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EFFECT OF PLANT EXTRACTED SMOKE AND REVERSION OF ABSCISIC ACID STRESS ON LETTUCE

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Abstract

The stimulation of seed germination by smoke and aqueous smoke extracts has received much attention in recent years. However, the combine effects of smoke with plant hormones on seed germination remain unknown. This investigation describes how lettuce (*Lactuca sativa*) seeds respond to smoke solution and how the smoke solution can alleviate abscisic acid (ABA) stress. The results showed that plant extracted smoke treated seeds had significantly quicker germination, higher germination percentage, fresh weight, relative seed germination percentage, peak value, germination value, shoot and root length, germination index and vigor index as compared to control. No germination was observed in ABA (0.1mM) treated seeds but significant number of seeds germinated when ABA and smoke solutions were mixed together. It was observed that smoke solutions overcome the inhibitory effects of ABA and improved germination and seedling vigor, indicating that smoke alleviated ABA stress not only at the germination level but also at seedling stage.

Introduction

The significance of the discovery in ninties that plant-derived smoke is responsible for promoting seed germination (De Lang & Boucher, 1990) has quickly captured the attention of both basic and applied plant scientists for its novel and surprising effects. It has now been well established that smoke is a broadly effective stimulant that enhances germination of approximately 1200 species in more than 80 genera worldwide (Dixon *et al.,* 2009). It is evident that the smoke has shown dynamic stimulatory effects on seed germination in species from both fire-prone habitats and fire-free habitats (Drewes *et al.,* 1995; Pierce *et al.,* 1995; Thomos & Van Staden, 1995). Studies of Sparg *et al.,* (2005) indicated that the effects of smoke extended beyond germination and enhanced seedling vigor. Similarly, Blank & Young (1998) showed that smoke improves emergence and seedling growth of different grasses. It has also been shown that smoke solutions stimulate flowering (Keeley, 1993), rooting (Taylor &Van Staden, 1996) and somatic embryogenesis (Senaratna*et al.,* 1999).Smoke contains thousands of different compounds and there have been many attempts to identify the active compound(s) (Preston *et al.,* 2004). In 2004, a potent bioactive compound in the cellulose-and plant-derived smoke was identified as 3-methyl-2H-furo [2,3-c]pyran-2-one (1) (Flematti *et al.,* 2004; Van Staden *et al.,* 2004), which was recently named karrikinolide (or KAR1) after the aboriginal noongar word for smoke, karrik (Flematti *et al.,* 2009). Dormancy in some vegetable seeds, e.g. *Lactuca sativa* (Drewes *et al.,* 1995) and *Oryza sativa* (Doherty & Cohn, 2000), is also broken by smoke solution. The dormancy breaking actions of combustion products has been identified as being similar to that of GAs, when applied to lettuce seeds (Drewes *et al.,* 1995), and cytokinins, using celery seeds (Thomas &Van Staden, 1995). The treatment of grand rapidslettuce seeds with different dilutions of an aqueous smoke extract produce a response curve similar to the phytohormone response curves (Drewes *et al.,* 1995).

The effect of a *Themedatriandra* smoke-derived extracts on the germination of grand rapids lettuce seed maintained in the dark indicated that high concentration, above 1:50 dilutions, were distinctly inhibitory to germinate. Higher dilutions, namely 1:100, 1:500, 1:1000 times dilutions, (Drewes *et al.,* 1995) significantly increased germination compared to water controls. This indicated that the smoke-derived extract was active over a wide concentration range. Thus, grand rapidslettuce seeds from different origins all responded positively to the smoke extracts and they were all smoke-sensitive over the same wide range of dilutions (Drewes *et al.,* 1995).

The plant hormone abscisic acid (ABA) belongs to a class of metabolites known as isoprenoids, also called terpenoids and derived from a common five-carbon (C5) precursor, isopentenyl (IDP). It is a universal plant hormone preventing growth and development in plants. ABA inhibits seed germination of many species (Gallardo *et al*., 1991; Divi & Krishna, 2010; Rehman *et al*., 2011; Tabur & Öney, 2012). ABA is also reported to inhibit seed germination by inhibiting GA biosynthesis directly under high temperature (Gonai *et al.,* 2004). Ihle & Dure (1970; 1972) demonstrated that ABA inhibits precocious germination of cotton embryos. ABA has been suggested to induce a dormant state during the later phase of seed maturation, after this point its function is limited because the concentration falls below an inhibiting level. GA is required to overcome this ABA induced dormant state. However, ABA levels increase upon imbibition in dormant seeds and not in non-dormant seeds. LePage-Degivry & Garello (1992), Wang *et al.,* (1995) and Grappin *et al.,* (2000) indicated that the actual level of ABA during imbibition is important. It has been shown that the fundamental processes such as cell division and the synthesis of DNA, RNA and protein become inhibited in plant system treated with ABA (Fountain & Bewley, 1976). In barley aleorone tissue and cotton embryos exogenous ABA prevents the permeation of enzymes which are involved in mobilizing storage material during germination (Dure, 1975; Varner, 1976). Although the molecular nature of the physiological block by which

ABA prevents the completion of germination is still unknown, it is generally believed that ABA functions by interfering with mRNA synthesis, processing or translation (Dure, 1975).

Seed germination and the establishment of a normal seedling are determining features for the propagation of plant species, which are of both economic and ecological importance. Because of its high vulnerability to injury, disease, and water/environmental stress, germination is considered to be the most critical phase in the plant life cycle (Nonogaki *et al.,* 2010). Rapid emergence and good seedling establishment is one of the most important aspect of seed production in the field. In the other hand germination and emergence are important issues in plant production because they have a significant effect on the next stages of plant growth in the field. Rapid and uniform seedling emergence are essential to obtain high yield with good quality and quantity in annual crops (Yari *et al.,* 2010).The purpose of this investigation was to study the effects of plant-derived smoke on seed germination and seedling vigor of *Lactuca sativa* and to find that whether different dilutions of above mentioned smoke solutions can alleviate ABA stress or not.

Materials and Methods

Plant collection and production of smoke solutions: *Cymbopogonjwarancusa* and *Zeamays* plants were collected from the outskirts of KUST, Kohat, Pakistan. Smoke was generated by burning semi dried plant material (333g) in a specially designed furnace and forced smoked to bubble out into 1 liter of DDW through a pipe. The derived smoke was diluted up to 1:10000 for further use. Abscisic Acid (ABA) solution of 0.1mM was prepared and the effect on seed germination of it was investigated.

