

# Smoke Induced Seed Germination in Maize in Response to Self and Other Plants Biomass-derived Smoke

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## ABSTRACT

Fire ecology plays an important role in germination and establishment of many plant taxa. Smoke induced germination and seedling vigor are well documented in many wild, crop, and weedy species. Karrikins (KAR) substances in smoke are reported to be responsible for these effects. However, only a few experiments have been conducted on different plant-derived smoke effects on particular plant species' seeds. This study was conducted to investigate effects of self-derived and other plants biomass-derived smoke on germination and post-germination processes in maize and its wild relative teosinte. Smoke derived from maize and a legume (*Vigna unguiculata* ssp. *sesquipedalis*) foliage burning was exposed to maize variety Guidan 162 and teosinte (*Zeamexicana* (Schrad.) Kuntze). Germination percentage in both maize and teosinte exposed to maize smoke was found to be significantly higher than unexposed and legume smoke exposure, however germination in legume smoke exposure was found to be slightly higher than control but not significantly so. Shoot length in maize seeds exposed to maize smoke was highest and differed significantly compared to control and legume smoke exposure, while control and legume smoke exposure showed approximately the same shoot lengths. Coleoptile and primary root lengths showed no significant variation among all treatments. Similarly, seminal root length didn't show much variation but legume smoke exposure seeds found to have the lowest seminal root length. Hence direct exposure to smoke without rinsing in water may not positively affect the shoot and root length in maize. Further studies should address morphological traits, transcriptome expression, and enzyme activity to clarify effects of self-derived and other plant-derived smoke on different plant species.

**Key words :** Karrikins, Plant-derived smoke, Teosinte, Fire ecology, Germination

## Introduction

The seed is required for the continuous existence of plant species. Plants have been variously adapted to

keep their seeds protected, dispersed, and germinated when adequate soil and climate conditions are attained. Seed germination is the most important checkpoint for annual plants' survival (Shu *et al.*,

2015), especially during unfavorable environmental conditions. But seed germination is affected by several factors including viability, dormancy, soil moisture, temperature, and light. Seed dormancy is a condition which prevents germination of seed or extension of tubers, even if necessary conditions are provided (temperature, moisture, oxygen, and light) (Bonner, 1984). An important role of seed dormancy is to prevent germination when conditions are favourable for germination, but the probability of seedling survival and establishment is low (Fenner and Thompson, 2005). A high level of seed dormancy is often considered inadmissible for field crops because rapid germination and emergence are generally required in these crops. To some extent, dormancy during seed or kernel development is advantageous to prevent pre-harvest sprouting or precocious germination (Bewley, 1997). Various environmental cues stimulate seed germination, and smoke from burning vegetation is also known to be important for some seeds' germination (Brown and Staden, 1997).

Fire ecology plays an important roles in the germination and establishment of some wild plants, fynbos species, and in pastures (Bradstock and Auld, 1995; Brown *et al.*, 1993 and De Lange and Boucher, 1993). Indeed, fire-ephemeral species known to be obligate fire followers, as they germinate, grow, and flower only after the fire, while their seeds remain dormant in the soil until the next fire event (Nelson *et al.*, 2012). Fire provides both physical and chemical cues to promote germination in various taxa. Heat produced by fire weakens hard seed coats and disrupts structures blocking radicle emergence (Thanos *et al.*, 1992), but besides heat, chemical cues from fire potentially act on soil seed banks (Soós *et al.*, 2019).

Seeds of *Audouinia capitata* (a fynbos species) usually germinate only after fires in natural conditions. It was demonstrated that germination of these seeds was initiated by chemical substances found in smoke derived from burning fynbos plant material (de Lange and Boucher, 1990). Historically poor farmers in South Africa placed maize cobs containing seeds over hut fireplaces for storage (Modi, 2002). Maize seeds exposed to unidentified wood smoke germinated faster and seedlings were more vigorous than those not exposed (Modi, 2002).

Similarly in India, maize and legume seeds are hung in the kitchen and exposed smoke, possibly to maintain seed health (Kumar *et al.*, 2009). Smoke

was also reported to increase seedling vigor of various indigenous medicinal plants (Sparg *et al.*, 2005). Seed responses to fire or smoke may be interpreted as an evolutionary adaptation to ensure germination following a fire, as post-fire reduces much above-ground vegetation (Brown *et al.*, 2003). Use of smoke for ecological restoration and improving germination of seeds of some agricultural and horticultural crops is well documented (Sparg *et al.*, 2005). Smoke acts as a stimulant to enhance germination of approximately 1,200 species in more than 80 genera worldwide (Dixon *et al.*, 2009).

