



**Full Length Article**

## Seed Invigoration with Paclobutrazol Improves Seedling Growth, Physiological, Biochemical Attributes and Fruit Yield in Okra

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### Abstract

The production of okra is affected by improper seedling growth and abiotic factors like temperature and soil. This study was aimed to examine the effect of paclobutrazol (PBZ) seed priming on the growth, seedling vigor and yield of okra. A pot experiment was conducted in completely randomized design under natural conditions and seeds of two okra varieties were soaked in 0, 4, 8, 10 and 20 mg L<sup>-1</sup> of PBZ solution. Results showed that PBZ seed soaking affected the germination rate and maximum reduction in germination rate was observed at 20 mg L<sup>-1</sup> of PBZ. A gradual decrease in plant height was recorded in PBZ treated plants as compared to control. A decrease in plant height of PBZ treated plants was accompanied with an increase in plant biomass and number of branches. PBZ seed soaking resulted in darker green leaves with higher chlorophyll, protein and total free amino acid contents than control plants. The activity of antioxidant enzymes, peroxidase and superoxide dismutase was also higher in PBZ treated plants. The improvement in all vegetative and physiological parameters ultimately had a positive effect on yield. PBZ treated plants showed an increase in the number of pods and pod length while seed weight remained unaffected. Thus, seed priming with optimum doses of PBZ could improve the seedlings and yield of okra. © 2021 Friends Science Publishers

**Keywords:** Paclobutrazol; Seed priming; Okra; Fruit yield; Antioxidants

### Introduction

Seed quality and vigor are crucial elements for the stand establishment and productive success of crops (Dotto and Silva 2017). Some techniques like priming, foliar application and soil/medium application of plant growth regulators are being employed to improve seedling vigor and crop productivity. Seed priming is considered to be the most useful strategy being cost-effective and convenient tool (Maiti and Pramanik 2013; Baskin and Baskin 2014; Paparella *et al.* 2015). Seed soaking in a specific solution triggers normal metabolic processes of germination before radical emergence (Chunthaburee *et al.* 2014; Ibrahim 2016), and can enhance the germination percentage, decrease germination time and ensures seedlings establishment (Heydariyan *et al.* 2014; Abiri *et al.* 2016). Reduction in imbibition time (Brocklehurst and Dearman 2008), and metabolic repair during imbibition, and improved metabolites and enzyme activities related to germination results in increased and synchronized germination of primed seeds.

Seed priming can be categorized into hydropriming,

halopriming, osmopriming, and hormone priming (Ibrahim 2016). Hormone priming has been widely used to enhance the germination rate. Previous studies indicated that seed priming with hormones like abscisic acid (Ali *et al.* 2012; Wei *et al.* 2017), salicylic acid (Farahmandfar *et al.* 2013; Ulfat *et al.* 2017) and gibberellic acid (Chunthaburee *et al.* 2014) proved effective for increasing seed germination, growth and yield of wheat (Afzal *et al.* 2005; Iqbal and Ashraf 2013; Ulfat *et al.* 2017), rice (Khaliq *et al.* 2015; Wei *et al.* 2017) beet (Dotto and Silva 2017) and sunflower (Jafri *et al.* 2015). In addition to these hormones, many reports on seed soaking with Paclobutrazol (PBZ) are also available. Application of PBZ in rooting medium also reduced the adverse effects of abiotic stresses (Anwar *et al.* 2017). However, the application of PBZ is not effective due to poor penetration into leaf surfaces (Still and Pill 2003).

Paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1H-1, 2, 4-triazol-1-yl)-pentan-3-ol] belongs to the triazole family of plant growth regulators. PBZ mediated growth-regulating properties in plants by changing the levels of plant growth regulators like gibberellins, cytokinins and abscisic acid (Jaleel *et al.* 2008). In broad

way, it influences the isoprenoid pathway, and alters the phytohormone levels by increasing CKs and ABA contents, whilst reducing the ethylene gibberellin synthesis (Kamounsis and Sereli 1999). Its application induces many physiological changes like enhanced production of photosynthetic pigments (Fletcher *et al.* 2000) and mineral absorption, abiotic stress tolerance, carbohydrate synthesis, flowering and seeds production (Davis *et al.* 1988; Gopi and Jaleel 2009). It also helps maintain chloroplast structure under water stress conditions by increasing antioxidant enzyme activities and maintaining the membrane stability (Mohamed *et al.* 2011; Soumya 2014).

