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# SEED PRIMING AND EXOGENOUS APPLICATION OF SALICYLIC ACID ENHANCE GROWTH AND PRODUCTIVITY OF OKRA (*Abelmoschus esculentus* L.) BY REGULATING PHOTOSYNTHETIC ATTRIBUTES

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#### KEYWORDS

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Hormonal priming

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# ABSTRACT

Low and uneven germination is a serious problem for the successful production of okra seedlings. Priming of seeds as well as supplementation of different plant growth regulators exhibited better response in successful seedling production which eventually results in higher yield. Therefore, the present study was conducted to evaluate the effects of seed priming and exogenous application of salicylic acid (SA) on okra seed germination and plant development. The okra seeds were primed by 1 mM and 2 mM of SA for 60 minutes whereas the seeds were washed several times with distilled water for the control treatment. Similar doses of SA have been exogenously sprayed to the 12 days okra seedlings for 4 days. The results of the study revealed that seed priming with SA enhanced germination percentage (GP), increased coleoptile length and weight, shoot and root length, and seed vigor index (SVI). Similarly, exogenous application of 1 mM SA increased relative water content (RWC), contents of chlorophyll a, chlorophyll b, total chlorophyll while a higher dose of SA (2 mM) degraded the leaf pigments. Supplementation of SA altered photosynthetic attributes, net photosynthetic (Pn) and transpiration rate (Tr), stomatal conductance (Gs), and water use efficiency (WUE). Moreover, SA

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yield per plant. The results of this study indicated that seed priming and exogenous application of SA enhanced germination and okra productivity by regulating RWC and photosynthetic attributes where 1 mM SA is more effective compared to 2 mM SA.

#### **1** Introduction

Okra (Abelmoschus esculentus L.) is a widely-grown dicotyledonous plant and popular summer crop throughout the tropics (Tania et al., 2020; Elkhalifa et al., 2021) under the family of malvaceae. It is a familiar vegetable crop in many countries and also known as lady's fingers, or gumbo that grows up to 1.0-2.1 meters tall. The immature pod is the edible part of okra which is harvested at the tender stage. Okra is an important source of vitamins, A, C, and K, and mineral salts including calcium (Gemede et al., 2015). However, successful okra production faces various problems due to certain factors like seed quality, climatic circumstances, and cultural operations (Kusvuran, 2012; Lamichhane et al., 2021). It has been reported that slow and uneven germination is the main hurdle in okra production due to the presence of hard seededness (Felipe et al., 2010; Pandita et al., 2010; Lamichhane et al., 2021). Also, the okra yield reduces due to the slow and erratic emergence of okra seedlings (Rahman et al., 2016). However, nowadays, researchers are concentrated on different approaches like seed priming, screening of best varieties, and organic fertilizer application, and exogenous applications of different plant growth regulators such as auxin, SA, abscisic acid, 5-aminolevulinic acid, citric acid, and so on to overcome this problem (Rhaman et al., 2016; Yakoubi et al., 2019; Abdel Latef et al., 2021; Rhaman et al., 2021a, b; Tahjib-Ul-Arif et al., 2021).

Supplementation of chemicals to plants, either as an exogenous foliar application or seed treatments, may prompt their physiological apparatuses, leading to plant growth enhancement (Vwioko, 2013). For instance, seed priming with plant growth regulators can bring changes in the phenotypes of plants from seed germination to senescence (Rhaman et al., 2021a). Salicylic acid (SA) is an endogenous growth regulator of phenolic nature, which influences a range of various progressions in plants such as seed germination and seedling growth, photosynthesis, and stomatal regulation (Khan et al., 2003; Prodhan et al., 2018; Koo et al., 2020). It has been reported that okra seeds priming with SA enhance the vegetative growth rate and leaf water contents of plants (Raza et al., 2013). Supplementation of SA increases the growth rate and photosynthesis of Rosmarinus officinalis plants (Najafian et al., 2009). In addition, the exogenous application of SA enhances the yield and yield contributing characters of Brassica juncea (Dugogi et al., 2012). Hussein (2015, 2017) reported that seed priming with SA enhances the viability of okra. Likewise, SA application enhances biochemical characteristics and plant developments of okra (Raza et al., 2013). In addition, supplementation of SA enhances the morphological parameters and growth rate of onion and soybean (Razmi et al., 2017; Bhasker et al., 2020).