Seed source: Lettuce (*Lactuca sativa*) seeds were purchased from Shehzad Seeds Store W-374 Fowara Chowk Raja Bazar Rawalpindi, Pakistan and were a product of Takii& Co., Ltd. Kyoto, Japan. The seeds were stored at 4°C for further use.

Germination test: All the Petri dishes, filter papers, forceps, pipettes, conical flasks, volumetric flasks beakers and scissors used in these tests were autoclaved. Three replicas of each treatment with 10 seeds in each were placed on a double layer of Whatman No.1 filter paper in 9cm Petri dishes, moistened with 3-5 ml of distilled water (control) or the respective test solutions. Petri dishes were kept in a dark chamber at 22±4°C. Germination counts were taken on a daily basis for 10 days. After 10 days, root shoot length, fresh and dry weight of roots & shoots were measured. Seed germination, root elongation and germination index (GI, a factor of relative seed germination and relative root elongation) were evaluated according to Tam &Tiquia (1994).

$$\text{Relative seed germination (\%)} = \frac{\text{Number of seeds germinated in test solution}}{\text{Number of seeds germinated in the control}} \text{ X } 100$$

$$\text{Relative root elongation (\%)} = \frac{\text{Mean root elongation in plants with test solutions}}{\text{Mean root elongation in the control}} \text{ X } 100$$

$$\text{Germination Index} = \frac{\text{\% Seed germination x \% Root elongation}}{100}$$

The vigor index was calculated according to (Orchard. 1977). Seedling vigor index (SVI) = [seedling length (cm) × germination percentage]

Shoot and root of each seedling was cut carefully, measured and averaged. For seed germination, method of (Ramana *et al.,* 2002) was followed. Seed parameters were recorded by following a formula:

Speed of germination=number of seeds germinated/ days offirst count + number of seeds germinated/ days offinal count

Peak value = cumulative percent germination on each day/number of days elapsed since initial imbibitions

Germination value = Peak value × Germination (%)

Alleviating solution: Alleviating solution was made by the combination of smoke and ABA solutions. Both of these solutions have contrasting effects, one promontory while other inhibitory. Therefore, it was hypothesized that the combination of both solutions could minimize the inhibitory effect of ABA. A combine solution of ABA (0.1mM) and *Cymbopogon* smoke (1:500) or maize smoke (1:5000) was made. Smoke dilutions were selected on their promotory performance in previous experiments. The effect of these alleviating solutions was studied on lettuce seed germination and seedling growth.

Statistical analysis: One-way analysis of variance (ANOVA) with multiple mean comparisons were carried out on data using Graph Pad Prism software (version 5.0, San Diego, California USA). The purpose of these tests was to identify statistically significant effect and interactions among various tests and control treatments. The significant difference between the mean values of various treatments were determined using Duncan's multiple range test (DMRT) at $p<0.05$ using Statistical Analysis System (SAS 9.1)

Results

Seed germination: *Lactuca sativa* respond differently to both smoke solutions in terms of germination parameters as is the characteristic feature of plant derived smoke. While studying germination, it was observed that 1:100,1:500 and 1:1000 v/v dilutions of *cymbopogon* smoke showed highly significant ($p<0.05$) increased the germination percentage of lettuce over control (Table 1). The results are different for maize smoke with almost decreased germination percentages as only 1:1000 v/v showed better germination percentage as compared to control (Table 2). 1:500 and 1:1000 dilutions v/v of *Cymbopogon* smoke showed highly significant ($p<0.05$) increased germination index, while 1:100 and 1:5000 showed significant ($p<0.05$) increase in germination index as compared to control (Fig. 1a). In case of maize derived smoke, 1:5000 and 1:10000 dilutions showed highly significant ($p<0.05$) increased germination index while 1:100 and 1:1500 significantly ($p<0.05$) increased germination index over control (Fig. 4a). With respect to control, Speed of germination of *Cymbopogon* derived smoke was observed to be higher than control however maize derived smoke showed similar results as a control. For peak value, no statically significant promoting effect was observed for any of the smoke solution (Figs. 1b-c & Figs. 4b-c). 1:100, 1:500 and 1:1000 v/v dilutions of *Cymbopogon* derived smoke and 1:1000 and 1:5000 of maize derived smoke showed highly significant ($p<0.05$) increase in terms of germination value over the control (Fig. 1d, Fig. 4d).

Relative seed germination (%) was significantly ($p<0.05$) improved by all dilutions of *Cymbopogon* derived smoke (except 1:10000) over control (Fig. 2a), while in maize derived smoke dilutions, only 1:1000 and 1: 5000v/v dilutions showed significant ($p<0.05$) increased relative root elongation as compared to control (Fig. 5a).

