



Defense Priming in Okra using Chemically Defined Elicitors

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Authors' contributions

This work was carried out in collaboration between both authors. Authors KK and RP conceived and designed the research. Author KK performed the experiments. Author RP directed the research. Authors KK and RP wrote the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Biotic and abiotic stresses pose a threat to the growth and yield of plants. With increasing population and decreasing viable land area, there is a dire need of stress-resistant crop plants. Bam-FX treatment provides a sustainable method of priming the immune systems of plants without the need of genetic modifications.

Okra (*Abelmoschus esculentus*) seeds and seedlings were treated with chemically defined elicitors - Bam-FX solutions of different concentrations to assess its effect on seed germination and plantlet morphological growth. The gas chromatography-mass spectrometry (GCMS) and High resolution liquid chromatography-mass spectrometry (HR-LCMS/MS) were used to assess various metabolites in treated samples. We also treated some seeds with fungus (*Aspergillus* sp. 100 cfu/mL) or organic acids to assess the priming effect in seeds.

We observed that, of the four Bam-FX concentrations initially selected, Bam-FX dilutions of 1:175, 1:250, 1:500 were most effective in inducing the most optimal seed germination and morphological growth of the seedlings. Bam-FX treatment led to most marked upregulation of carboxylic acid and fumaric acid in both Okra seeds and seedlings. The fungal infection seemed to exhibit synergistic effect against Bam-FX treatment. The treatment with organic acids seemed to induce certain metabolites, such as carbamic acid, phenylboronic acid, anthranilic acid, etc., that were not otherwise induced in the non-Bam-FX-treated samples. Bam-FX, though originally designed for optimal plant growth in space, exhibited a high potential in priming the defense mechanisms of Okra against abiotic stresses.

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1. INTRODUCTION

Similar to animals, plants also suffer from several biotic and abiotic stresses. Such stresses adversely affect the plant metabolism, which ultimately reduces their yield and productivity [1,2]. Abiotic stresses, such as unfavorable temperature, water deficit/excess, soil salinity, etc., are known to affect the physiological reactions occurring inside the plant body, which drastically reduces the plant growth and yield by more than 50% [3]. Biotic stresses, such attack by herbivores and pathogens lead to plant injury and diseases [1]. Stress tolerance and resistance is a crucial part of agriculture to enable better growth and yield of the crops and other useful plants.

Several studies have shown that both abiotic and biotic stresses trigger the activation of several transcription factors and effector proteins, and induce the accumulation of various types of secondary metabolites that help protect the plant against such stresses [4]. In the past few decades, several researchers have focused on “priming” of plant defense responses against different types of biotic and abiotic stresses [5]. Defense priming, using organismal and non-organismal stimulants, can help in sustainable agricultural production by reducing the use of agrochemicals for the protection of crops from pests and diseases. Prime defenses could be both physical and chemical, and include morphological barriers, such as trichomes, cell wall lignification, and silica deposition, and syntheses of toxic chemicals (alkaloids, terpenoids, and phenolics), which act as repellents, deterrents, anti-nutrients, and anti-digestive compounds. Once primed, a plant defends itself more rapidly, strongly and/or enduringly, against subsequent threats. Priming agents could be live organisms (e.g. microorganisms or arthropods), chemicals (e.g. vitamins or plant hormones), or components thereof, and can be applied to various tissues and at diverse developmental stages (for example, to foliage or roots of mature plants, or to seeds). For instance, soaking of seeds in zinc or any other metal containing solution for a specified time, after which the seeds are re-dried and sown, is a low-cost technique of seed priming [6]. Zn priming of seeds, in turn, lead to a higher enhancement in vigor, growth, and yield when compared to using zinc application techniques for soil [7].

Bam-FX® is a proprietary Elicitor composition developed by Zero Gravity Solutions Inc . It directly affects the physiological mechanisms and innate immune system of plants to help them overcome both abiotic and biotic stresses without the need for any form of genetic modification. It helps in reducing the pest and microbial infestations and help in obtaining better yield even in regions with cold, heat, or water stress. It enhances nutrient absorption, chlorophyll content, and antioxidant activity, and induces systemic responses within the plant body.

In this study, We hypothesized that, not only will the priming treatment enhance the germination rate and reduce the seed germination time, but will also promote the synthesis of different carboxylic acids as well as the benzoic acid pathways.

2. METHODS

2.1 Bam-FX Solutions and Okra Seeds

The standard Bam-FX solution was purchased from Zero Gravity Solutions Inc, USA, and diluted using distilled water to obtain four concentrations of Bam-FX solutions, that is, 1:175 (least diluted), 1:250, 1:500, and 1:1000 (most diluted). The pH of the solutions was kept at 5.8 ± 0.2 . Intact Okra (*Abelmoschus esculentus*) seeds were purchased from a local vendor.