Recently, many studies have been reported that seed priming is a promising technique that enhances seed germination and fastens plant growth under normal and as well as in stress conditions by regulating different physiological processes (Muhei, 2018; Hasanuzzaman & Fotopoulos 2019; Rhaman et al., 2020). Similarly, many reports showed that supplementation of SA enhances the growth and yield of many plant species by regulating photosynthetic attributes (Tahjib-Ul-Arif et al., 2018; Gul et al., 2020). Though, some studies reported that SA priming of okra seeds enhances morphological parameters and yield of okra. However, the regulatory role of SA priming and exogenous application of SA on okra crop enhancement still remains unclear. Therefore, the present study was conducted to assess the effects of seed priming and the exogenous application of SA on okra production.

#### 2 Materials and Methods

## 2.1 Plant Materials and Chemicals

The experiment was carried out at the Department of Seed Science and Technology of Bangladesh Agricultural University, Mymensingh, using BARI dharosh-2, a recently developed high yielding variety that is widely cultivated in many regions of Bangladesh. The BARI dharosh-2 is very soft, tasty, and produced fruit at every single node. The chemicals, SA (Sigma-Aldrich), sodium hypochlorite (Sigma-Aldrich), and Hyponex (Osaka, Japan) nutrient solution were used as an analytical grade in this study.

#### 2.2 Experiment at pre-seedling stage

Uniform in appearance okra seeds were sorted out and surface sterilized with 1% sodium hypochlorite for 5 min and then washed 3-4 times with dH<sub>2</sub>O. For the seed priming experiment, the seeds were soaked in 1mM and 2 mM SA for 60 minutes, and the control experiment seeds were washed in distilled water for several times in a normal laboratory (the room temperature was  $25\pm1^{\circ}$ C and relative humidity was 95%) conditions. After that, treated seeds were placed in a petri-dish (150×20 mm diameter) having three layers of wetted-Whatman filter papers and incubated for 10 days for the germination study. Fifteen treated seeds were placed in each petri-dish. Thus there were three treatments in this study;

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control; 1mM SA, 2 mM SA. The experiment was conducted with a completely randomized block design having three replicates.

Morphological parameters, coleoptile length, and coleoptile weight at the germination period were collected after the 10<sup>th</sup> day. Germination percentage (GP) and seed vigor index (Tania et al. 2020) were computed with the following equations:

Germination percentage (GP)=

 $\frac{\text{Total number of seeds germinated}}{\text{Total number of seeds placed in germination}} \times 100$ 

Seed vigor index (SVI) =  $GP \times seedling length (cm)$ 

## 2.3 Experiment at seedling stage

Uniformly germinated seeds were placed in plastic pots (22 cm in height and 25 cm in diameter) filled with soil (6 seedlings per pot). The soil is properly mixed with nutrient solution Hyponex (Osaka, Japan) containing nitrogen, phosphorous, potassium, and other micronutrients. The nutrient solution (2 ml mixed with water for per pot) was applied twice in a week in the pots. After 12 days, seedlings were exogenously treated with 1 mM and 2 mM SA for four times in four days (single spray per day at 11 am; 8 ml per plant per spray). After 4 days of SA treatment, morphological and physiological data were collected from three plants while several healthy plants from each pot were kept to record reproductive stage data and yield per plant.

#### 2.4 Relative water content measurement

Relative water content (RWC) was determined followed by the standard procedure of Mostofa & Fujita (2013). In the case of RWC measurement, leaf samples were collected after 21 days of planting and then fresh weight (FW) of leaves were taken and immersed in dH<sub>2</sub>O and kept for 4 hr. After that, excess water was removed from the turgid leaves with a paper towel and turgid weight (TW) was recorded instantly. After that leaves were oven dried at 70 °C for 48hrs and dry weight (DW) was recorded. The RWC was calculated according to the following formula:

RWC (%) =  $(FW - DW)/(TW - DW) \times 100$ 

#### 2.5 Estimation of photosynthetic and gas exchange parameters

The net photosynthetic (Pn) and transpiration rate (Tr), stomatal conductance (Gs), were measured using a portable photosynthetic machine (LCi-SD System, ADC Bioscientific Ltd., Hoddesdon, UK) data were recorded from the fully expanded leaf at 11.00 am to 2.00 pm. The measurements were performed at a 600-700  $\mu$ mol mol<sup>-1</sup> CO<sub>2</sub> concentration, leaf temperature was 28 °C, and photosynthetic photon flux was 600-700  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, the flow rate at 200 mL min<sup>-1</sup>, and the area inside the leaf chamber being at 5.8 mm<sup>3</sup>.

## 2.6 Measurement of leaf chlorophyll contents

The contents of photosynthetic leaf pigments chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), total chlorophyll, and carotenoids were determined spectro-photometrically based on the method described by Lichtenthaler (1987). For this, 0.5g fresh leaves were collected into a small vial containing 10mL of 80% acetone. The containers were covered by aluminum foil and preserved in the dark for 7 days for extraction of pigments. The absorbance was measured from leaf extraction at 663, 645, and 663 nm wavelength for *Chl a*, *Chl b*, and total chlorophyll contents by using a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan). The *Chl a*, *Chl b*, total chlorophyll, and carotenoids contents were calculated using the following equations:

Chlorophyll a =  $0.999 \times A663 - 0.0989 \times A645$ 

Chlorophyll b =  $-0.328 \times A663 + 1.77 \times A645$ 

Carotenoids =  $480 + (0.114 \times 663 - 0.638 \times 645)$ 

### 2.7 Principal component analysis (PCA)

To assess the relationship between the morpho-physiological traits and SA treatments, a PCA was performed using Minitab 17 statistical software

# 2.8 Statistical Analysis

Data collected for each parameter were subjected to one way ANOVA using Minitab 17 statistical software (Minitab Inc., State College, PA, USA). The statistical differences among the mean values of different treatments were compared using Tukey's pairwise comparisons (P < 0.05).

#### **3 Results**

## 3.1 Exogenously applied SA enhances seedling traits of Okra

This study examined the effects of exogenous SA on seedling traits of okra. Data revealed that the growth traits of okra plants were significantly enhanced by SA treatment (Figure 1). Supplementation of 1 mM SA showed a significant increment in germination percentage (GP), coleoptile length (CL), coleoptile weight (CW), shoot length (SL), root length (RL), and seed vigor index (SVI) by 44.3, 74.7, 122.2, 46.7, 84.1, and 131.6%, respectively, over control (Figure 1), while 2 mM SA did not significantly increase the seedling traits. The germination percentage was higher in 1mM SA (86.6%) compared with 2mM (70%) and control (60%), respectively (Figure 1a). Similarly, coleoptile length (6.31 cm) and weight (0.121g) were highest at 1mM concentration of SA (Figure 1b, c). A similar trend was found in the shoot and root length, and seedling vigor index. These results indicate that supplementation of SA enhances the seedling traits of okra.

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Figure 1 Effects of seed priming with SA on okra seed germination (a), coleoptile length (b), coleoptile weight (c), shoot length (d), root length (e), and seed vigor index (f). The error bar represents standard error. Differences among treatments were analyzed by Tukey's test: P<0.05.

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**3.2** Supplementation of SA regulates the relative water content and leaf pigments of okra

This study investigated the water status of okra plants by measuring relative water content (RWC) with or without SA. Supplementation of 1 mM and 2 mM SA increased RWC 13.9 and 21%, respectively, compared with control (Figure 2a).

Results of pigment analysis revealed that there were no significant differences in *Chl a* and *Chl b* content with or without 1 mM SA application. However, they exhibited an increasing trend of *Chl a* (2.7%), *Chl b* (4.3%), and total chlorophyll (3.7%) while carotenoids contents did not affect that concentration of SA compared with control (Figure 2b-e). On the other hand, 2 mM SA reduced the *Chla, Chl b*, total chlorophyll, and carotenoids contents compared with control (Figure 2b-e).





