

SMOKE INDUCED PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR CHANGES IN GERMINATING RICE SEEDS

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Abstract

Smoke produced by fire is an important environmental stimulus that plays a major role in improving the germination of plant species, including crops. However, smoke induced biochemical and molecular mechanisms involved in seed germination during imbibition remains unknown. Here, we explored the physiological, biochemical and molecular changes in the rice seed imbibed for 48 h in smoke-water (1:500 and 1:1000 dilutions (v/v)), Gibberellic acid (GA₃) (50 and 100 μM) and Abscisic acid (ABA) (5 and 10 μM). Increased the smoke concentration showed a significant increase in the germination percentage. It was also observed that smoke (1:1000) and GA₃ (100 μM) treated seeds had more water uptake as compared to other treatments. Interestingly smoke (1:1000) significantly induced carbohydrate, protein and lipid level of the imbibed seeds. However, macro and micro elements concentrations were decreased in seeds treated with smoke, GA₃ and ABA as compared with seeds treated with water. Furthermore, the expression of GA₃ and ABA responsive cis-elements genes was up-regulated by low or high dilution of smoke during seed imbibition, while the transcript abundance of some genes were up-regulated by GA₃ at seedling stage.

Key words: Smoke, Germination, Ion analysis, Cis-elements, Hormone.

Introduction

Germination is the start of plant's life cycle, where the emergence timing of seedling from the protective coat is critical for plant growth and development at later stage (Rajjou *et al.*, 2012). Despite the extensive investigation, seed germination is still a multifaceted physiological process that is not well elucidated. The phase transition of a seed from imbibition to germination is regulated by peripheral environment including light, temperature and nutrients in addition to the internal growth regulators such as gibberellic acid (GA₃) and abscisic acid (ABA) which play central role in the regulation of seed germination (Leung & Giraudat, 1998; Wolnyet *et al.*, 2018), furthermore, GA₃ has been involved in the initiation and completion of germination, while ABA is involved in the later stages of seed maturation (An & Lin, 1998; Leung & Giraudat, 1998; Van Staden *et al.*, 2000; Vishal, & Kumar, 2018).

In many ecosystems, fire events give an important opportunity for plant regeneration by providing essential resources such as light, temperature and nutrients (Delange *et al.*, 1990; Dixon *et al.*, 2009). This regeneration was considered initially due to the direct effect of heat. However, now it became evident that smoke is one of the most important stimulator of germination (Brown *et al.*, 1993; Baldwin *et al.*, 1994; Jain *et al.*, 2008) as it is a mixture of active phytochemicals and their breakdown products (Pierce *et al.*, 1995).

It has been reported that smoke affects activity of α amylases and β-tubulin in dormant seeds of *Avena fatua* L (Cembrowska-Lech & Kepczynski, 2017). Furthermore,

the active compound present in smoke reduced the level of ABA and regulated GA during seed germination of *Arabidopsis* (Chiwocha *et al.*, 2009). There are few reports available which showed that smoke affects initial water uptake in tomato (Ghebrehiwot *et al.*, 2008) and water homeostasis during germination in *Eragrostis (tef)* (Livak *et al.*, 2001), but still the genes regulating the effect of smoke and the molecular aspects of smoke regulated germination during imbibition are unexplored. The objective of the current study was to monitor the physiological and biochemical changes in the rice seeds imbibed in smoke water for 48 h in comparison with GA and ABA treatments. We also present a comprehensive comparative analysis of the changes in GA and ABA responsive cis-elements that occur in rice embryos after treatment with smoke-water.

Materials and Methods

Plant material and production of smoke: Rice (*Oryza sativa* L. *Indica*. cv. Basmati-385) seeds were obtained from National Agriculture Research Center, Pakistan. Smoke was prepared by burning of 333 grams of *Cymbopogon jwarancusa* leaves and bubbling it through one liter of distilled water (Tieu *et al.*, 1999).

Seed Imbibitions: Rice grains (500 mg) were weighted before imbibition for 48 hr in distilled water (as control), smoke solution (1:500 and 1:1000 dilution (v/v)), 50 and 100 μM GA₃ and 5 and 10 μM of ABA at 25°C. After imbibitions, the seeds were blotted dry and weighed. Percentage imbibition was calculated as $\{(W2-W1)/W1\} * 100$, where W1 is the initial weight of seeds and W2 is the weight of seeds after imbibitions. The imbibed seeds

were germinated on double filter paper in Petri dishes (90 mm) and incubated in a growth chamber at 30±2°C in dark condition until the germination was observed (with visible radicles and coleoptile) and were scored on the 5, 6 and 7th days in triplicate.