The application of PBZ through seed soaking is considered as an alternative, safe and valuable method to improve germination, reduce plant height and no residual impact on fruits (Magnitskiy *et al.* 2006). Seed priming in 50 or 500 ppm (Still and Pill 2006). PBZ in both tomato and cucumber (Pasian and Bennett 2001; Cho *et al.* 2002) was effective in reducing plant height and increasing yield. Studies have also shown that seed priming with PBZ (4 ppm) improved seed germination, seedling quality, yield and winter hardiness of rapeseed (Anwar *et al.* 2017). It enhanced soluble proteins, proline and lignin contents and reduce the transpiration rate by stomatal closure in many crops (Özmen *et al.* 2003; Jaleel *et al.* 2006; Wang *et al.* 2015; Kamran *et al.* 2018). PBZ has also been found to affect plant growth and development by modifying photosynthetic parameters, which are associated with broader canopy and enhanced light interception (Tesfahun and Menzir 2018). It also delayed leaf senescence and improved flowering and seed yields in different plant species (Davis *et al.* 1988).

Okra [*Abelmoschus esculentus* (L.) Moench] is a popular summer vegetable of many tropical countries including Pakistan. However, many factors like adverse environment, poor nutrition, soil physical properties like soil compaction and mechanical hindrance resulted in poor germination and seedling establishment finally affecting the growth and yield of okra (Kusvuran 2012). Most commonly used okra varieties are tall and have short fruiting period. Hard seed coat is another hindrance for okra seed germination (Felipe *et al.* 2010). In agriculture, rapid and even growth is necessary for maximum yield; the reason why priming is an important solution.

Different growth regulators such as cytokinins, GA<sub>3</sub> and auxins have been applied to many vegetables like tomato (Khan *et al.* 2006), potato (Panwar *et al.* 2006), spinach (Akhtar *et al.* 2008) and citrus (Nawaz *et al.* 2008). But only a few reports are available deciphering the role of PBZ in seedling growth and yield of okra and other vegetables. Keeping in view these facts, the current study was planned to investigate the influence of seed priming with PBZ on germination percentage, seedling growth, physiological condition of the plant and ultimately on yield. This paper is the first report on the use of PBZ as a potential approach to significantly decrease okra plant height with an

increase in the number of branches, which results an increase in plant yield under semi-arid climatic conditions.

## Materials and Methods

### Experiment site, plant material and growth conditions

The experiment was conducted in the wire house of Government College University, Faisalabad, Pakistan, during summer season (April–June, 2018), under natural climatic conditions. Average relative humidity was 44.53%, average temperatures during day and night were 38.9 and 24.13°C, respectively during experiment (PMD 2015). The seeds of two okra varieties namely, Punjab-selection (PS) and Green-gold (GG) were obtained from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan. Seeds of both varieties were primed with 50 mL each of 0, 4, 8, 10 and 20 mg L<sup>-1</sup> PBZ concentrations for 12 h. After priming, seeds were blotted dried and 10 seed were sown in each plastic pot having 20 cm diameter. Pots were filled with mixture of soil, sand and debris (2:1:1). The design of experimental was completely randomized with four replicates per treatment. Fresh leaf samples of okra were harvested after 30 days of sowing kept in a freezer at -20°C for determination of different growth and biochemical attributes.

### Data collection

**Growth and yield attributes:** Pots were examined daily to count number of seeds germinated every day. Germination percentage was calculated by following formula:

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{total number of seeds}} \times 100$$

Seedlings were harvested after 30 days of germination and fresh weights of shoot and root were examined. Dry mass was calculated after drying in an oven at 80°C. Seedling length was measured after separating plant roots from shoots. Number of branches was also calculated. Yield parameters were measured at maturity. Number of pods per plant, pod length, number of seeds per pod and 100 seed weight was calculated.

**Analysis of chlorophyll contents:** For the estimation of chlorophyll contents, 0.5 g fresh leaf samples were grinded in 80% acetone and chlorophyll *a*, *b* and total chlorophyll was determined using Arnon method (1949). Absorbance of the extract was taken at 480, 645, 663 nm was measured using spectrophotometer.

**Total soluble proteins and total free amino acids:** For total soluble protein in fresh leaves of okra was estimated by Bradford method using Coomassie brilliant blue dye (Bradford 1976). The fresh leaf (0.25 g) was grinded in 10 mL chilled potassium phosphate buffer (50 mM). The grinded material was centrifuged for 15 min at 10,000 rpm. Then reaction mixture (100 µL) mixed with Bradford reagent (5 mL) in test tubes. These test tubes were incubated

in dark for 20 min and then absorbance at 595 nm was measured. Hamilton and Van Slyke (1943) method was used for estimation of amino acids. The fresh leaves were grinded in 0.2 M phosphate buffer with pH (7.0). Then, 1% pyridine, 2% ninhydrin was added in a test tube having 1 mL plant extract. Test tubes were placed in boiling water bath for 30 min. Reading was taken at 570 nm on a spectrophotometer. Leucine was used for standard curve and following formula was used for calculation of total free amino acids using the formula:

$$\text{Leucine equivalent TFAA} = \frac{\text{Reading of sample} \times \text{volume of sample} \times \text{dilution factor}}{\text{Fresh leaf weight} \times 1000}$$