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# 3.3 Exogenous SA regulates photosynthetic parameters of okra plant

The present study investigated the effects of exogenous SA on photosynthetic parameters such Pn, Tr, Gs, and WUE of okra plants. The results showed a significant reduction in Pn (28.09%), Tr (26.07%), and Gs (48.57%) with the application of 2 mM SA compared with control (Figure 3a-c). On the other hand, supplementation of 1 mM SA did not show any effect on Pn, and Tr compared with control (Figure 3a, b). However, the Gs (28.57%) and WUE (27.3%) showed a reduction with 1 mM SA

(a)

application while 2 mM SA application did not change WUE compared with control (Figure 3c-d). These results indicate that supplementation of different concentrations of SA acid regulates the photosynthetic parameters of okra.

# 3.4 The SA enhances plant growth, yield contributing characters, and yield of okra

This study examined the effects of exogenous SA application on the growth of okra plants. The results showed that application of 1 mM SA significantly increased 70.9% plant height (31.3 cm) while



(b)



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2 mM of SA (20.6 cm) did not show any significant increases in plant height as compared to the control (18.3 cm) (Figure 4a). Similarly, supplementation of 1 mM SA reduced the duration of flower bud initiation and the days to first flowering compared to 2 mM SA and control (Figure 4b, c). It has been observed that first flower bud initiation and days to first flowering were observed at 49.67 days and 53.67 days, respectively at 1 mM SA supplementation. A similar trend was reported in the case of final yield, the highest number of fruits per plant (32.6) was found in the case of 1 mM SA (23.6) (Figure 4d).

# 3.5 Estimate the treatment-variables interactions through PCA

The PCA was performed to assess the relationship between the

morpho-physiological traits and SA treatments (Figure 5). PC scores separated three treatments for their positive and negative values across PC1 and PC2 (Figure 5). The PC1 and PC2 conjointly exhibited 81.2% of the data variability among the morpho-physiological traits of okra. PC1 exhibited 48.9% data variability and separated 1.0 mM SA treatment from the other two treatments (control and 2.0 mM SA) for their positive and negative PC scores, respectively (Figure 5). PC1 separated 1.0 mM SA treatment from the two other treatments due to higher positive coefficients of GP, CL, CW, SL, RL, SVI, plant height, number of fruits per plants, RWC, transpiration rate, and contents Chl a, Chl b, total Chl (Figure 5). The PCA2 exhibited 32.3% data variability and this PC separated the control treatment from the 2.0 mM SA treatment largely due to higher negative coefficients of stomatal conductance (Gs) and net photosynthetic rate (Pn) (Figure 5).



Figure 4 Effects exogenous application of SA on plant height, yield and yield contributing characters of okra. (a) Plant height (b) Days to flower bud initiation (c) Days to first flowering (d) total number of fruits/plants. The error bar represents standard error. Differences among treatments were analyzed by Tukey's test: P<0.05.

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Figure 5 Principal component analysis of morpho-physiological parameters of okra. The studied parameters were germination percentage (GP), coleoptile length (CL), coleoptile weight (CW), shoot length (SL), root length (RL), seed vigor index (SVI), plant height, Chl a, Chl b, total Chl, carotenoids, net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), water use efficiency (WUE), days to flower bud initiation (DFBI), days to first flowering (DFF), and fruits per plant.

#### **4** Discussion

It is well-known that proper germination of the seed is the fundamental and crucial step in the plant development cycle, as it plays an important role in the acclimatization of seedlings to the ever-changing environment and its subsequent productivity (Sonya et al., 2019; Shiade & Boelt, 2020). In this era, different pretreatment application techniques are widely being used on the emergence and establishment of seedlings. Seed priming is one of the most widely used techniques that promote seed germination, enhance morphological parameters, and improve plant growth and development under non-stress as well as in stress conditions (Muhei, 2018; Rhaman et al., 2020, 2021a). Therefore, the present study was conducted on the evaluation of the effects of seed priming and exogenous application SA on the okra plant. Many studies reported that okra seed priming enhances germination and SVI (Hussein 2015; Tania et al., 2020; Lamichhane et al., 2021). Results of the current study showed that okra seed priming with 1 mM SA significantly increased GP, CL, CW, SL, RL, and SVI (Figure 1), while 2 mM SA did not show any significant change in the germination rate and seedling traits. The PCA analysis also revealed that the majority of morpho-physiological traits were closely associated with 1 mM SA treatment (Figure 5). These results indicated that a lower concentration of 1 mM SA is more effective than a higher concentration of 2 mM SA on the enhancement of seed germination and seedling traits of okra.