Elemental Analysis: After completion of imbibition process, a part of grains were dried in oven at 80°C for two days. Well-grounded dried grains (25 mg) were digested with conc.H₂SO₄ and H₂O₂ (2:1 ratio v/v). After digestion, all samples were diluted with deionized distilled water up to 25 ml. The concentrations of macro and micro elements in the acid-digested materials were determined using an Inductively Coupled Plasma (ICP-MSAglient7500a).

FT-IR Spectroscopy: Small beads were prepared by mixing grains powder (2 mg) with KBr (1:100 p/p) for Fourier transformed infrared (FTIR) spectroscopy (THERMO- NICOLET AVTAR-370, USA) and spectra were recorded between 400 and 4000 cm⁻¹. Three spectra were collected from each sample and only one representative spectrum was shown in the results. Curve-fitting of the original spectra was done with Origin 7 software. The FT-IR spectra between 3000 and 2800 cm⁻¹ was chosen to analyze lipids, the range of 1800 and 1500 cm⁻¹ was chosen to analyze proteins, whereas the spectra between 1200 and 1000 cm⁻¹ was used to analyze carbohydrates.

RNA Isolation from different Tissues: Total RNA was isolated from seed embryos (after imbibitions of 48 h) using TRIPure reagent (Biotek, China) as described by the manufacturer. Synthesis of cDNA was performed by using Promega first-strand synthesis system (M-MLV). Gene-specific primers (Supplementary Table 1) were used for amplification of complete coding sequence (CDS) from the cDNA.

Expression analysis using real-time PCR: The SYBR Premix Ex Taq kit (TaKaRa) was used for real-time PCR analysis, with the primers listed in Supplementary Table 1. PCR was performed with a BioRad-CFX96t thermal cycler under the following conditions: pre-denaturing at 95°C for 2 min, followed by 40 cycles of 10 s at 95°C and 20 s at 60°C in 25 µL of reaction mixture containing 1×SYBR Premix Ex Taq, 0.2 µM of each primer, and 1×ROX Reference Dye II. The relative expression levels were calculated using the $2^{-\Delta \Delta C_T}$ method (Bewley *et al.*, 1997).

Statistical analysis

Analysis of Variance was done by using the Statistix 9 software. Mean values of various parameters were significant differences from their respective control at $p < 0.05$. All the results were shown as mean ± standard deviation (S.D) for three replications.

SupplementaryTable 1. Specific primers used for semi-quantitative RT-PCR analysis.

Gene name (Accession No.)	Primer	Sequence (5'→3')
LOC_Os01g0328400	F	AGATGCAGATCTTCGTGAAG
Ubiquitin	R	CTCCTTGTCCTGGATCTTCG
LOC_Os02g0530600	F	GAGATCCCTGATGCTCGTCG
Putative seed maturation protein	R	TCTTCTTGGGAAGCCGCTAG
LOC_Os03g0159600	F	CACATTCACAGCGCAGACCA
Putative abscisic acid-induced protein	R	ACACGTCGCCATACCGGATC
LOC_Os06g0341300	F	ACGTCCGATCATACTACTC
Putative Late embryogenesis abundant protein	R	ACGTCCGATCATACTACTCTC
LOC_Os08g0127900	F	ACCACCACCACCACCAACAC
Similar to Globulin 1	R	AGGAGGAGGATGCAGAGGCA
Putative early embryogenesis protein		
LOC_Os12g0529400	F	CTAGAAGAAAAGAGATGGAGA
Auxin-binding protein 4 precursor (ABP)	R	CTTTCACAAGCGAATTGTCT
LOC_Os05g0218100	F	ACTCCACGACGACGACATCC
Putative cold regulated protein	R	GGACGACATCGACTTCACGT
LOC_Os02g0765600	F	AATCATCCATCTCCGAAGTG
Alpha-amylase precursor	R	TGTCAAGTTGGAGGAGAGGC
LOC_Os01g0323600	F	CACCCCCGTGCAGAATCACA
S-adenosylmethioninesynthetase 2	R	TATCTGCCCTTCTTCGTCCGCC
LOC_Os08g0136700	F	ACATCTCTATAGCTCATCA
DUF26 domain containing protein	R	AAGGCTGTAAGTAGTACCAG
LOC_Os08g0430500	F	GAACAAAAAGCATTTCGCATC
14-3-3-like protein S94	R	CTCCCGAGACATTTTGACCA