2.1.1 Treatment of pre-germinated seeds

The Okra seeds were washed using sterile distilled water for 5 to 10 s. These seeds were then soaked in the different diluted Bam-FX solutions for 5, 10, 20, 30, and 40 min at room temperature. After incubation, the seeds were removed from the treatment solutions and aseptically placed on wet tissue papers in tissue culture dishes. The culture dishes were then incubated at room temperature. After 12, 24, 48, and 72 h, seed germination and plantlet growth were recorded. The optimum exposure duration was used for further experiments.

2.1.2 Treatment of germinated seeds

The Okra seeds were washed using sterile distilled water for 5 to 10 s and placed on wet tissue paper in sterile tissue culture plates. The plates were then incubated at room temperature

for 72 h. At this stage, germinated sprouts were obtained. These sprouts were then treated with the different diluted Bam-FX solutions for 5 min, 10 min, 20 min, 30 min, and 40 min at room temperature. After 12, 24, 48, and 72 h, seed germination and plantlet growth were recorded. The optimum exposure duration was used for further experiments.

2.2 Fungal infection with Bam-FX Treatment

Seeds: The washed pre-germinated seeds were soaked with Bam-FX under optimal conditions, as determined earlier. The treated seeds were placed on wet tissue paper in sterile tissue culture plates in two sets. One set of seeds were infected with fungus (*Aspergillus* sp. 100 cfu/mL). All the seeds were incubated at room temperature for 72 h.

Seedlings: The germinated sprouts were treated with Bam-FX under optimal conditions, as determined earlier. The treated seeds were incubated for 24 h and subjected to metabolite extraction.

2.3 Effect of Organic Acids on Plant Growth

The washed Okra seeds were kept on sterile tissue paper in four different plates. Then, each plate was filled with 1 mL of an organic acid. We used succinic, fumaric, benzoic, salicylic acids for our study. The plates were then incubated at room temperature for 48 h. Next, the seeds were crushed and subjected to cold methanolic extraction to obtain their metabolites.

2.4 Metabolite Extraction

The pre-germinated (seeds) and germinated seeds (seedlings) were again treated under optimum conditions (as determined above). The metabolites of treated samples were extracted using the hot and cold methanolic extraction methods.

- Hot methanolic extraction: 10 mL methanol:water (1:1) was mixed with 1 g of crushed seeds/seedlings and kept at 70 °C for 15 min. Then, the mixture was incubated for room temperature and used for the preparation of esters.
- Cold methanolic extraction: 10 mL methanol was mixed with crushed seeds/seedlings

and kept at 4 °C for 14 h. After incubation, the mixture was used for the ester preparation.

- Methyl ester preparation: 250 µg or 50 µL of lipid samples were added to 1 mL of 1% methanolic NaOH (freshly prepared). The samples were heated at 55 °C for 15 min. In hot tubes, 2 ml of 5% methanolic HCl (freshly prepared) was added and the mixture was again heated for 15 min at 55 °C. The fatty acid methyl esters (FAME) were eluted by adding 1 mL of hexane to the above mixture. FAME were then stored at 8-10 °C.

2.5 GCMS

The methyl esters obtained from the samples (as described in previous subsection) were used for GCMS. GC-MS analysis (Agilent 5975C gas chromatography system) was performed following the users' guide to calibrate method with FAME standards, available on Fiehn GC-MS Metabolomics library-2008 (Agilent Chem Station, Agilent Technologies Inc., Wilmington, USA), with slight modifications, using retention time locking method. HP-5MS capillary column (30 m length, 10 µm Duraguard, 0.25mm diameter; narrow bore and 0.25µm film) manufactured by Agilent J&W GC columns, USA, was used for the analyses. Following oven temperature program was maintained: 60 °C (1 min), followed by 325°C at 10°C/min as final hold for 10 min before cool-down. Run time was 37.5 min. Injection temperature was set at 250°C, MSD transfer line at 290°C, and ion source at 230 °C. Helium was used as carrier gas (constant flow rate of 0.723 mL/min; carrier linear velocity 31.141 cm/s). Sample (1 µL) was injected onto the column via split mode (split ratio was 1:5). Chromatograms were analyzed using Automated Mass Spectral Deconvolution and Identification System (AMDIS). Metabolite identification was performed by comparing the retention times (Rt), retention indices (RI), and mass spectral fragmentation pattern of compounds using the references present in Agilent Fiehn Metabolomics library. Many of the metabolites were also identified by comparing chromatographic and spectral properties with that of standard compounds.

2.6 HR LCMS/MS

Metabolites were analyzed using a Vanquish UHPLC system (Thermo, USA) connected to a Q Exactive Plus - Orbitrap MS (Thermo, USA). The

system was operated in positive electrospray ionization mode. Metabolites were separated on a C-18 reverse-phase column (2.1 × 150 mm, 1.8 μm particle size). The auto-sampler and column temperature was kept constant at 4°C and 40°C, respectively. Mobile phase A contained 0.1% formic acid in water and mobile phase B contained water. The flow rate was 300 μL/min. Solvent B was held at 5% for 2 min, and then, increased to 95% between 2 and 20 min, lowered to 5% from 20 to 25 min and held at 5% between 25 and 26 min. Run was stopped at 30 min. The limit of detection was set at 3 times the signal-to-noise ratio, whereas the limit of quantitation was set at 10 times the signal-to-noise ratio.