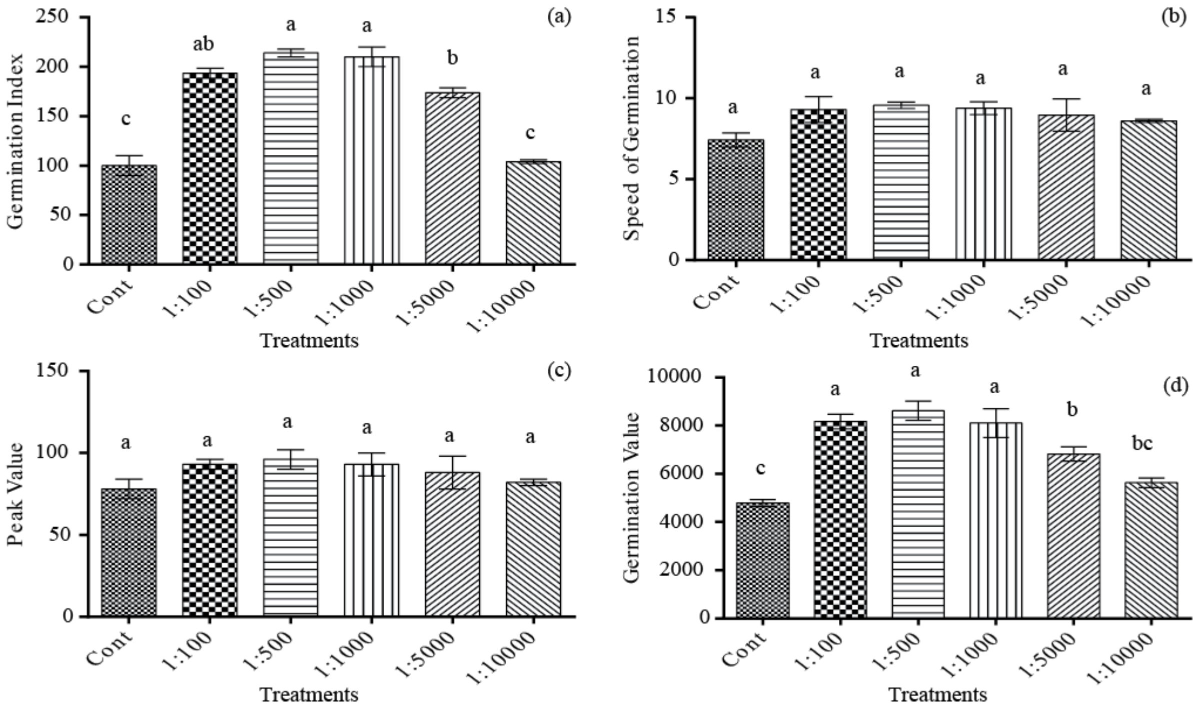

Fig. 1. Effect of *Cymbopogon* derived smoke with different dilutions on germination index (a), speed of germination (b), peak value (c) and germination value (d) of *Lactuca sativa*. Each value is the mean ±SE of 3 replicates per treatments. Different letter indicates siginificant ($p<0.05$) differences between control and smoke treated plants as evaluated by DMRT.

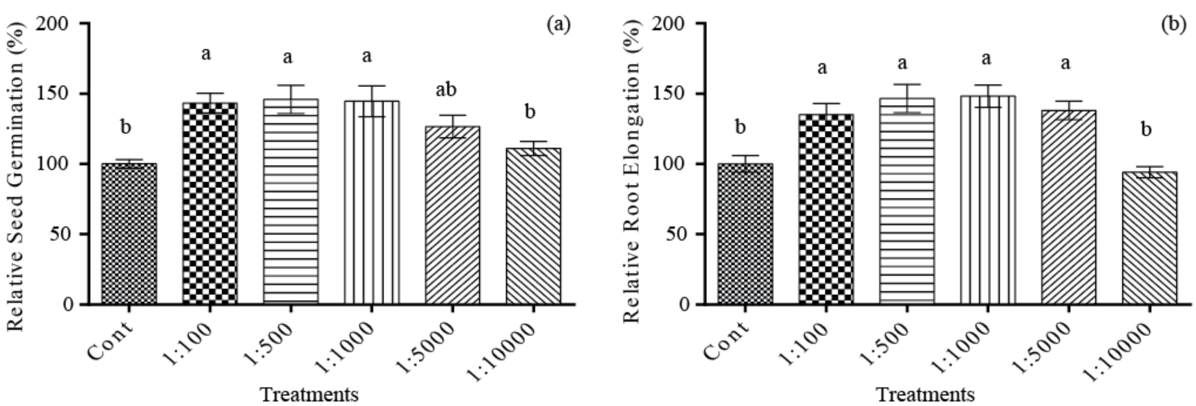

Fig. 2. Effect of *Cymbopogon* derived smoke with different dilutions on relative seed germination % (a) and relative root elongation % (b) of *Lactuca sativa*. Each value is the mean ± SE of 3 replicates per treatments. Different letter indicates significant ($p<0.05$) differences between control and smoke treated plants as evaluated by DMRT.

Table 1. Effect of *Cymbopogon* derived smoke with different dilutions on growth attributes of *Lactuca sativa*.

Growth attributes	*Cymbopogon* derived smoke with different dilutions					
	Control	1:100	1:500	1:1000	1:5000	1:10000
Germination (%)	61.6 ± 1.6c	88.3 ± 1.7a	90 ± 4a	87.3 ± 1.5a	78 ± 1.5b	68.3 ± 1.5c
Shoot length (cm)	4.07 ± 0.12c	4.20 ± 0.06bc	4.50 ± 0.12a	4.40 ± 0.06ab	4.23 ± 0.07ab	4.13 ± 0.09bc
Root length (cm)	2.00 ± 0.06b	2.70 ± 0.06a	2.95 ± 0.03a	2.97 ± 0.03a	2.75 ± 0.08a	1.88 ± 0.49b
Shoot fresh weight (g)	0.23 ± 0.04c	0.32 ± 0.02ab	0.36 ± 0.02a	0.29 ± 0.02ab	0.28 ± 0.01bc	0.30 ± 0.01ab
Root fresh weight (g)	0.0373 ± 0.01c	0.0627 ± 0.01ab	0.0560 ± 0.02a	0.0541 ± 0.02ab	0.0654 ± 0.01bc	0.0521 ± 0.02ab

In each row, different litters indicate significant (p<0.05) difference between control and *Cymbopogon* derived smoke treated *Lactuca sativa* plants according to DMRT. Values in the table refer to mean ± SE

Table 2.Effect of maize derived smoke with different dilutions on growth attributes of *Lactuca sativa*.

Growth attributes	maize derived smoke with different dilutions					
	Control	1:100	1:500	1:1000	1:5000	1:10000
Germination (%)	62.5 ± 2.04ab	52.5 ± 2.04b	60 ± 4.08ab	67.5 ± 2.04ab	70 ± 2.05a	62.5 ± 6.12ab
Shoot length (cm)	2.4 ± 0.2b	3.2 ± 0.14a	2.85 ± 0.3ab	2.9 ± 0.2ab	3.1 ± 0.05a	2.8 ± 0.3ab
Root length (cm)	1 ± 0.1b	1.5 ± 0.25ab	1.5 ± 0.3ab	0.95 ± 0.05b	1.7 ± 0.4ab	2 ± 0.3a
Shoot fresh weight (g)	0.15 ± 0.02c	0.18 ± 0.01bc	0.25 ± 0.02ab	0.17 ± 0.01c	0.26 ± 0.04a	0.25 ± 0.01ab
Root fresh weight (g)	0.02 ± 0.001b	0.025 ± 0.002ab	0.03 ± 0.001a	0.029 ± 0.002a	0.032 ± 0.002a	0.032 ± 0.004a

In each row, different litters indicate significant (p<0.05) difference between control and *Cymbopogon* derived smoke treated *Lactuca sativa* plants as evaluated by DMRT. Values in the table refer to mean ± SE

Seedling vigor: Shoot and root length was improved with plant derived smoke but at different dilutions. Except 1:100 and 1:1:10000 of *Cymbopogon* derived smoke, all dilutions of both smoke solutions significantly (p<0.05) increased shoot length over control (Tables1&2). In case of root length, 1:10000 of *Cymbopogon* derived smoke dilution and 1:1000 of maize derived smoke dilution showed no significant difference over control while the rest of dilutions of the both smokes showed significant (p<0.05) increased root length as compared to control (Tables 1&2). Similarly all dilutions of both smokes (except 1:100 and 1:1000 for shoot length) showed significantly (p<0.05) increased shoot and root fresh weight (Tables 1&2) as compared to control. Those plants having higher shoot length was observed to have higher fresh weights of shoots and roots. The above situation was also observed in the case of relative root elongation. All dilutions of both smoke solutions showed significantly (p<0.05) higher relative root elongation except 1:1000 of maize derived smoke and 1:10000 of *Cymbopogon* derived smoke (Figs. 2b& 5a). Vigor index of plants treated with both smoke solutions showed significantly increased (p<0.05) vigor index as compared to control (Figs. 3& 6), however 1:10000 v/v of *Cymbopogon* derived smoke showed no significant increase in vigor index.