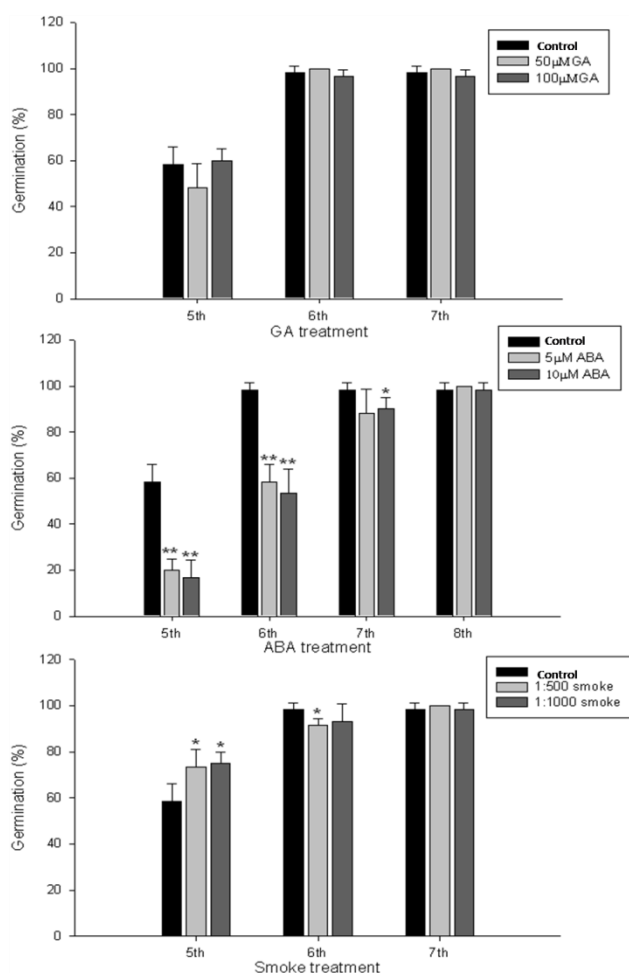


Fig. 1. Germination percentage of rice seed imbibed in different concentration of GA_3 , ABA and smoke for 48 hours. Germination rates were scored on the 5th and 6th, 7th and 8th days in triplicate. Figures show mean \pm SD ($n = 3$) while asterisks show significant differences from their respective control at $p < 0.05$.

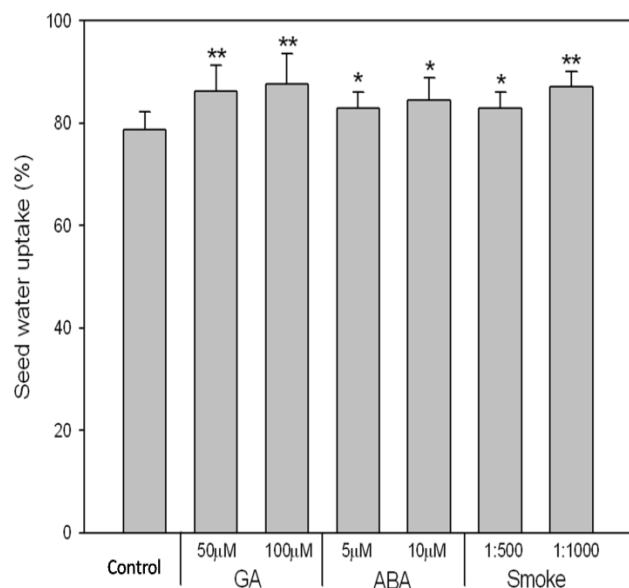


Fig. 2. Seed water uptake of rice seed imbibed in different concentration of GA_3 , ABA and smoke for 48 hours. Figures show mean \pm SD ($n = 3$) while asterisks show significant differences from their respective control at $p < 0.05$.

