further suggest that higher concentrations of smoke-water, although inhibitory to germination, may positively improve growth of papaya seedling, although prescreening showed that low-concentration smoke-water was ineffective. Papaya seedlings treated with smoke-water improved all growth parameters, including shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, the number of leaves, chlorophyll content, and the seedling vigor index. Ten percent (v/v) smoke-water resulted in significantly greater growth parameter results than the control (Table 2). However, the 5% treatment had the highest root fresh weight. In addition, smoke-water treatments consistently and significantly increased the levels of percent N in roots and shoots and also significantly increased levels of percent magnesium in shoots (Table 3). Roche et al. (1997) suggested that high doses of smoke-water may also confer protection against predation and microbial attack to seeds and perhaps also to seedlings.

Several other studies revealed that smoke-water or smoke-derived compounds can possibly be used in improving and promoting the growth of agricultural and horticultural crops such as red rice (Doherty and Cohn, 2000), indigenous maize (Modi, 2004), indigenous rice (Kulkarni et al., 2006), commercial bean (Van Staden et al., 2006), okra (Kulkarni et al., 2007), tomato (Kulkarni et al., 2007), and onion (Kulkarni et al., 2010). Furthermore, Kulkarni et al. (2008) reported that smoke-water-treated tomato plants increased growth until the fruiting stage, fruited earlier, and increased the number of fruit. These results indicate that smoke-water has the potential to be used in promoting the growth of horticultural and agricultural crops and for the production of healthy and vigorous seedlings (Light and Van Staden, 2004).

Conclusions

Low concentrations of smoke-water (0.1% and 0.2%, v/v) clearly showed significantly potent germination activity in 'Tainung No. 2' papaya seeds and reduced the time to germination. Additionally, all smoke-water concentrations (1% to 10%, v/v) promoted multiple growth attributes of seedlings. Smoke-water enhanced the growth of papaya seedlings by promoting the uptake of some mineral nutrients. The use of smoke technology can be helpful in minimizing the use of chemical fertilizers as well as improving the seed germination rate, shortening germination time, and promoting growth of seedlings and crops.

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