Being a water related trait, RWC is a well-known pointer of water status in plants (Li et al., 2017). It has been reported that

supplementation of different exogenous chemicals enhances the water status of different plant species under stress and normal conditions (Saboon et al. 2015; Ahmad et al. 2017; Tahjib-Ul-Arif et al. 2018). Results of this study also showed that supplementation of SA significantly increased RWC in plants (Figure 2a) and indicates that SA may be involved in up-taking extra water from the soil to fine-tune water status inside plant tissues. Exogenous application of SA enhances leaf pigments in B.napus, wheat, and maize (Baghai et al., 2002; Tahjib-Ul-Arif et al., 2018; Sumaiya et al., 2020). We found that supplementation of 1 mM SA significantly increased Chl a, Chl b, Total chlorophyll contents, and carotenoids of okra leaves. On the contrary, supplementation of 2 mM SA reduced the leaf pigments (Figure 2b-e). These results indicated that a lower concentration of SA may enhance the activities of enzymes related to pigments biosynthesis and/or reduce the oxidative stress which reduces the degradation of leaf pigments. On the other hand, a high concentration of SA may inhibit the biosynthetic enzymes of leaf pigments and/or enhance the oxidative stress which may decline the leaf pigments status (Moharekar et al., 2003; Janda et al., 2014; Ma et al., 2017).

Enhancement of photosynthesis capacity is a crucial step for successful crop production as photosynthetic attributes are the primary determinant of crop yield (Simkin et al., 2019). Plant regulates photosynthetic attributes including Gs, and Tr to enhance photosynthetic capacity (Nazar et al., 2015; Maswada et al., 2018). In the present study, supplementation of 2 mM SA decreased the photosynthetic efficiency which may be associated with decreased Tr and Gs (Figure 3b, c). Similar results have been reported by

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Figure 6 Simplified model showing SA-regulated okra crop enhancement by seed priming and exogenous application of SA.

various previous researchers and suggested that reduced photosynthetic efficiency might be associated with decreased photosynthetic attributes (Maswada et al., 2018; Tahjib-Ul-Arif et al., 2018). Moreover, supplementation of 1 mM SA did not change the Pn and Tr but that supplementation reduced the Gs and WUE (Figure 3c, d). These results indicated that a low concentration of SA induces stomatal closure (Prodhan et al., 2018) and reduces WUE, which may help to enhance RWC (Figure 2a) for the physiological function of plants.

Among various physiological processes, photosynthetic attributes are directly related to crop production. In the present study, supplementation of 1 mM SA significantly enhanced leaf pigments (Figure 2b-e) and regulated Pn, Tr, Gs, and WUE (Figure 3). Thus, low concentration of SA contributed to improved seedling traits (Figure 1), increased plant height, reduced time duration on flower bud initiation and days to first flowering, and increased number of fruits per plant (Figure 4a-d) by regulating photosynthetic attributes. On the other hand, a high concentration of SA reduced the germination and seedling traits (Figure 1) and the number of fruits per plant by degrading the photosynthetic attributes. The PCA analysis also strengthen that supplementation of 1 mM SA enhanced the morpho-physiological parameters of okra (Figure 5). Taken together with all the results in the study, we proposed a simple model for the SA-regulated okra crop enhancement by seed priming and exogenous application of SA (Figure 6). Our results are in agreement with previous reports where seed priming and exogenous application of SA enhance seed germination and productivity of okra by regulating RWC and photosynthetic attributes (Rhaman et al., 2021a).

#### Conclusion

It is concluded that seed priming and exogenous application of SA enhance okra seed germination and productivity by regulating leaf water contents, leaf pigments, and photosynthetic attributes. Further, the results of the current study also suggest that 1 mM SA can be used for successful okra production. However, it is highly recommended to experiment with the field level to validate the results of the current study.

# **Conflict of interest**

The authors declare that they have no conflict of interest.

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