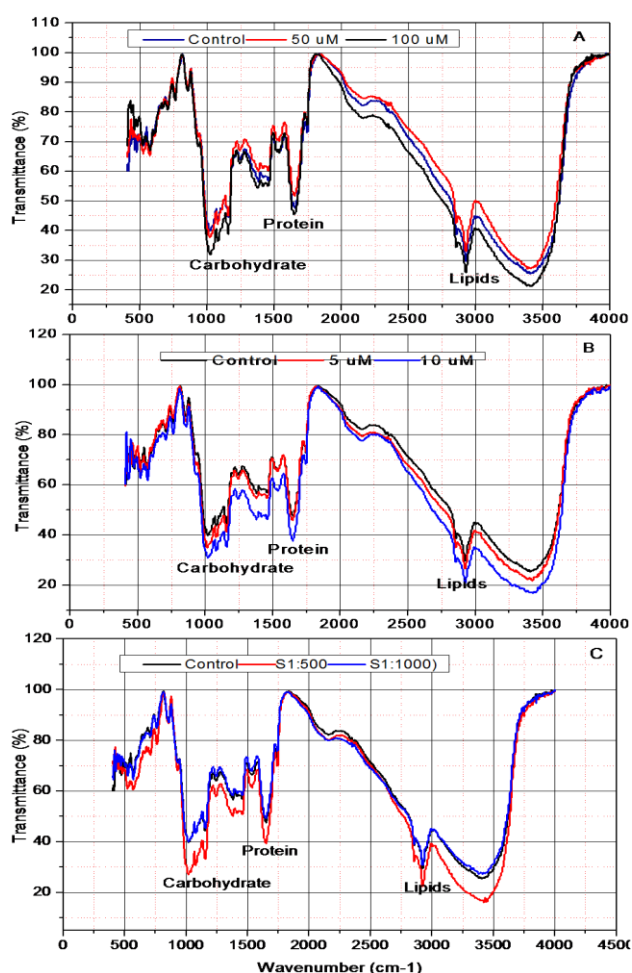


Fig. 3. FT-IR absorption spectra of the rice seeds imbibed in water, GA_3 , ABA and smoke for 48 hours. The major chemical constituents that contribute to the formation of bands in the particular wave numbers are carbohydrate, protein and lipids. At least three spectra were obtained from each sample and only one representative spectrum of each treatment is shown.

Results

Treatment of smoke-water significantly improved the germination of the imbibed seeds in 5 days, whereas comparatively less germination was recorded in GA_3 , ABA and water treated control seeds (Fig. 1). The first appearance of radicle was observed after 3 days in the smoke treated samples. Seed imbibed in smoke and GA_3 took less time to germinate than ABA imbibed seeds. Interestingly, during further growth period after 6 and 7 days, no significant difference in germination percentage was detected in control, smoke and GA_3 treated seeds (Fig. 1). It was observed that smoke (1:1000) and GA_3 (100 μ M) had more water uptake as compared to other treatments, when seeds were imbibed for 48 hr (Fig. 2).

Induction of metabolites alteration of imbibed seeds was done using FT-IR. The seeds were placed for 48 hr in different solution produced large numbers of sharp absorption peaks in the mid-IR region (2,000-1000 cm^{-1}) and end region (3000-2800 cm^{-1}) indicating a rich chemical composition of carbohydrates, proteins and lipids (Fig. 3). The IR spectra decreased the absorbance ratio of carbohydrate in treated seeds with the increasing

ABA and GA₃ level from lower to higher concentrations. However, FT-IR spectra showed slight increase in smoke induced carbohydrate accumulation at higher dilution (1:1000) but decreased at lower dilution (1:500) (Fig. 3).

In protein, an increase in band intensity was observed, indicating a considerable increase in protein accumulation in treated seeds with increasing GA₃, while this increased was more at 50 μ M GA₃ (Fig 3). However, in ABA, the absorbance peak of protein was decreased with increasing ABA level but the decreased was more pronounced at 10 μ M (Fig. 4). The IR spectra increased the absorbance ratio of protein in seeds treated with 1:1000 dilutions but decreased at 1:500 dilutions (Fig. 3).

The lipids IR spectrum mainly occurred between 3000 and 2800 cm^{-1} , where a IR peak fall was observed for lipids indicating a significant decrease in seed treated with ABA, where this decrease was more prominent at 10 ABA (Fig. 3). These IR spectra showed that GA₃ and smoke induced band intensity at 50 μ M and 1:1000 dilution, but this value decreased with increasing level of GA₃ and smoke (Fig. 3). Data indicated that the protein, carbohydrate and lipid synthesis was sensitive to ABA during imbibition than GA₃ and smoke while smoke upheld a higher ordered form of protein, carbohydrate and lipids in the imbibed seeds at higher dilution which could be one factor of early germination.