Effect of Abscisic acid and smoke solutions on seed germination: After studying the germination and post germination responses of *Lactuca sativa* towards *Cymbopogon* and maize derived smoke solutions, another experiment was conducted to check the ABA effects on lettuce seed germination. As usual, Results from experiment showed very strong inhibitory effects of ABA on lettuce seed germination (Fig. 7) and up to 6 days, there was no germination in ABA treated plates. Then the same concentration of ABA (.1mM) was made in 2 most responsive smoke dilutions of both plants (one dilution of each). Alleviating solutions (Smoke + ABA) was observed to overcome the negative effects of ABA stress. Alleviating solutions significantly improved germination percentage of lettuce when compared with ABA dilution in DDW treated seeds. In ABA dilution in DDW treated seeds, no emergence of radical was observed while seeds treated with Alleviating solution significantly improved seed germination and shoot length compared to ABA dilution in DDW treated seeds in which it was zero (Figs. 8 & 9).

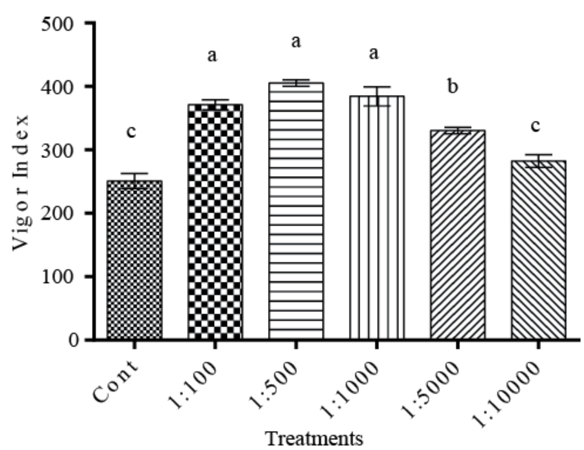

Fig. 3. Effect of *Cymbopogon* derived smoke with different dilutions on vigor index of *Lactuca sativa*. Each value is the mean ± SE of 3 replicates per treatments. Different letter indicates siginificant (p<0.05) differences between control and smoke treated plants as evaluated by DMRT.

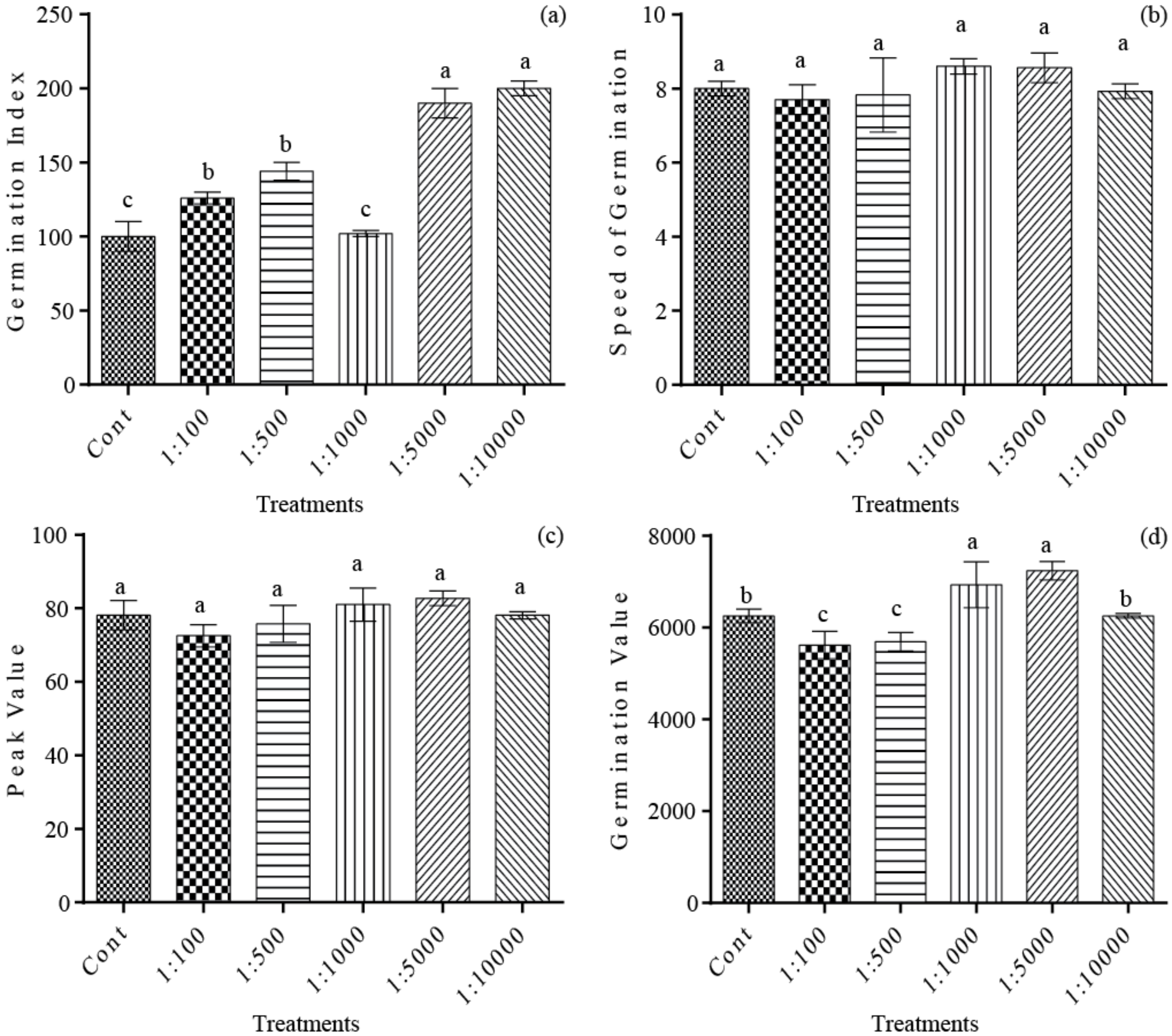

Fig. 4. Effect of maize derived smoke with different dilutions on germination index (a), speed of germination (b), peak value (c) and germination value (d) of *Lactuca sativa*. Each value is the mean ± SE of 3 replicates per treatments. Different letter indicates significant ($p<0.05$) differences between control and smoke treated plants as evaluated by DMRT.