Flematti *et al.*, (2004) Identified a compound present in plant and cellulose-derived smoke as the butenolide 3-methyl-2H-furo[2,3-c]pyran-2-one (1) that promotes germination of various taxa. This compound is stable at high temperatures and same is named as karrikinolide (KAR 1) to distinguish it from the other karrikins and to acknowledge the lactone functional group as a key feature (Flematti *et al.*, 2009). It has been found active at  $10^{-10}$  molar concentration for many plant species (Flematti *et al.*, 2004). However several otheralkyl-substituted 2H-furo[2,3-c]pyran-2-ones are also reported, in addition to butenolide, 3-methyl-2H-furo[2,3-c]pyran-2-one (1) as germination stimulants in smoke (Flematti *et al.*, 2009). The name karrikin has been suggested for this family of butenolides able to break seed dormancy, stimulate germination, and to promote seedling vigor (Dixon *et al.*, 2009). Six major karrikin compounds have been identified and annotated as KAR1-KAR6 in plant-derived smoke, which mainly differ in their methyl substitution (Nelson *et al.*, 2012).

Smoke water containing KAR1 has been found to enhance seedling vigor of several plant species including crops (Jain *et al.*, 2006; Daws *et al.*, 2007). Generally, Karrikins and Strigolactones (SL) are known to be structurally related and share common substituted butenolide moieties (Nelson *et al.*, 2011; Ghebrehiwot *et al.*, 2008). SLs and Karrikins are recognized by two closely related receptors DWARF 14 (D14) and KARRIKIN INSENSITIVE 2 (KAI2), in plants (Walters *et al.*, 2012), and they are both known to interact with the same F-box protein MORE AUXILIARY BRANCHES 2 (MAX2) (Nelson *et al.*, 2011). Many plant species have been reported to be respond to smoke or karrikins, including *Arabidopsis thaliana* (Nelson *et al.*, 2009), lettuce (Drewes *et al.*, 1995), tomato (Jain and Staden, 2006; Kulkami *et al.*, 2008), rice (Kulkami *et al.*, 2006), and maize (Sparg *et al.*, 2006).

Seedling vigor is an important trait in maize, and seedlings with high vigor may escape drought temporarily. A few studies have been done on the effect of smoke and smoke water on maize seed germination and seedling vigor. Both aerosol smoke and smoke water treatment increased germination and seedling vigor in maize (Sparg *et al.*, 2006). Soós *et al.* (2009) reported that smoke water treatment to maize seeds induces stress and ABA related genes are overexpressed in the early post-germination phase, which may improve adaptation of seedlings to their environment. Few studies have compared effects of smoke derived from maize plant itself, or from other plants, on maize and its progenitor teosinte. Hence this investigation is performed to better understand the effect of different plant biomass-derived smoke on seed germination and seedling vigor of maize and its wild relative.

## Materials and Methods

### Plant material and biomass for smoke

Seeds of Guidan 162 corn variety (developed by Maize Research Institute, Guangxi Academy of Agricultural Science, China) and Teosinte (*Zea mexicana* (Schrad.) Kuntze) were cleaned with 3 % Sodium hypochlorite (NaOCl) for 7 minutes and soaked in distilled water for 3 hours before exposure to smoke. For smoke preparation, maize and legume (Yardlong bean, *Vigna unguiculata* ssp. *sesquipedalis*) leaves were shade dried for 14 days, and a 2-meter chimney was prepared by using a clay pot and steel pipe. The temperature of smoke at the top of the chimney was pre-tested and it was approximately 29°C.

### Smoke exposure, growth conditions, and germination test

Both corn and teosinte seeds were exposed to the smoke of maize and legume leaves separately for 20 minutes by holding mesh strainer with seeds at the top of the chimney (taking care to avoid mesh strainer contact with chimney). For control, seeds were exposed to 20 minutes of 29 °C air in an incubator. Immediately after smoke exposure, treatment and control seeds were sprayed with 5 ml water and transferred to 9-cm base diameter plant tissue culture bottles containing 2 layers of wet germination paper. All the treatments viz., maize seeds unexposed to smoke (M-C), maize seeds exposed to

maize smoke (M-M), maize seeds exposed to legume leaves smoke (M-L), teosinte seeds unexposed to smoke (T-C), teosinte seeds exposed to maize smoke (T-M), teosinte seeds exposed to legume leaves smoke (T-L) were replicated 8 times with 26 seeds each. Seeds were incubated in an incubation chamber at 25 °C and 75% relative humidity with 12 hours light/dark cycles for 7 days and germination paper were re-moistened whenever required. The germination percentage initially was taken at 4 and 7 days after incubation for maize and teosinte respectively. Seeds were considered germinated whose radicle and plumule length was 2 mm or more. After 7 days of incubation, shoot length (seed to the tip of the shoot), coleoptile length (distance between first node and point where the first leaf broke), primary root length (embryonic radicle root), and seminal root length (second embryonic root type emerge from the scutellar node) of maize seedlings were measured and recorded.