In order to get further insight, we performed ICP-MS analysis for macro and micro nutrients in seeds treated with ABA, GA₃ and smoke for 48 hr. It was observed that macro and micro elements were decreased, when treated with GA₃, ABA and smoke as compared to seeds treated with water (Table 1). Interestingly, GA₃ treated seeds had more Na, Ni, Fe and Cr content at 50 μ M as compared to control while other elements decreased except Cd with increasing level of GA₃. In seeds treat with ABA had the least amount of nutrients as compared to control. Similarly seed imbibed in smoke water had the lowest amount of nutrient at 1:1000 dilutions except Cd and Zn than GA₃, ABA and water imbibed seeds (Table 1).

To get better understanding of smoke effects on gene expression during seed imbibition, we executed real-time PCR analysis for GA and ABA responsive cis-elements genes (Supplementary Table 1) with rice ubiquitin as the internal control. Transcriptional variations were estimated during the first 48 h of imbibition, before seeds begin to germinate (Fig. 2B), in order to identify early regulatory response of GA and ABA responsive cis-elements genes that may happen in germination. The data indicated that the expression of S-adenosylmethionine synthetase 2 (Os01g0323600), Alpha-amylase precursor (Os02g0765600), Putative seed maturation protein (Os02g0530600), Putative abscisic acid-induced protein (Os03g0159600), Putative cold regulated protein (Os05g0218100), Putative late embryogenesis abundant protein (Os06g0341300), 14-3-3-like protein S94 (Os08g0430500) and Globulin 1 Putative early embryogenesis protein gene (Os08g0127900) were up-regulated by low or high dilution of smoke while the

transcript abundance of DUF26 domain containing protein gene (Os08g0136700) and an Auxin-binding protein 4 precursor (ABP)(Os12g0529400) was decreased by smoke treatment as compared to control (Fig. 4).

The result showed that the expression of S-adenosylmethionine synthetase 2 (Os01g0323600), Alpha-amylase precursor (Os02g0765600), Putative seed maturation protein (Os02g0530600), Putative Late embryogenesis abundant protein (Os06g0341300) and Auxin-binding protein 4 precursor (ABP)(Os12g0529400) were also induced by low and high concentration of GA₃ as compared to control. In contrast, the level of transcript in Putative abscisic acid-induced protein (Os03g0159600), Putative cold regulated protein (Os05g0218100), 14-3-3-like protein S94 (Os08g0430500) and Globulin 1 Putative early embryogenesis protein gene (Os08g0127900) and DUF26 domain containing protein gene decreased with GA₃. For the ABA treatment, the expression level significantly decreased in all the genes except DUF26 domain containing protein gene (Os08g0136700) and Auxin-binding protein 4 precursor (ABP) (Os12g0529400) at 5 or 10 μ M ABA (Fig. 4).

To further examine, whether these genes were regulated by smoke at seedling stage under same conditions, the data reveals that Alpha-amylase precursor and Putative cold regulated protein gene were induced by low or high dilution of smoke at seedling stage while the transcript abundance of other genes were reduced at seedling stage. Most of these genes were up-regulated by GA₃ at seedling stage (Fig. 5).

Discussion

In last decade, the physiological response of smoke-water treatments on seed germination has been observed (Iqbal *et al.*, 2016; Jamil *et al.*, 2014) however, less information is available about the possible mechanisms of smoke action in seed during germination. In this study, we presents the detailed report to observe the effects of smoke on carbohydrate, protein, lipids, micro/macro nutrients, GA₃ and ABA responsive cis-elements genes in rice grain imbibed for 48 hr in smoke water.

Seeds imbibition in smoke water indicates that the promotion of germination by smoke solutions is highly dependent on the initial period of rapid imbibition. Smoke-water may actually support water uptake during imbibition, which permits the commencement of metabolic activities that ends in radicle emergence (Wei *et al.*, 2009). It may be suggested that the smoke triggered an early, partial enhancement of seed germination rates, signifying that imbibed seed may use most of the nutrients to start metabolic activities earlier than other treatments. Van Staden *et al.*, (1995) suggested that smoke water triggers the enzymes which are involved in the mobilization of stored materials in seeds or sometimes it changes the membrane permeability to ease the translocation of regulators. The water uptake starts the metabolic activity of the seed from the dormant position and leads to physiological and biochemical variations (Wei *et al.*, 2009), which may improve seed germination.