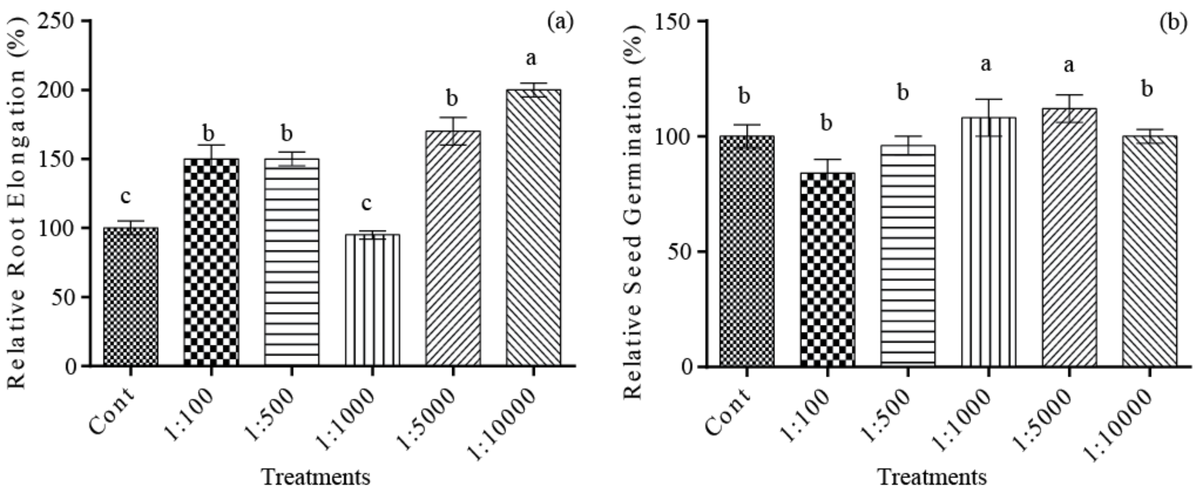

Fig. 5. Effect of maize derived smoke with different dilutions on relative seed germination % (a) and relative root elongation % (b) of*Lactuca sativa*. Each value is the mean ±SE of 3 replicates per treatments. Different letter indicates siginificant ($p<0.05$) differences between control and smoke treated plants as evaluated by DMRT.

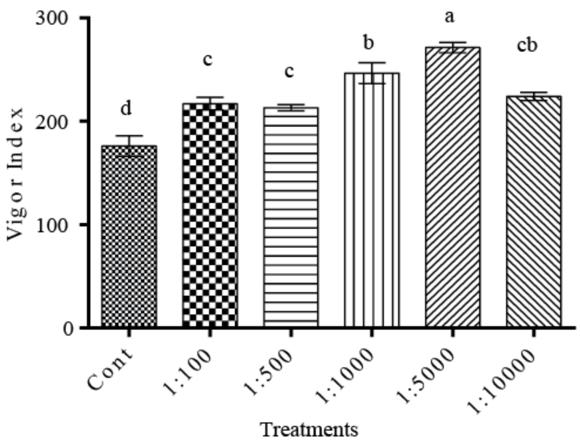

Fig. 6. Effect of maize derived smoke with different dilutions on vigor index of *Lactuca sativa*. Each value is the mean ±SE of 3 replicates per treatments. Different letter indicates significant ($p<0.05$) differences between control and smoke treated plants as evaluated by DMRT.

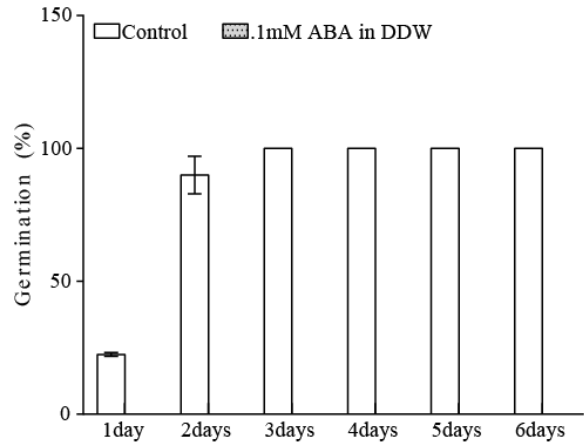

Fig. 7.Comparative effect of 0.1mM ABA dilution and DDW (control) on seed germination percentage of *Lactuca stiva* from 1[st]day to 6[th] day respectively. Each data point shows mean ± SE of three replicates. Up to 6days no germination was recorded.

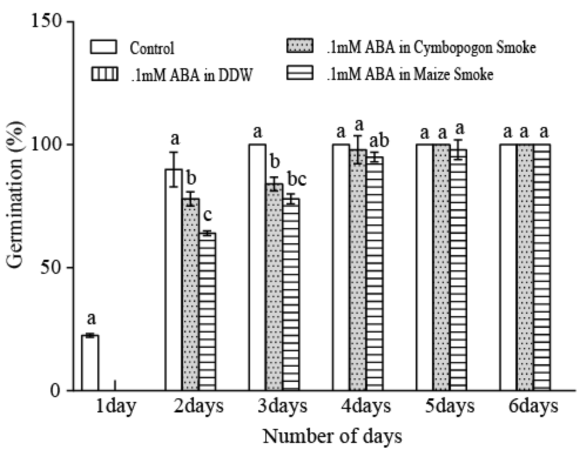

Fig. 8. Comparative effect of control, 0.1mM ABA in DDW, 0.1mM ABA in *Cymbopogon* derived smoke and .1mM of ABA inmaize smoke solutions on seed germination percentage of *Lactucasative*. In each row, different litter indicates significant ($p<0.05$) difference between control and other three different ABA dilutions according to DMRT. Values in the figure refer to mean ± SE of three replicates.