3. RESULTS

3.1 Effect of Bam-FX on Seed Germination and Seedling Growth

The Okra pre-germinated (Fig:1) and germinated (Fig:2) seeds were treated with four different concentrations of Bam-FX. As shown in Fig 1, ≥80% germination rate and optimum root and leaf growth was observed when the seeds were treated using solutions with 1:175 (10 min exposure) and 1:500 (30-40 min exposure) dilutions. On the other hand, in case of post-germinated seeds, the most optimum growth of seedlings was observed when they were treated using solutions with 1:175 (10 min exposure) and 1:250 (5 min exposure) dilutions. Solution with lower concentration (1:1000 dilution) needed to be exposed for longer durations (40 min or higher) to achieve acceptable germination and growth.

3.2 Effect of Bam-FX Treatment on the Carboxylic Acid Content

We observed that treatment with a concentrated solution of Bam-FX (1:175 dilution) led to an initial increase in the carboxylic acid content in Okra seeds at 12 h, followed by a decrease at 24 h, compared to the control samples. In contrast, treatment with a diluted solution (1:500 dilution) led a marked rise in the carboxylic acid content at 24 h compared to the control samples. We obtained a 16.85% and 19.3% carboxylic acid content at 24 h following hot and cold methanolic extraction methods, respectively (Fig. 1).

3.3 Analysis of Secondary Metabolites in Okra Seeds using GCMS

As shown in Fig 2, for seeds treated with 1:175 Bam-FX, we observed an increase in the levels of 2,6-dichloro-4-nitrophenol, succinic acid, 5-(Heptafluoropropyl) uridine, and cyclohexanepropanoic acid at 12 h, and tris (methoxydimethylsilyl) silane, 2,5,8,11,14,17-hexaoxaoctadecane, dimethyl 2-butyl-1-(2-trimethylsilylethoxymethyl)imidazole-4,5-dicarboxylate at 24 h, compared to corresponding control samples.

Furthermore, for seeds treated with 1:500 Bam-FX, we observed an increase in the levels of pentanoic acid, ethyl homovanillate (TMS derivative), and cyclohexanepropanoic acid at 12 h, and tris (methoxydimethylsilyl) silane and 3-ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy) trisiloxane at 24 h, compared to corresponding control samples. When the same analysis was repeated following the cold methanolic extraction method, at 24 h, we observed an increase in the levels of 2'-hydroxypropiophenone, silicic acid, and dimethyl 2-butyl-1-(2-trimethylsilylethoxymethyl)imidazole-4,5-dicarboxylate, and fumaric acid (eicosyl 2-methylpentyl ester) in seeds treated with 1:175 and 1:500 Bam-FX, respectively (Fig 3).

3.4 Analysis of Secondary Metabolites in Okra Seeds using LCMS

Next, we assessed the levels of seeds treated with 1:175 and 1:500 Bam-FX followed by incubation for 24 h. At 24 h, we observed an elevation in the levels of B glyceric acid, S 10 Hydroxycamptothecin, erythromycin, turmerone, aspartic acid, taxifolin, and epimedin A after treatment with 1:175 Bam-FX, and in levels of arachidonic acid, artemisinin, gedunin, ginsenosides, guanidosuccinic acid, picrotoxinin, quinine, taxifolin, ricinine, and linoleic acid after treatment with 1:500 Bam-FX (Figs. 2a and b).

3.5 Effect of Bam FX on Germinated Okra Seeds

In search of the effect of BamFX on seedlings, we used 48 hours grown untreated seedlings. These seedlings were sprayed with BamFX dilutions 1:175 and 1:500. Samples were taken out at 12 hours and 24 hours after BamFX treatment. In these seedlings, we observed elevation in the levels of anthranilic acid,

propanoic acid, 4 nitrothalic acid, and octadecanoic acid, and at 24 h, we observed elevation in levels of cyclohexaneacetic acid, 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptylester, triazole carboxylic acid, 2-ketoisocaproic acid, dimethyl malonic acid, fumaric acid, and butanoic acid (Fig. 3).

For seeds treated with 1:500 Bam-FX, at 12 h, we observed elevation in the levels of sebacic acid, dimethylmalonic acid, butanoic acid, and silicic acid, and at 24 h, we observed elevation in levels of 2- ketoisocaproic acid, fumaric acid, benzoic acid, and silicic acid (Fig. 3).

3.6 Effect of Fungal Infection on Secondary Metabolites of Bam-FX-Treated Seeds

We observed an elevation in the levels of jervine, picrotoxinin, quinine, rescinnamine, ricinine, taxifolin, linolenic acid and ascorbic acid in the Bam-FX treated seedlings infected with *Aspergillus* sp. (Figure 4a). In case of seeds, we observed elevated levels of psoralidin, erythromycin, aconitine, gedunine, ginsenosides, 18 B glycyrrhetic acid, arachidonic acid and aspartic acid (Fig. 4b).