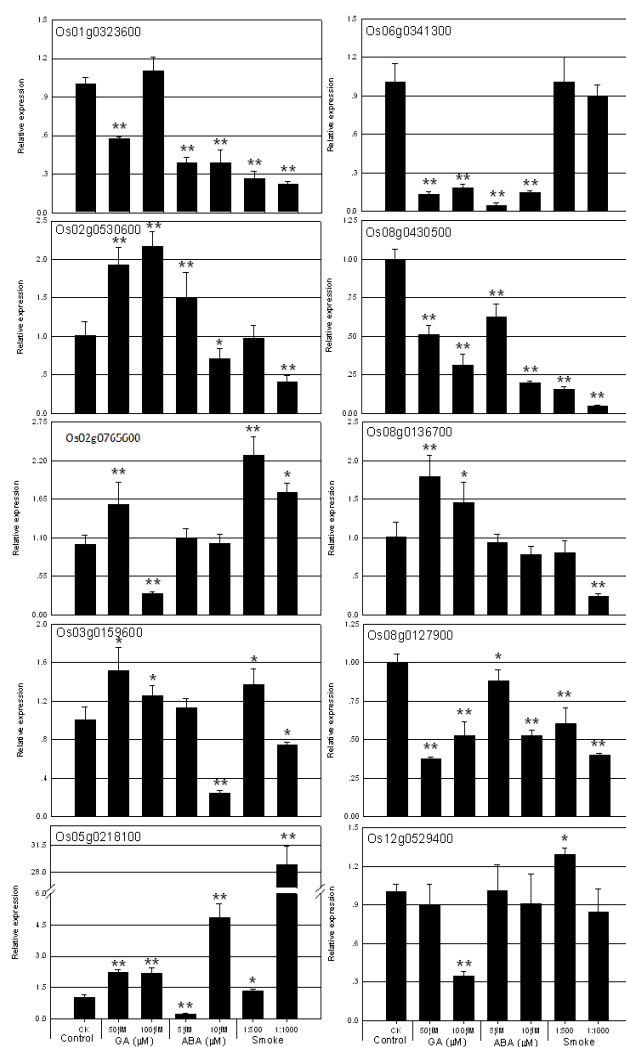
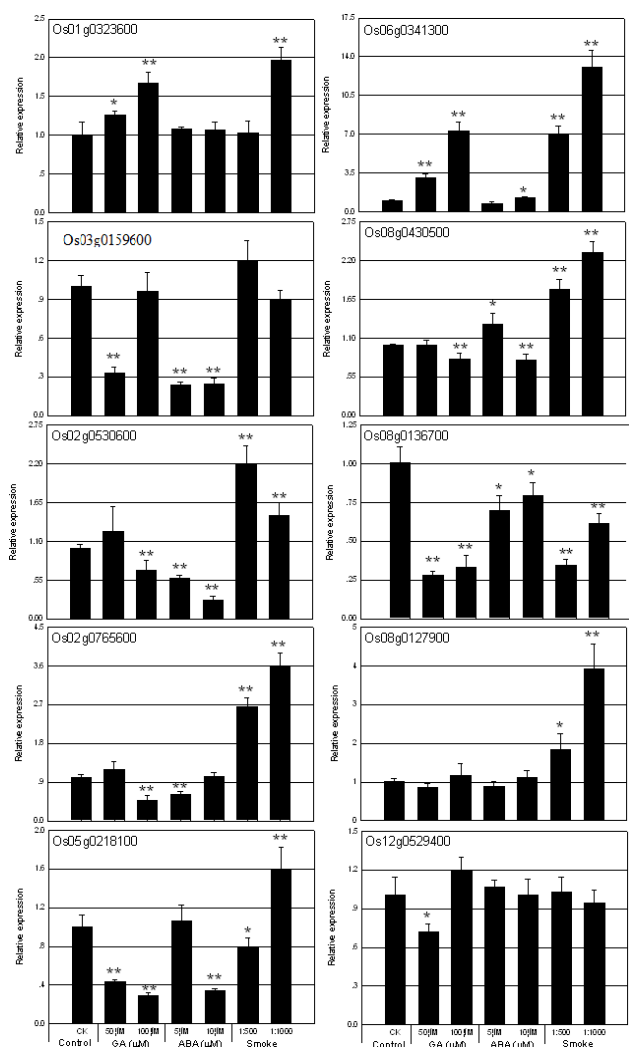