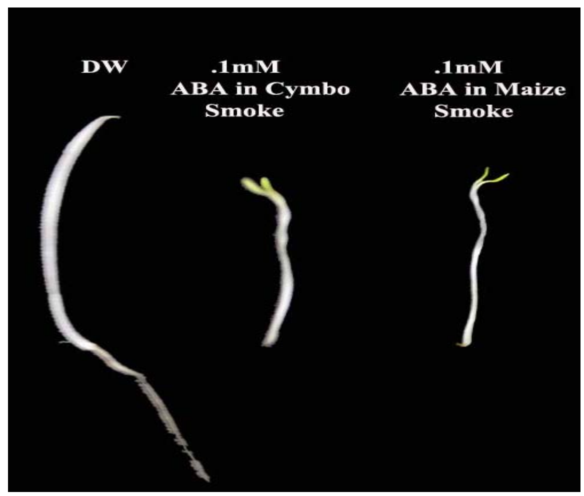

Fig. 9. Comparative effect of control, .1mM ABA in *Cymbopogon* derived smoke and .1mM of ABA in Maize smoke solutions on seedling vigour of *Lactuca sativa*, shows that plant derived smoke have alleviated ABA not only at germination level but also at seedling vigor.

Discussion

Plant-derived smoke is one of the most convenient means of application. The biologically active compounds present in smoke readily dissolve in water and when this smoke-extract is used as diluted solution, treated seeds of many species show a marked improvement in germination. De Lange & Boucher (1990) were the first to report on this phenomenon. Now it has been well established that smoke is a broadly effective stimulant that enhances seed germination (Brown & Van Staden, 1997; Roche *et al.,* 1997; Lloyd *et al.,* 2000; Wills & Read, 2002; Dixon *et al.,* 2009). This study highlights the effect of *Cymbopogon* and maize extracted smoke on the germination and seedling vigor of lettuce (*Lactuca sativa*) and describes combine effects of smoke and abscisic acid.

This study confirms and extends previous findings (Clark & French, 2005; Dixon *et al.,* 1995) by showing that the degree of smoke responsiveness is a specific attribute. In this study, smoke extract up to 1:10000 times dilution was used. While studying germination, it was observed that 1:100,1:500 and 1:1000 v/v dilutions of *Cymbopogon* smoke showed highly significant ($p<0.05$) increased the germination percentage of lettuce over control. This provides a justification to Brown & Van Staden (1997) who reported that more dilute solution improve seed germination. The results were different for the maize smoke with almost decreased germination percentages as only 1:1000 v/v showed better germination percentage as compared to control. However it was in accordance with Sparg *et al.,* (2005) who stated that although smoke treatment may not necessarily have an effect at the germination stage, it may play a role in the post germination stage and suggested many species not respond to smoke treatments, these species may show some responses at their post germination stages, i.e. improved seedling vigor.

Similar is the case here with the speed of germination and peak value parameters of both plants derived smoke solutions.This study has shown that smoke solutions of both plants (*Cymbopogon* and maize) enhance growth of lettuce seedlings. Shoot and root length was improved with plant derived smoke but at different dilutions. Except 1:100 and 1:1:10000 of *Cymbopogon* derived smoke, all dilutions of both smoke solutions significantly ($p<0.05$) increased shoot length over control (Tables 1&2).In case of root length, 1:10000 of *Cymbopogon* derived smoke dilution and 1:1000 of maize derived smoke dilution showed no significant difference over control while the rest of dilutions of the both smokes showed significant ($p<0.05$) increased root length as compared to control (Tables 1&2).The results are similar to Sparg *et al.,* (2006) who stated that seedlings grown from seeds exposed to plant derived smoke produced longer roots and shoots.Similarly all dilutions of both smoke solutions (except 1:100 and 1:1000 for shoot length) showed significantly ($p<0.05$) increased shoot and root fresh weight (Tables 1&2) as compared to control. Those plants having higher shoot length was observed to have higher fresh weights of shoots and roots. Zhou *et al.,* (2011) also stated the similar results. The same effect was also observed in the case of relative root elongation. All dilutions of both smoke solutions showed significantly ($p<0.05$) higher relative root elongation except 1:1000 of maize derived smoke and 1:10000 of *Cymbopogon* derived smoke (Figs. 2b & 5a). These results also conform findings of Abdollahi *et al.,* (2011).

As known, exogenous ABA is a potent inhibitor of seed germination and seedling growth in many species (Kabar 1997; Gallardo *et al.,* 1991; Divi & Krishn, 2010). It inhibits continuation of embryo development during germination and related developmental process (Borrow, 1974). Ilhe & Dur (1970; 1972) demonstrated that ABA inhibits precocious germination of cotton embryos. Our results also indicated that ABA at 0.1, 0.5 and 1.0 mM dilutions strongly inhibited germination of lettuce seeds. In this investigation. As stated earlier, smoke promotes seed germination and seedling vigor. So it was of a great interest whether smoke can alleviate ABA stress or not.

The results were quite surprising. Both dilutions of ABA within smoke alleviated ABA stress and seed germination occurred. With the passage of time also improved seedling vigor was observed, indicating that smoke has alleviated ABA stress not only at the germination level but also at seedling vigor. This suggests three possibilities. One Possibility may be that exogenous ABA prevents the formation of enzyme which are involved in mobilizing storage materials during germination (Dure, 1975;Varner, 1976) so may be the case smoke compounds present in ABA dilutions prevent ABA to stop the formation of that particular enzyme which are involved in mobilizing storage material during germination.

Secondly, It has been shown that fundamental process such as cell division and the synthesis of DNA, RNA and protein become inhibited when exogenous ABA is applied during seed germination (Fountain & Bewly, 1976; Borrow, 1974; Pilet, 1978). This suggests that compounds in smoke do not allow Absisic acid to inhibit cell division and synthesis of protein by bringing some structural or genetical changes in the ABA.

Thirdly and the most important aspect of smoke is, it contains nitrogenous compounds, nitrates, nitric oxides and cyanides (Gavin *et al.,* 2011), which reduce seed dormancy and increase seed germination (Oracz *et al.,* 2008; Alboresi *et al.,* 2005). As ABA is a positive regulator of dormancy induction and most likely also maintenance, while it is a negative regulator of germination. GA releases dormancy, promotes germination and counteract ABA effects. Here plant derived smoke plays the same role as of GA through nitrogenous compounds, nitrates and cyanides. Nitrates have the capability to release dormancy independently of nitrate reductase and act as a signal not the nutrient. Exogenous application of nitrates to plants reduces ABA contents of mature dry seeds, whereas addition of nitrate to a germination medium leads to faster decline in the ABA in imbibed seeds than a water imbibed control (Matakiadis *et al.,* 2009) which results in a uniform seed germination. These changes in ABA content are caused primarily by CYP7A2, the gene for which is induced by nitrate both in developing and imbibed germinating seeds (Matakiadis *et al.,* 2009). NO breaks seed dormancy in many species. NO also reduces the ABA sensitivity of dormant Arabidopsis seeds.