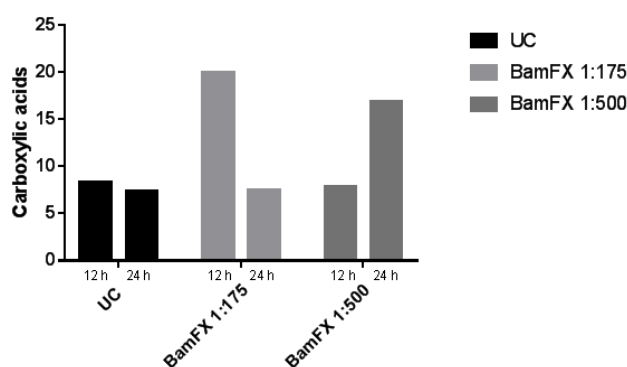


Fig. 1. Carboxylic acid induction in Bam-FX treated Okra seeds

The UC – untreated seeds , BamFX1:175 – BamFX dilution in water 1:175 , BamFX 1:500 – BamFX dilutions in water 1:500. The carboxylic acids were estimated after 12 h and 24 h in treated and untreated seeds

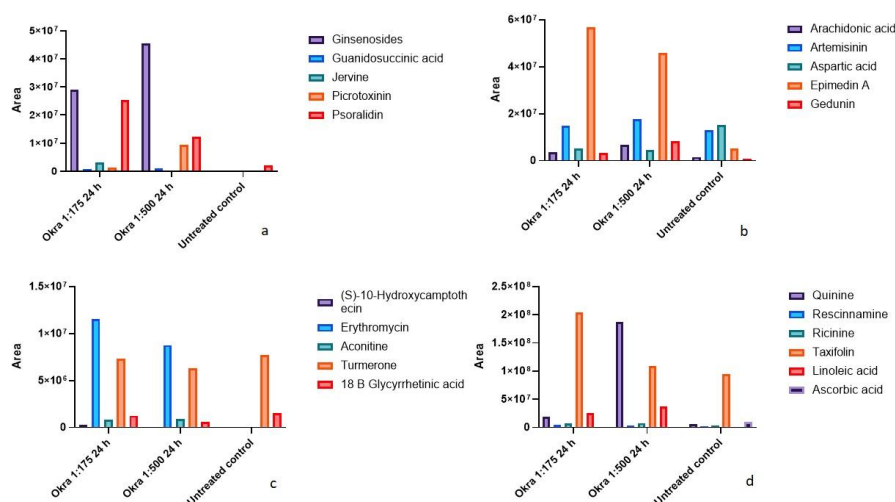


Fig. 2. Enhancement of the carboxylic acid production in the seeds and seedlings treated with Bam FX

The HR LCMS/MS was used for the analysis of the Carboxylic acids in the samples. 1:175 24 h- carboxylic acids extracted after 24 h from Seeds treated with BamFX dilution 1:175. 1:500, 24 h- carboxylic acids extracted after 24 h from Seeds treated with BamFX dilution 1:500. 1:175,