Fig. 4. The expression analyses of GA and ABA responsive cis-elements (S-adenosylmethioninesynthetase 2 (Os01g0323600), Alpha-amylase precursor (Os02g0765600), Putative seed maturation protein (Os02g0530600), Putative abscisic acid-induced protein (Os03g0159600), Putative cold regulated protein (Os05g0218100), Putative Late embryogenesis abundant protein (Os06g0341300), 14-3-3-like protein S94 (Os08g0430500) and Globulin 1 Putative early embryogenesis protein gene (Os08g0127900),DUF26 domain containing protein gene (Os08g0136700) and Auxin-binding protein 4 precursor (ABP) (Os12g0529400)) in seeds imbibed for 48 hr in different concentration of GA₃, ABA and smoke. The detection was done based on three independent samples and ubiquitinewas used as internal control. Figures show mean ± SD (n = 3) while asterisks show significant differences from their respective control at *p*<0.05.

Fig. 5. The expression analyses of GA and ABA responsive cis-elements (S-adenosylmethioninesynthetase 2 (Os01g0323600), Alpha-amylase precursor (Os02g0765600), Putative seed maturation protein (Os02g0530600), Putative abscisic acid-induced protein (Os03g0159600), Putative cold regulated protein (Os05g0218100), Putative Late embryogenesis abundant protein (Os06g0341300), 14-3-3-like protein S94 (Os08g0430500) and Globulin 1 Putative early embryogenesis protein gene (Os08g0127900),DUF26 domain containing protein gene (Os08g0136700) and Auxin-binding protein 4 precursor (ABP) (Os12g0529400)) in seedlings raised from seeds imbibed for 48 hr in different concentration of GA₃, ABA and smoke. The detection was done based on three independent samples and ubiquitinewas used as internal control. Figures show mean ± SD (n = 3) while asterisks show significant differences from their respective control at *p*<0.05.

Table 1. Micro and macro element in rice seeds imbibed in different concentration of GA₃, ABA and smoke for 48 hours.

Elements	Control	GA3 (uM)		ABA (uM)		Smoke (dilution)	
		50	100	5	10	1:500	1:1000
Na	0.195±0.002	0.215±0.01	0.211±0.007	0.104±0.01	0.120±0.01	0.102±0.01	0.116±0.02
K	2.87±0.02	1.46±0.01	1.47±0.01	1.61±0.01	19±0.01	1.15±0.01	1.881±0.04
Ca	2.85±0.03	2.285±0.01	2.77±0.01	2.32±0.04	2.096±0.04	2.059±0.02	2.679±0.06
Mg	154±0.03	143±0.04	1.25±0.01	1.21±0.03	1.29±0.01	1.27±0.05	1.405±0.03
Zn	0.092±0.002	0.067±0.001	0.096±0.001	0.0636±0.002	0.0706±0.001	0.0785±0.007	0.0815±0.001
Cd	0.00091±0.0005	0.00084±0.001	0.00091±0.001	0.00060±0.002	0.00119±0.001	0.00073±0.001	0.00095±0.001
Cu	0.00520±0.0001	0.00477±0.0001	0.00217±0.001	0.00274±0.0006	0.00402±0.001	0.0403±0.001	0.00414±0.001
Ni	0.00302±0.0001	0.00304±0.0001	0.00294±0.001	0.00217±0.0001	0.001712±0.001	0.00142±0.001	0.00278±0.001
Fe	0.00083±0.0001	0.00092±0.0001	0.00078±0.001	0.00074±0.0007	0.00064±0.0004	0.00069±0.0006	0.00061±0.0006
Cr	0.01203±0.001	0.01322±0.0006	0.01365±0.001	0.01338±0.0001	0.01159±0.0001	0.01236±0.0005	0.01153±0.001

We observed a decrease in the absorbance ratio of IR spectra for the carbohydrate, protein and lipids regions, in treated seeds as affected by an increasing concentrations of ABA. The protein absorption peaks generally located between 1800 and 1500 cm^{-1} consisted of amide-I and amide-II (Stehfest *et al.*, 2005), but sometimes intermingle with other absorption peaks within this region (Surewicz *et al.*, 1993). The absorbance peak of protein and lipids were increased at 50 μM GA_3 but decreased in case of carbohydrate. However, smoke induced significant increase in carbohydrate, protein and lipid accumulation at higher dilution (1:1000) but decrease in these components at lower dilution (1:500) (Fig. 3). The results demonstrated that the protein, carbohydrate and lipid synthesis was sensitive to ABA during imbibition, while smoke at higher dilution accumulated these components in the imbibed seeds which could be one factor of early germination. It has also been previously reported that processes of seed germination such as cell division and the synthesis of carbohydrate and protein become repressed when exogenous ABA is applied (Vidal-Valverde *et al.*, 2002). This suggests that the application of smoke solution may trigger cell division and synthesis of carbohydrate and protein by bringing some biochemical changes in the seed which leads early germination.