Cyanide is generally reported as an important signaling molecule for a variety of plant processes, including seed germination (Siegien & Bogatek, 2006).There are also some seeds which are known to evolve hydrogen cyanide during the germination process and helps the seed to accomplish the mission getting the normal seedling stage (Esashi *et al.,* 1991a, Esashi *et al.,* 1991b). The mode of action of cyanide in stimulating seed germination is thought to involve reactive oxygen species. It has also been proposed that cyanide acts at an initial step in dormancy alleviation and nitrogen oxide is required at a later step (Bethke *et al.,* 2006). Thus Plant derived smoke with the help of cyanides, nitrates and nitrogen oxides, plays a vital role in releasing dormancy caused by increased exogenous or endogenous levels of Abscisic acid. The discovery of this phenomena created the possibility of studying the physiological roles of new natural compounds present in smoke and their role in hormonal pathways. It also allowing a better understanding of the role of smoke in releasing dormancy and possible mechanism behind alleviation of Abscisic acid stress. However, physiological and biochemical investigations are essential to unravel the facts through which smoke alleviate Abscisic acid stress.

Conclusion

It is concluded from present work that *Cymbopogon* and maize derived smoke solutions significantly increased seed germination, seedling vigour and furthermore, reported for the first time that plant derived smoke alleviated abscisic acid (ABA) stress which indicates that plant derived smoke could alleviate the effect of other environmental stress e.g. drought etc.

Acknowledgement

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References

Abdollahi, M.R., B. Mehrshad and S.S. Moosavi. 2011. Effect of method of seed treatment with plant derived smoke solutions on germination and seedling growth of milk thistle (*Silybummari anum* L.). *Seed Sci. & Technol.*, 39: 225-229.

Alboresi, A., C. Gestin, M.T. Leydecker, M. Bedu, C. Meyer and H.N. Truong. 2005. Nitrate a signal relieving seed dormancy in Arabidopsis. *Plant Cell and Environment*, 28: 500-512.

Bethke, P.C., I.G.L. Libourel, V. Reinohl and R.L. Jones. 2006. Sodium nitroprusside, cyanide, nitrite and nitrate break Arabidopsis seed dormancy in a nitric oxide-dependent manner. *Planta*, 223: 805-812.

Blank, R.R. and J.A. Young. 1998. Heated substrate and smoke: influence on seed emergence and plant growth. *J. Range Management*, 51: 577-583.

Brown, N.A.C. and J. Van Staden. 1997. Smoke as a germination cue: A review. *Plant Growth Regulation*, 22: 115-124.

Clark, S. and K. French. 2005. Germination response to heat and smoke of 22 Poaceae species from grassy woodlands. *Austr. J. Bot.*, 53:445-454.

De Lange, J.H. and C. Boucher. 1990. Autecological studies on *Audouinia capitata* (*Bruniaceae*). I. Plant-derived smoke as a seed germination cue. *South African J. Bot.,* 56: 700-703.

Divi, U.K. and P. Krishn. 2010. Over expression of the brassino steroid biosynthetic gene AtDWF4 in arabidopsis seeds overcomes abscisic acid-induced inhibition of germination and increases cold tolerance in transgenic seedlings. *Plant Growth Regulation*, 29: 385-393.

Dixon, K.W., D.J. Merritt, G.R. Flematti and E.L. Ghisalberti. 2009. Karrikinolide: A phytoreactive compound derived from smoke with applications in horticulture, ecological restoration, and agriculture. *Acta Horticulturae,* 813: 155-170.

Dixon, K.W., S. Roche and J.S. Pate. 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia*, 101: 185-192.

Doherty, L.C. and M.A. Cohn. 2000. Seed dormancy in red rice (*Oryza sativa).* XI. Commercial liquid smoke elicits germination. *Seed Sci. Res.*, 10: 415-421.

Drewes, F.E., M.T. Smith and J. VanStaden. 1995. The effect of plant-derived smoke extract on the germination of light sensitive lettuce seed. *Plant Growth Regulation,* 16: 205-209.

Dure, L.S. 1975. Seed formation. *Annual Review of Plant Physiology*, 26: 259-278.

Esashi, Y., K. Isuzugawa, S. Matsuyama, H. Ashinoand and R. Hasegawa. 1991b. β-Glucosidase activities and HCN liberation in unimbibed and imbibed seeds, and the induction of cocklebur seed germination by cyanogenic glycosides. *Physiologia Plantarum*, 83: 34-40.

Esashi, Y., K. Isuzugawa, S. Matsuyama, H. Ashinoandand R. Hasegawa. 1991a. Endogenous evolution of HCN during pre-germination periods in many seed species. *Physiologia Plantarum*, 83: 27-33.

Flematti, G.R., E.L. Ghisalberti, K.W. Dixon and R.D. Trengove. 2004. A compound from smoke that promotes seed germination. *Science*, 305: 977.

Flematti, G.R., E.L. Ghisalberti, K.W. Dixonand and R.D. Trengove. 2009. Identification of alkyl substituted 2H-furo[2,3-c]pyran-2-ones as germination stimulants present in smoke. *J. Agric. Food Chemist*, 57: 9475-9480.

Fountain, D.W. and J.D. Bewley. 1976. Lettuce seed germination. Modulation of pregermination protein synthesis by gibberellic acid, abscisic acid, and cytokine. *Plant Physiolo.*, 58: 530-536.

Fountain, D.W.and J.D. Bewley. 1973. Polyribosome formation and protein synthesis in imbibed but dormant lettuce seeds. *Plant Physiolo.*, 52: 604-607.

Gallardo, M., M.M. Delgado, I.M. Sánchez-Calle and A. Matilla. 1991. Ethylene production and 1- amino-cyclopropane-1-carboxylic acid conjugation in thermo inhibited Cicerarietinum seeds. *Plant Physiolo.*, 97: 122-127.

Gavin, R., G.R. Flematti, D.J. Merritt, M.J. Piggott, R.D. Trengove, S.M. Smith, K.W. Dixon and E.L. Ghisalberti1. 2011. Burning vegetation produces cyanohydrins that liberate cyanide and stimulate seed germination*. Nature communication., DOI:* 10.1038/ncomms 1356: 1-5.