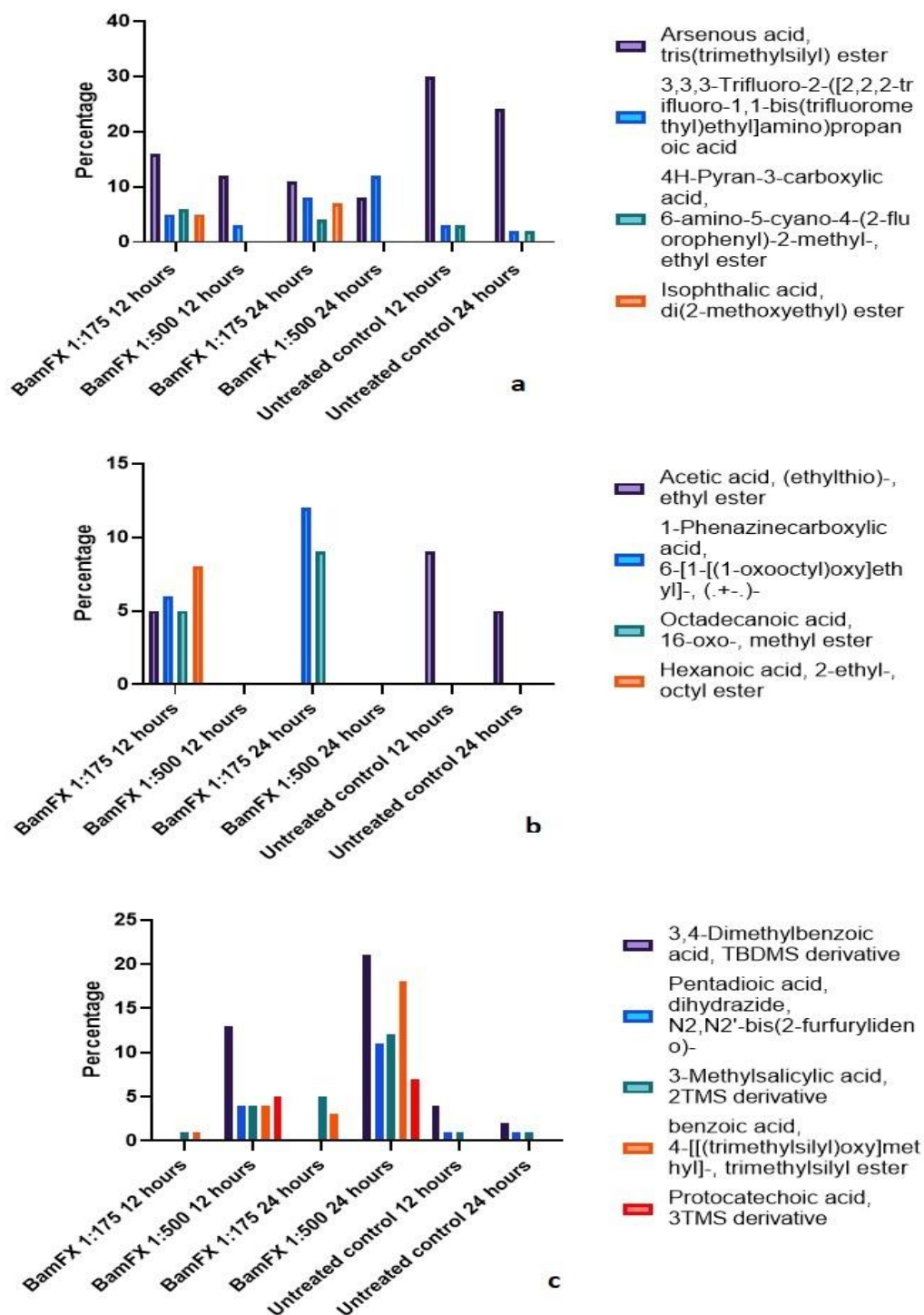
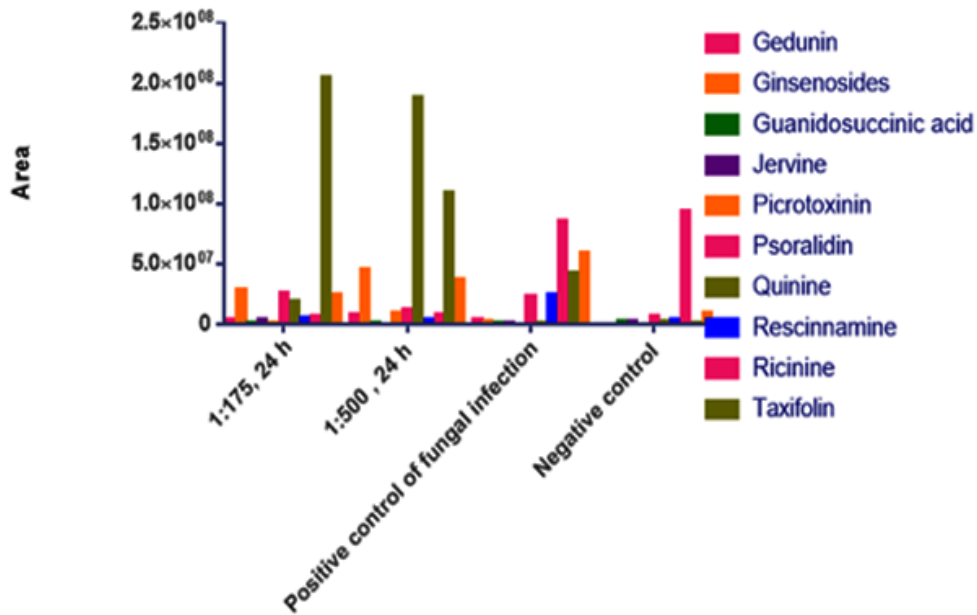