The results also demonstrated a decrease in macro and micro elements by the application of GA_3 , ABA and smoke as compared to seeds treated with water. Where, seed imbibed in smoke water had the lowest amount of nutrient at 1:1000 dilutions except Cd and Zn (Table 1). The smoke initiated an early enhancement of seed germination rates, suggesting that imbibed seed may use most of the nutrients to start metabolic activities earlier than other treatments. Similarly, Vidal-Valverde *et al.*, (2002) suggested that during seed germination, stored reserves were degraded normally which were used for respiration and synthesis of new cells. The process of seed germination starts with the uptake of water by the dormant dry seed and stop with the emergence of the radicle (El-Adawy *et al.*, 2003). Similarly, germination was also reported to be linked with the bioavailability of trace elements and minerals (Layer *et al.*, 2004).

ABA and GA are the classical phytohormones, which antagonistically regulate various plant growth processes such as seed germination, seed dormancy and maturation (Shu *et al.*, 2018). The process of seed germination consists of a system of gene expression and many ABA and GA-responsive functional genes are involved in the regulation of seed germination. It is well known that the interaction between abscisic acid (ABA) and gibberellins (GAs) is an essential factor controlling the transition from embryogenesis to germination in seeds. The results demonstrated that the expression of S-adenosylmethionine synthetase 2, Alpha-amylase precursor, Putative seed maturation protein, Putative abscisic acid-induced protein, Putative cold regulated protein, Putative Late embryogenesis abundant protein, 14-3-3-like protein S94 and Globulin 1 Putative early embryogenesis protein gene were up-regulated by low or high dilution of smoke (Fig. 4). Gomez-Porras *et al.*, (2007) reported that putative late embryogenesis abundant protein genes were regulated by ABA during seed maturation and development. Similarly, GA_3 also induced the expression of S-adenosylmethionine synthetase 2, Alpha-amylase

precursor, Putative seed maturation protein, Putative Late embryogenesis abundant protein and Auxin-binding protein 4 precursor (ABP). In contrast, the expression level significantly decreased in all the genes except DUF26 domain containing protein gene and Auxin-binding protein 4 precursor (ABP) at 5 or 10 μM ABA (Fig. 4). The involvement of most of these genes in seed germination has been evident from the literature. It has been reported that S-adenosylmethionine synthetase 2 have a regulatory effect on DNA transcription and chromosome structure and also involved in the biosynthetic pathway of various secondary metabolites (Kanzakiet *et al.*, 1993; Layer *et al.*, 2004). Alpha-amylase has been reported to play an important role in the mobilization of the carbohydrates, required for embryo growth during the seed germination (Sugimoto *et al.*, 1998; David *et al.*, 2007). The effect of GA_3 and smoke solution on seed germination, increasing alpha-amylase activity (Fig. 4) suggests that GA_3 and smoke are probably involved in the regulation of amylase activity. Auxin-binding protein (ABP) is involved in the auxin transport within the cell and can activate early modification of ion fluxes across the plasma membrane (Schoonheim *et al.*, 2009). As an important regulating factor, 14-3-3 protein participated in various signaling pathways (Shibasaki *et al.*, 1979; Layer *et al.*, 2004). It has been reported that allergenicity of rice is partially dependent on globulin and albumin fraction proteins (Nelson *et al.*, 2009). Likewise, Nelson *et al.*, (2009) detected that Karrikin in smoke water improved seed germination of *Arabidopsis* more efficiently than well-known phytohormones by improving the expression of the GA_3 biosynthetic genes $\text{GA}3\text{ox}1$ and $\text{GA}3\text{ox}2$ during seed imbibition.

The expressions of these genes were further checked at seedling stage under same condition. It was observed that Alpha-amylase precursor and Putative cold regulated protein gene were induced by low or high dilution of smoke while the transcript abundance of most of these genes were up-regulated by GA at seedling stage (Fig. 5).

Seed germination is a complex physiological process in which various biochemical and genetic changes occur. Beside these storage proteins, many GA and ABA responsive proteins may be involved during germination. In contrast to exogenous GA_3 or ABA, smoke seems to be involved in the stimulation of ABA and GA-responsive functional genes.

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