**Antioxidant enzymes assays:** For the antioxidant enzyme, fresh leaf (0.1 g) of okra was homogenized with pestle and mortar in 10 mL of ice-cold potassium phosphate (50 mM; pH 7.5). The homogenate was transferred to a plastic centrifuge tube and centrifuged at 10,000 g for 15 min at 4°C and supernatant obtained. For the estimation of superoxide dismutase (SOD) activity, Giannopolitis and Ries (1977) method was used. The principle of this method is to inhibit photochemical reduction of nitrobluetetrazolium (NBT) at 560 nm. The reaction mixture contains extracted enzyme (50 µL) and NBT (50 µL), riboflavin (1.3 µL), methionine (13 mM) and EDTA (75 mM) in glass test tube (Giannopolitis and Ries 1977). Then the reaction mixture was subjected to 15 watts fluorescent lamps at 79 µmol m<sup>-2</sup> s<sup>-1</sup> for 15 min. Peroxidase (POD) activities were measured by using Chance and Maehly (1955) method. The reaction volume (3 mL) contains 100 µL enzyme extract, 50 mM phosphate buffer (pH 7.5), 20 mM guaiacol, and 5.9 mM H<sub>2</sub>O<sub>2</sub>. The POD activity was assessed at 470 nm after every 20 seconds for 2 min using spectrophotometer.

### Statistical analysis

Pots were placed in completely randomized design with three replicates. The collected data were analyzed by two-way analysis of variance (ANOVA) and correlations using Statistix 8.1. software. Means were compared using least significant difference test LSD at  $P < 0.05$ .

## Results

### Germination rate and morphological traits

Priming with PBZ significantly ( $p < 0.001$ ) decreased seed germination rate in both okra varieties (Fig. 1a). Germination rate decreased with increasing PBZ concentration. PBZ20 (20 mg L<sup>-1</sup>) caused 76% decline in germination rate, while PBZ4 showed a 10% decrease in PS as compared to control. GG showed 87% decrease in germination rate at PBZ20 and 8% decrease at PBZ4. Decrease in germination rate was more in GG as compared to PS. PBZ priming significantly reduced ( $p < 0.001$ ) plant height and root length of both the okra varieties (Fig. 1). Maximum plant height and root length were recorded in the

control (PBZ0), while minimum plant height was observed at PBZ20 but root length was minimum at PBZ10. Maximum decrease in shoot length was 75% in PS, while 65% in GG at PBZ20. Root length reduction was 45% in PS and 47% in GG at PBZ10. Reduction in shoot length was more in PS than GG. Biomass of okra plants were increased at 4 and 8 mg L<sup>-1</sup> PBZ concentrations, while reduced at higher PBZ (10 and 20 mg L<sup>-1</sup>) concentrations (Fig. 1). Shoot dry biomass was increased by 32% at PBZ8 in PS. The maximum increase (32%) in shoot dry biomass was observed at PBZ8, which was 32% in PS and 19% in GG. Root dry biomass was also increased at lower PBZ levels. GG showed the highest increase (89%), while PS exhibited 80% increase. Maximum numbers of branches were observed at PBZ8 followed by PBZ4 and PBZ10 (Fig. 1). However, at higher concentration (20 mg L<sup>-1</sup>) maximum reduction in the number of branches was observed. PS showed an increase in the number of branches by 51%, while GG showed increase by 31% at PBZ8.

### Chlorophyll contents

PBZ priming did not significantly affect chlorophyll *a* content of both cultivars (Fig. 2). Maximum chlorophyll *a* content was observed at PBZ8. A maximum increase (15%) was in total chlorophyll contents were noted in GG and 9% in PS as compared to control. PBZ priming showed a significant effect ( $p < 0.05$ ) on chlorophyll *b* and total chlorophyll content in both okra varieties (Fig. 2). The maximum increase in chlorophyll *b* (10%) and total chlorophyll content (18%) in GG was observed at PBZ10, while lowest value was observed at PBZ20 in both parameters. GG accumulated higher photosynthetic pigments as compared to control.