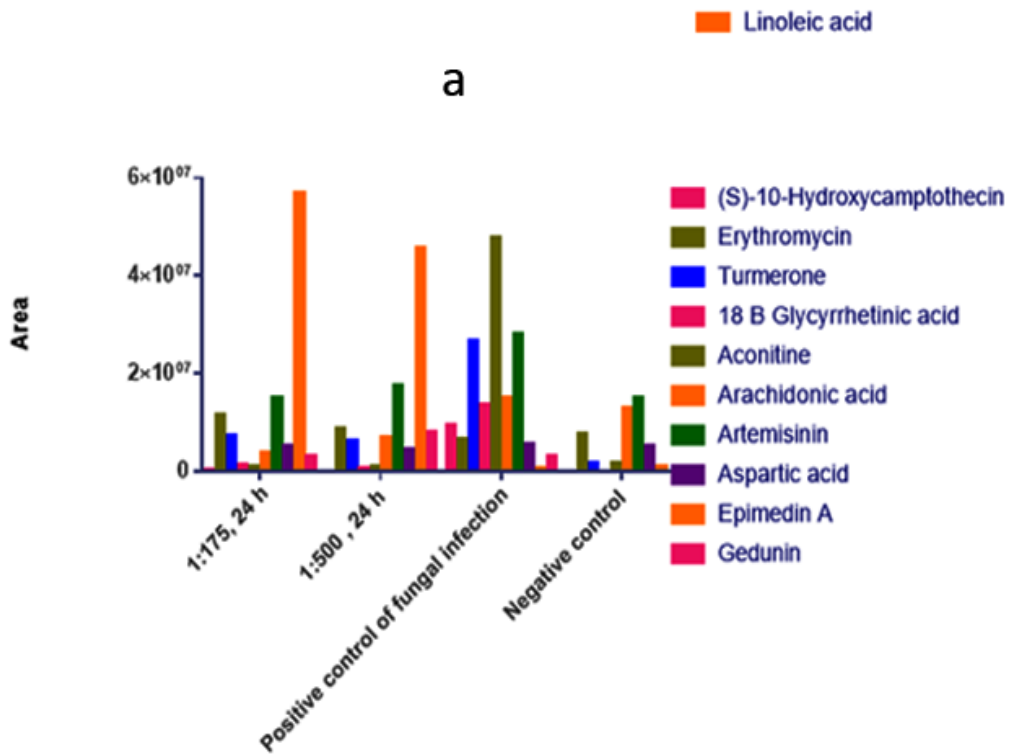