Gonai, T., S. Kawahara, M. Tougou, S. Satoh, T. Hashiba, N. Hirai, H. Kawaide, Y. Kamiya and T. Yoshioka. 2004. Abscisic acid in the thermo inhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. *J. Experi. Bot.*, 55: 111-118.

Grappin, P., D. Bouinot, B. Sotta, E. Miginiac and M. Jullien. 2000. Control of seed dormancy in *Nicotianaplumbaginifolia*: A post imbibition abscisic acid synthesis imposes dormancy maintenance. *Planta*, 210: 279-285.

Ihle, J.N. and L Dure, III. 1972. The developmental biochemistry of cotton seed embryogenesis and germination. III. Regulation of Biosynthesis of enzymes utilized in germination. *J. Biolo. Chemist*, 247: 5048-5055.

Ihle, J.N. and L. Dure, III. 1970. Hormonal regulation of translation inhibition requiring RNA synthesis*. Biochemical and Biophysics Res. Communi.*, 38: 995-1001.

Kabar, K. 1997. Comparison of reversal of abscisic acid-induced inhibition of seed germination and seedling growth of some Gramineae and Liliaceae members by kinetin and gibberellic acid. *Turkish J. Bot.*, 21: 203-210.

Keeley, J.E. 1993. Smoke-induced flowering in the fire-lily *Cyrtanthusventricosus*. *South African J. Bot.*, 59: 638-639.

Lloyd, M.V., K.W. Dixon and K. Sivasithamparam. 2000. Comparative effects of different smoke treatments on germination of Australian native plants. *Austra. J. Ecolo.*, 25: 610-615.

Matakiadis, T., A. Alboresi, Y. Jikumaru, K. Tatematsu, O. Pichon, J.P. Renou, B. Sotta, Y. Kamiya, E. Nambara and H.N. Troung.2009. The Arabidopsis abscisic acid catabolism gene CYP707A2 plays a key role in nitrate control of seed dormancy. *Plant Physiolo.*, 149: 949-960.

Nonogaki, H., G.W. Bassel and J.D. Bewley. 2010. Germination-still a mystery. *Plant Sci.*, 179: 574-581.

Oracz, K., H. El-Maarouf Bouteau, R. Bogatek, F. Corbineau and C. Bailly. 2008. Release of sunflower seed dormancy by cyanide: cross-talk with ethylene signaling pathway. *J. Experi. Bot.*, 59: 2241-2251.

Orchard, T. 1977. Estimating the parameters of plant seedling emergence. *Seed Sci. & Techonol.*, 5: 61-69.

Pierce, S.M., K. Esler and R.M. Cowling. 1995. Smoke-induced germination of succulents (Mesembryanthemaceae) from fire-free habitats in South Africa. *Oecologia*, 102: 520-522.

Preston, C.A., R. Becker and I.T. Baldwin. 2004. Is 'NO' news good news? Nitrogen oxides are not components of smoke that elicits germination in two smoke-stimulated species, Nicotianaattenuata and Emmenanthependuli flora. *Seed Sci. Res.*, 14: 73-79.

Ramana, S., A.K Biswas, S. Kundu, K.J. Saha and R.B.R .Yadava. 2002. Effect of distillery effluent on seed germination in some vegetable crops. *Bioresorce Technology,* 82: 273-275.

Rehman, S., H.R. Choi, M. Jamiland and S.J. Yun. 2011. Seed Germination or Seed Hatching? Effect of GA and ABA on germination behavior of black raspberry (*Rubuscoreanus* Miquel) seeds. *Pak. J. Bot.,* 43: 2811-2816.

Roche, S., K.W. Dixon and J.S. Pate. 1997. Seed ageing and smoke: partner cues in the amelioration of seed dormancy in selected Australian native species. *Austra. J. Bot.*, 45: 783-815.

Senaratna, T, K. Dixon, E. Bunn and D. Touchell. 1999. Smoke-saturated water promates somatic embryogenesis in geranium. *Plant Growth Regulation*, 28: 95-99.

Siegien, I. and R. Bogatek. 2006. Cyanide action in plants from toxic to regulatory. *ActaPhysiologiaPlantarum*, 28: 483.

Sparg, S.G., M.G. Kulkarni and J. van Staden. 2006. Aerosol smoke and smoke-water stimulation of seedling vigor of a commercial maize cultivar. *Crop Science Society of America*, 1336-1340.

Sparg, S.G., M.G. Kulkarni, M.E. Light and J. Van Staden.2005. Improving seedling vigour of indigenous medicinal plants with smoke. *Bioresource Technology,* 96: 1323-1330.

Tabur, S. and S. Öney. 2012. Comparison of cytogenetic antagonism between abscisic acid and plant growth regulators. *Pak. J. Bot.*, 445: 1581-1586.

Tam, N.F.Y. and S.M. Tiquia. 1994. Assessing toxicity of spent pig litter using seed a germination technique. *Resource Conservation Recycling*, 11: 261-274.

Taylor, J.L.S. and J. Van Staden. 1996. Root initiation in *Vignaradiata* (L.) Wilczek hypocotyl cuttings is stimulated by smoke-derived extracts. *Plant Growth Regulation*, 18: 165-168.

Van Staden, J., A.K. Jäger, M.E. Light and B.V. Burg. 2004. Isolation of the major germination cue from plant-derived smoke. *South Afri J. Bot.*, 70: 654-659.

Varner, H.D.J. 1976. Response of barley aleurone layers to abscisic acid. *Plant Physiolo.*, 57:175-178.

Wang, M., S. Heimovaara-Dijkstra and B. Van Duijn. 1995. Modulation of germination of embryos isolated from dormant and non-dormant barley grains by manipulation of endogenous Abscisic acid. *Planta*, 195: 586-592.

Wills, T.J. and J. Read. 2002. Effects of heat and smoke on germination of soil-stored seeds in a south-eastern Australian sand heathland. *Austral. J. Bot.*, 50: 197-206.

Yari, L., M. Aghaalikhani and F. Khazaei. 2010. Effect of Seed priming duration and temperature on seed germination behavior of bread wheat (*Triticum aestivum* L.). *J. Agricu. Biolo. Sci.*, 1-6.

Zhou , J., J. Van Staden, L.P. Guo and L.Q. Huang. 2011. Smoke-water improves shoot growth and indigo accumulation in shoots of Isatisindigotica seedlings. *South African J. Bot.*, 77: 787-789.

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