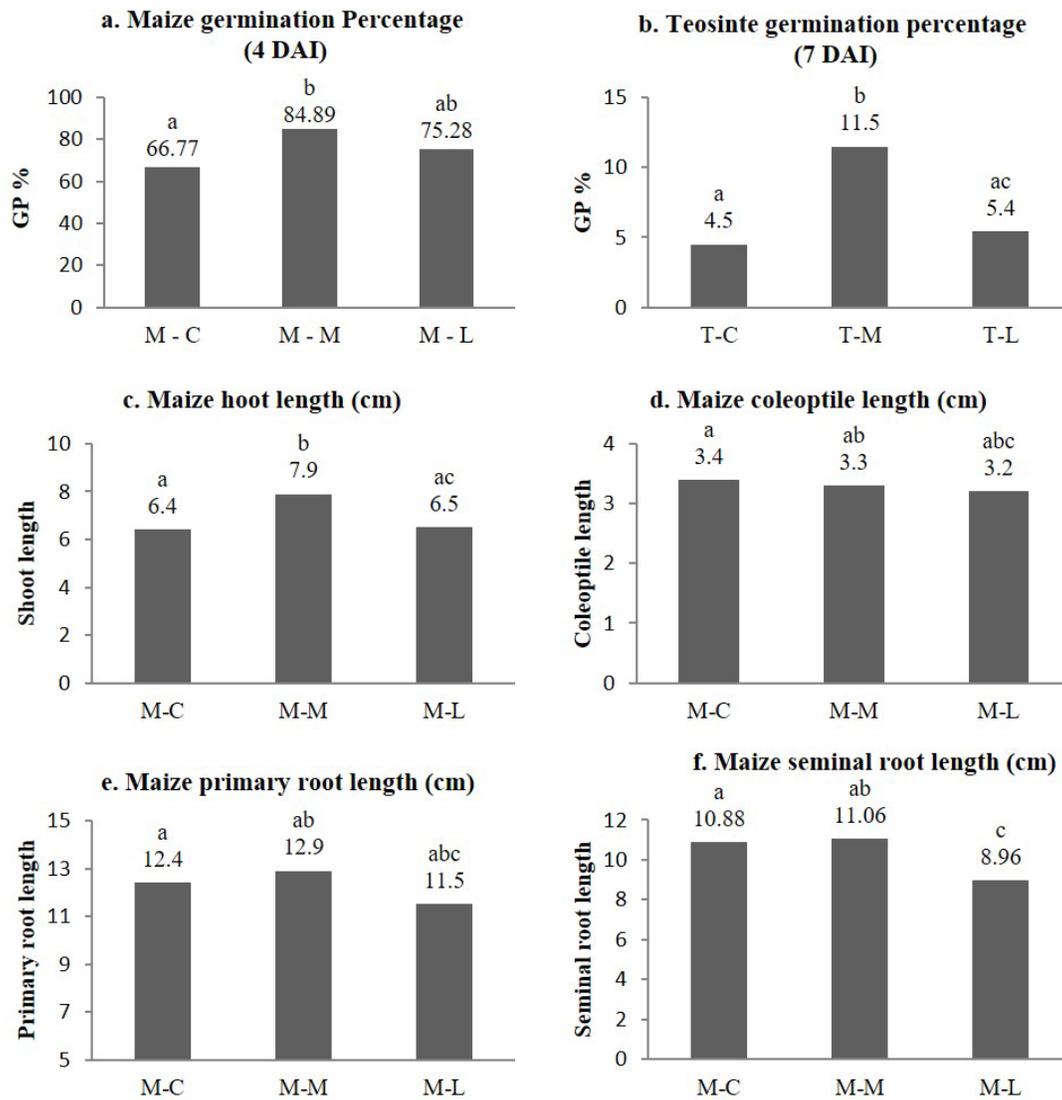
### Statistical analysis and calculations

Germination percentage (GP %) was calculated as (seeds germinated / total seeds) × 100. Shoot length, coleoptile length, primary and seminal root length, and germination percentage were analyzed by single-factor ANOVA at the significant difference ( $p < 0.05$ ) in Excel, and Tukey-Kramer Multiple Comparisons were done for significant difference between treatments.