Fig. 3. Induction of carboxylic acid in seedlings treated with the Bam FX
Untreated control 12 hours- untreated control sample of 12 hours growth,
Untreated control 24 hours – Untreated control sample of 24 hours growth.
BamFX1:175 12 hours- BamFX 1:175 sprayed and samples collected after 12 hours ,
BamFX 1:500 12 hours- BamFX1:500 sprayed and samples collected after 12 hours ,
BamFX1:175 24 hours- BamFX1:175 sprayed and samples collected after 24 hours,
BamFX1:500 24 hours- BamFX1:500 sprayed and samples collected after 24 hours.



a



b

Fig. 4. Cumulative effect of fungal infection and Bam-FX treatment
 1:175 24 h- BamFX1:175 sprayed and samples collected after 24 hours,
 1:500 24 h- BamFX1:500 sprayed and samples collected after 24 hours,
 Positive control of fungal infection – *Aspergillus sp* treated Okra seedlings
 Untreated control – Untreated control sample of 24 hours growth.

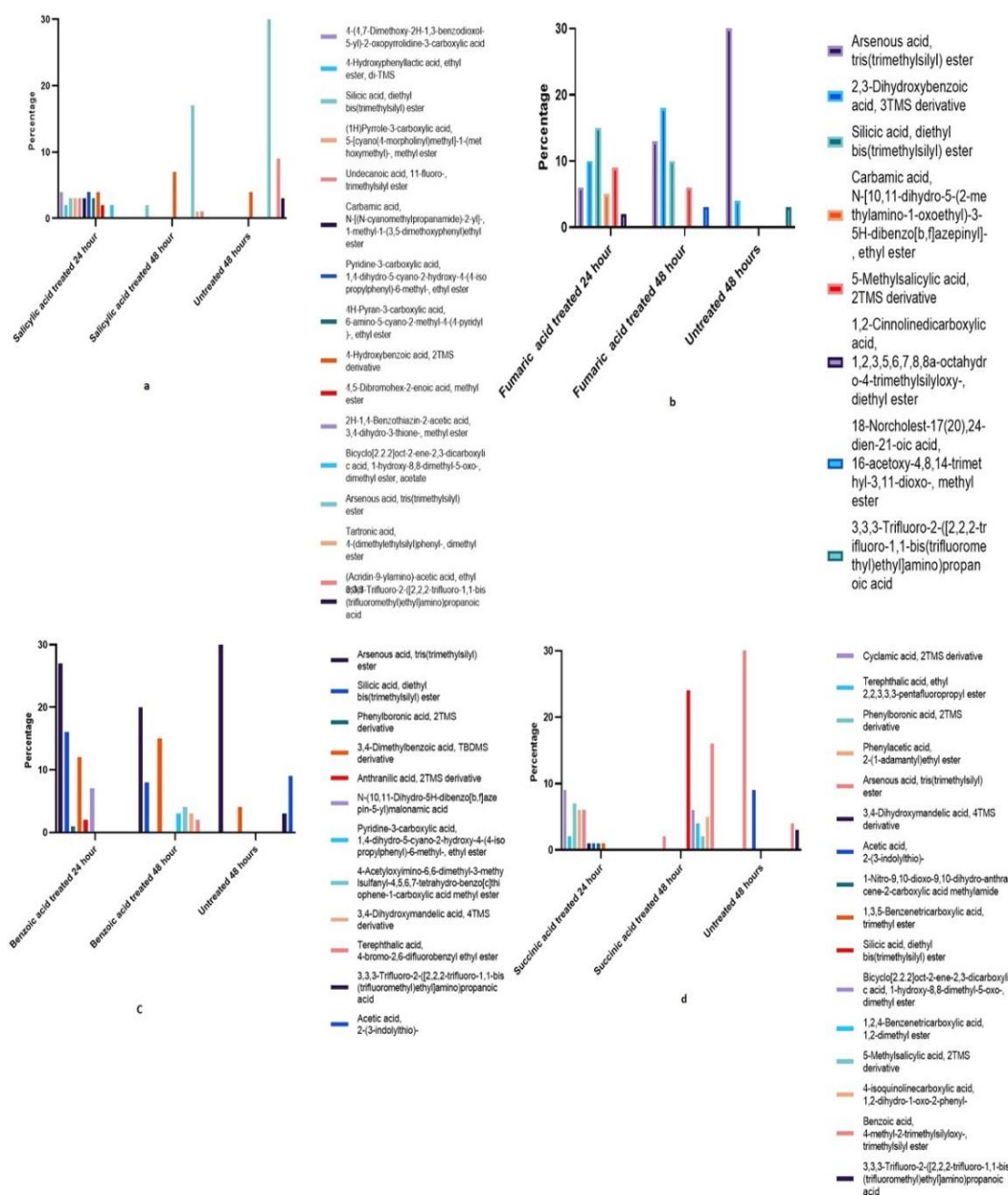


Fig. 5 . Effect of different organic acids on the metabolism of Bam-FX-treated seeds
 (a) Seeds treated with Succinic acid , (b) Seeds treated with Salicylic acid , (c) Seeds treated with Fumaric acid ,
 (d) Seeds treated with Benzoic acid

3.7 Effect of Organic Acid Treatment on Bam-FX Treated Seeds

Bam-FX treated seeds were treated with succinic, fumaric, benzoic and salicylic acids and incubated for 24 h and 48 h after treatment. We assessed the metabolic profiles of the samples at

both time points. We observed that, after treatment with succinic acid, the levels of cyclamic acid, terephthalic acid, phenylboronic acid, phenylacetic acid, 3,4 dihydroxymandelic acid, 1, Nitro, 9,10-dioxo-9-10-dihydroanthracene-2-carboxylic acid, 1,3,5 benzenetri carboxylic acid were elevated at 24 h, and those of silicic

acid, bicyclooct-2-ene-2,3-dicarboxylic acid, 1,2,4-benzenetricarboxylic acid, 5 methylsalicylic acid, 4-isoquinoline carboxylic acid, and benzoic acid were elevated at 48 h (Fig. 5a).

After treatment with salicylic acid, the levels of salicylic acid, undecanoic acid, carbamic acid, 4-(4,7-dimethoxy-2H-1,3-benzodioxol-5-yl)-2-oxopyrrolidine-3-carboxylic acid, pyridine-3-carboxylic acid, 1,4-dihydro-5-cyano-2-hydroxy-4-(4-isopropylphenyl)-6-methyl-4H-pyran-3-carboxylic acid, 6-amino-5-cyano-2-methyl-4-(4-pyridyl), 4,5-dibromohex-2-enoic acid were elevated at 24 h, and those of arsenous acid, tartronic acid, acridin-9-yl-amino-acetic acid, and benzoic acid were elevated at 48 h (Fig. 5b).

After treatment with fumaric acid, carbamic acid and 1,2-cinnolinedicarboxylic acid, 1,2,3,5,6,7,8,8a-octahydro-4-trimethylsilyloxy-diethyl ester were induced at 24 h, while 18-norcholest-17(20),24-dien-21-oic acid and 16-acetoxy-4,8,14-trimethyl-3,11-dioxo-, methyl ester were found at 48 h only (Fig. 5c). At both time points, we observed the generation of arsenous acid and silicic acid; however, their levels were higher at 48 h compared to the levels at 24 h (Fig. 5c).

In case of benzoic acid, phenylboronic acid, anthranilic acid, and dibenzoazepinmalonamic acid were induced at 24 h, while pyridine-3-carboxylic acid, 1,4-dihydro-5-cyano-2-hydroxy-4-(4-isopropylphenyl)-6-methyl-ethyl ester, 4-acetyloxymino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo [c]thiophene-1-carboxylic acid methyl ester, 3,4-dihydroxymandelic acid, 4TMS derivative, terephthalic acid, 4-bromo-2,6-difluorobenzyl ethyl ester were found at 48 h only (Fig. 5c). At both time points, we observed the generation of silicic acid and 3,4-dimethylbenzoic acid, TBDMS derivative; however, their levels were higher at 48 h compared to the levels at 24 h (Fig. 5d).

4. DISCUSSION AND CONCLUSION

Environmental stresses, both biotic and abiotic, pose a major challenge in adequate growth and development of plants. Such stresses ultimately lead to a decrease in the yield of the plants, including agricultural crops and economical plants. The immune responses of plants to such stressors are extremely complex and changes constantly based on the type of stress. Several chemical compounds, such as fungicides,

insecticides, and fertilizers, are used to promote better plant growth and yield and thwart the attack of adverse elements, such as pests, harmful pathogens, and environmental stresses. A plethora of signal transduction pathways and networks act and interact with each other to mediate the plant responses against the different types of stresses [8]. An important part of plant defenses include rapid stress perception and deployment of adequate counter-measures [1]. The basal constitutive defense mechanisms of plants play an important role in the recognition of the type of stress and activation of the corresponding signaling cascades [9,10]. Each stress is countered with a specific type of signaling response. However, when facing more than one type of stress, the plant's overall response cannot be predicted from its respective responses to individual stresses [4]. Several researchers are still attempting to elucidate such multiple responses of plants in both lab and field conditions. Furthermore, unlike animals, plants do not possess a somatic immune system or mobile immunity cells [11]. Instead, plants are dependent upon the innate immune system and the signaling molecules that are released by the cells located in adversely affected areas on plant bodies [12,11]. The innate immunity system of plants is broadly divided into two parts. One part of this immune system employs the pattern-recognition receptor (PRRs), which are present on the cell surfaces and are used to recognize the damage-associated molecular patterns (DAMPs) derived from the host body and microbe-associated molecular patterns (MAMPs) or pathogen-associated molecular patterns (PAMPs) that are found in a wide range of pathogenic microbes [13,14]. The other part employs the disease resistance (R) proteins that act on the pathogen-released effector molecules that are responsible for the infections [15]. Previous studies have shown that exposure to these elicitors, such as DAMPs, PAMPs, chemical or physical stimulating agents, could aid in modification in the expression of the "defensive genes" of plants. Such genes are generally involved in the induced systemic resistance and systemic acquired resistance. The process of inducing such genes using artificial elicitors is essentially termed as priming [16]. Here, we used a proprietary chemical cocktail mixture, Bam-FX, developed by Zero Gravity Solutions Inc. to induce defensive responses in Okra.

The active ingredient in BAM-FX is zinc sulphate (6.9%) and copper sulphate pentahydrate

(2.1%), mixed with inert carriers (91%), containing water(86%) and other sulphates (5%) . Its application helps in the movement of necessary ions to deficiency sites, which, in turn, improve plant yield, growth, and resistance against pests and pathogens. Although its efficiency has been proved in previous studies, the mechanism of its action in different plants species and in various environmental and soil conditions has not yet been completely elucidated. To the best of our knowledge, this is the first study elucidating the effects of optimum concentrations of Bam-FX solution on Okra growth, yield, and metabolism.

Here, we observed that the application of Bam-FX promoted the seed germination and morphological growth of Okra. However, we observed that both very low and high concentrations of Bam-FX were less efficient than the concentrations in-between. Among all the concentrations, the Bam-FX solution with 1:175 dilution was found to be most effective in promoting the morphological traits of both pre- and post-germinated Okra seeds.

Several studies have previously shown that, apart from being primary metabolites, carboxylic acids are potent priming agents in plants (Gamir et al, 2012; [16]. Here, our results indicated an increase in the production of carboxylic acids in Okra seeds and seedlings following treatment with all the concentrations of Bam-FX. However, the highest synthesis of carboxylic acids was observed when Okra was treated with Bam-FX with 1:175 and 1:500 dilutions.

Organic acids are mainly a part of the Krebs cycle in mitochondria and, to some extent, the glyoxylate cycle in glyoxysomes. They are generally present in small amounts, but, under stress, their levels increase. Such increase in levels of organic acids is then encountered by plants via the production of several secondary metabolites, such as flavonoids and phenolic compounds [17]. Our results indicated that exposure to organic acids led to an increased synthesis of such compounds in the plants treated with Bam-FX.

It is noteworthy that, to the best of our knowledge, this is the first study involving optimization of adequate solution concentration and duration of exposure to Bam-FX, and previous literature in this respect is highly limited. However, our results indicate the Bam-FX holds great potential in improving the growth and

tolerance of the plants via natural mechanisms, hence reducing the need to use harmful fertilizers and pesticides and other alternative techniques, such as genetic manipulation. The main limitation of this study was that we did not compare the effects of Bam-FX with those of conventionally used fertilizers. In their recent attempt to establish a pest management program in tomato, Mauch-Mani *et al* [16] reported that, although Bam-FX promoted the growth and yield of the plants, it was still less effective than the conventional N-P-K fertilizer. However, they reported better pest resistance in the plants treated with Bam-FX. These findings also warrant the need for further studies on the optimizing the use of Bam-FX on various plants under different soil conditions.

DISCLAIMER

Dr. Raveendran Pottathil is Chief Technology Officer and COO of Zero Gravity Solutions Inc. USA and he invented a Bam-FX induced multigenerational priming in plants. The Bam-FX is a product of Zero Gravity Solutions Inc. USA.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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