## Results

### Germination percentage in maize and teosinte

The germination percentage of maize (4 days after incubation) was highest in treatment exposed with maize-derived smoke (85 %) followed by legume smoke exposure (75%) (M-L) and there was a significant difference between control and maize smoke exposure seeds, while no significant difference found between maize smoke and legume smoke exposure seeds, or between control and legume smoke exposure. However, there was a significant difference between no smoke exposure (T-C) and maize smoke exposure (T-M) with 11.5% germination after 7 days after incubation in teosinte. However, T-C and T-L showed nearly the same germination percentage and T-M differed significantly from T-L.



**Fig. 1.** Effects of maize and legume (*Vigna unguiculata* ssp. *sesquipedalis*) derived smoke on a. germination of maize, b. germination of teosinte (*Zea mexicana* (Schrad.) Kuntze), c. shoot length, d. coleoptile length, e. primary root length, and f. seminal root length. Data labels depict significant differences between treatments. (M-C: Maize seeds untreated (control); M-M: Maize seeds treated with maize leaves smoke; M-L: Maize seeds treated with legume leaves smoke; T-C: Teosinte seeds untreated (control); T-M: Teosinte seeds treated with maize leaves smoke; M-L: Teosinte seeds treated with legume leaves smoke). (DAI: Days after Incubation).

### Shoot and Coleoptile length in maize

Shoot length was highest in M-M (7.9 cm), followed by M-L (6.5 cm) and lowest in unexposed (M-C). There was a significant difference between M-C and M-M, but not between M-C and M-L. Coleoptile length was highest in M-C (3.4 cm) and M-M (3.3 cm) but there were no significant differences among treatments.

### Primary and seminal root length

The primary root length was highest in M-M (12.9 cm) and followed by M-C (12.4 cm), but there were no significant differences among treatments. Seminal root lengths were similar in M-C and M-M and lowest in M-L.

### Discussion and Conclusion

This study showed effects of self-derived (maize

leaves) and other plant-derived (legume leaves (*Vigna unguiculata* ssp. *sesquipedalis*)) smoke on the germination and post-emergence morphological parameters of maize (variety Guidan 162) and teosinte (*Zea mexicana* (Schrad.) Kuntze). Self (maize) derived smoke significantly increased germination percentage (85%) in maize compared to unexposed (66.8 %). Similarly, teosinte seeds unexposed and exposed to maize derived smoke differed significantly, but germination percentage in legume derived smoke is slightly higher than control but didn't differ significantly. A similar phenomenon appeared in maize, where germination in control and legume derived smoke exposure is not significant but later (M-L) showed higher germination percentage over control. This supports the previous finding that exposure to aerosol smoke for 30 minutes followed by rinsing significantly increased germination percentage than control in maize (Sparg *et al.*, 2006) where low concentration rice smoke water promoted germination in papaya seeds (Chumpookam *et al.*, 2012). The shoot length of maize smoke exposure is highest with 7.9 cm with compared control (6.4 cm), while shoot length was approximately similar in both control and legume smoke exposure (6.5 cm).

However, coleoptile lengths in all treatments were similar without significant differences. Primary and seminal root lengths were higher for maize smoke-exposed seeds, but there was no significant difference. But both values were lowest in legume smoke-exposed treatment. Maize seeds exposed to smoke followed by rinsing showed the highest shoot and root length than unexposed, but smoke-exposed unrinsed showed approximately same root length and very slightly higher shoot length with respect to control (Sparg *et al.*, 2006). Smoke water treated rice seeds showed higher shoot length and root length than untreated (Kulkarni *et al.*, 2006). Hence direct exposure to smoke without rinsing in water may not positively affect the shoot and root length in maize. One shortcoming in this experiment was that post-germination measurements were made in an intentionally non-stressful environment to mainly focus on effect of smoke on germination without the interaction with stress conditions. Hence future experiments can include interaction effect of stress and smoke on germination.

Karrikins are usually produced burning of different plant materials, straw, filter paper, and even sugar; their production from polysaccharides and sugar was proposed as source of pyran ring in

karrikins and which may be derived directly from pyranose sugars in plant material (Flematti *et al.*, 2015). Karrikins in both maize and legume derived smoke increased germination percentage in both maize and teosinte here. Fire and smoke would have played an important role during evolution of germination, as teosinte with hard seed coat responded positively to maize derived smoke exposure. The highest germination and shoot length in maize derived smoke exposure seeds suggests self-derived smoke exposure may have an extra effect, but root and coleoptile lengths appeared unaffected. Hence, additional studies should be performed concerning morphological traits, transcriptome expression, and enzyme activity to fully understand effects of self- and other plant-derived smoke on this and other crop species.

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