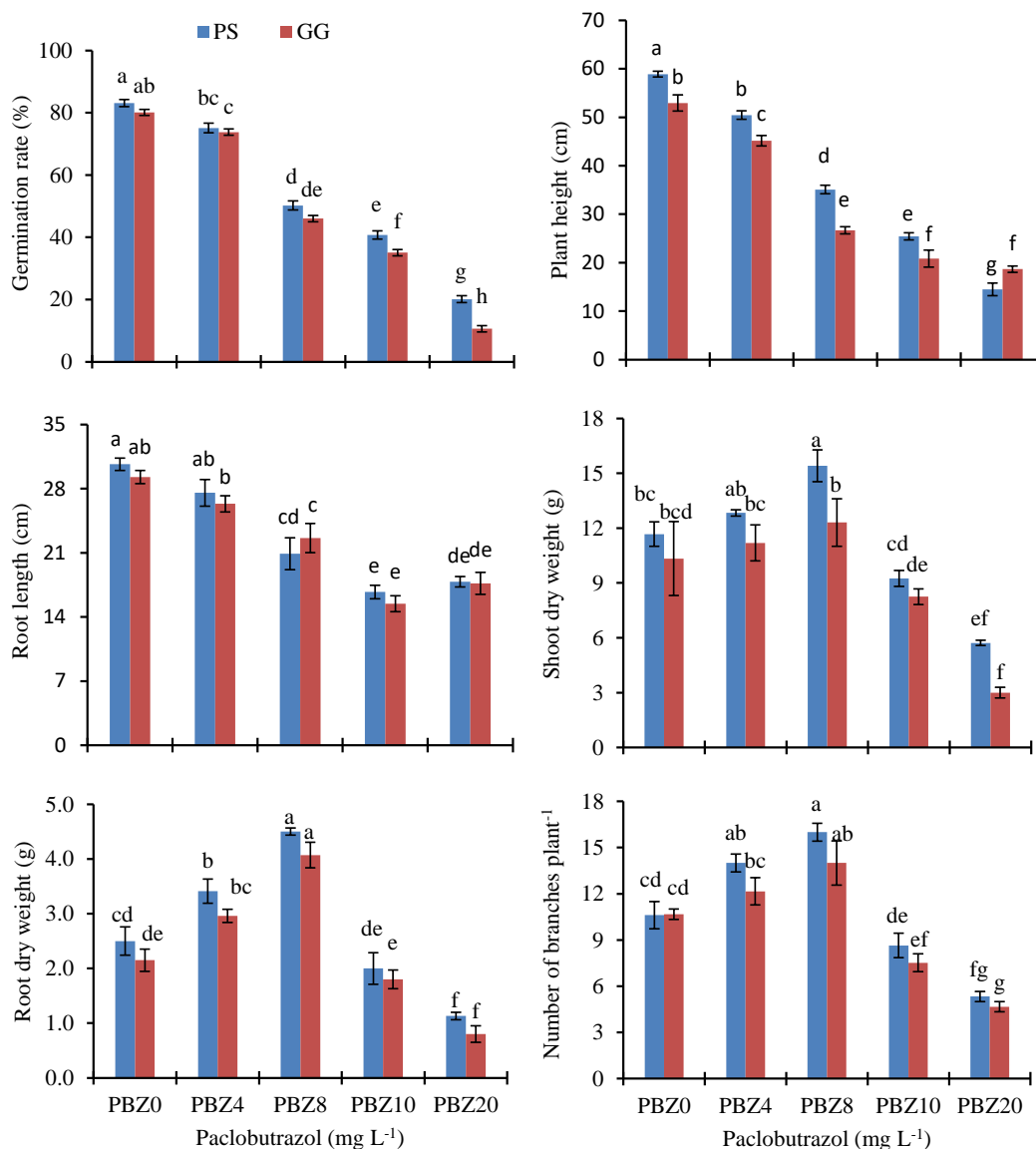
### Protein, total free amino acids and antioxidants

PBZ priming also showed a significant effect ( $p < 0.01$ ) on total free amino acids in both cultivars (Fig. 3a). The highest increase in this attribute (33%) was observed in GG at PBZ8. PBZ priming resulted significant increase ( $p < 0.001$ ) in protein contents in both okra varieties (Fig. 3b). Protein content was increased with an increase in PBZ concentration. A higher protein content in PS (27%) and GG (33%) was observed at PBZ20 as compared to control. PBZ priming showed a significant effect ( $p < 0.001$ ) on POD and SOD dismutase activity (Fig. 3c-d). The maximum increase in POD activity was observed at PBZ20 followed by PBZ10, PBZ8 and PBZ4. PBZ20 showed an increase in POD contents by 27% in PS, while the 33% increase in this activity in GG compared to control. Similarly, SOD activity was also significantly ( $p < 0.001$ ) enhanced by the PBZ application, while the variety × treatment was non-significant ( $P > 0.05$ ). The highest increase in SOD (28%) was observed at PBZ8 in GG. Increase in antioxidant activity was more in GG as compared to PS.

**Table 1:** The effect of seed priming with PBZ on the yield attributes of okra

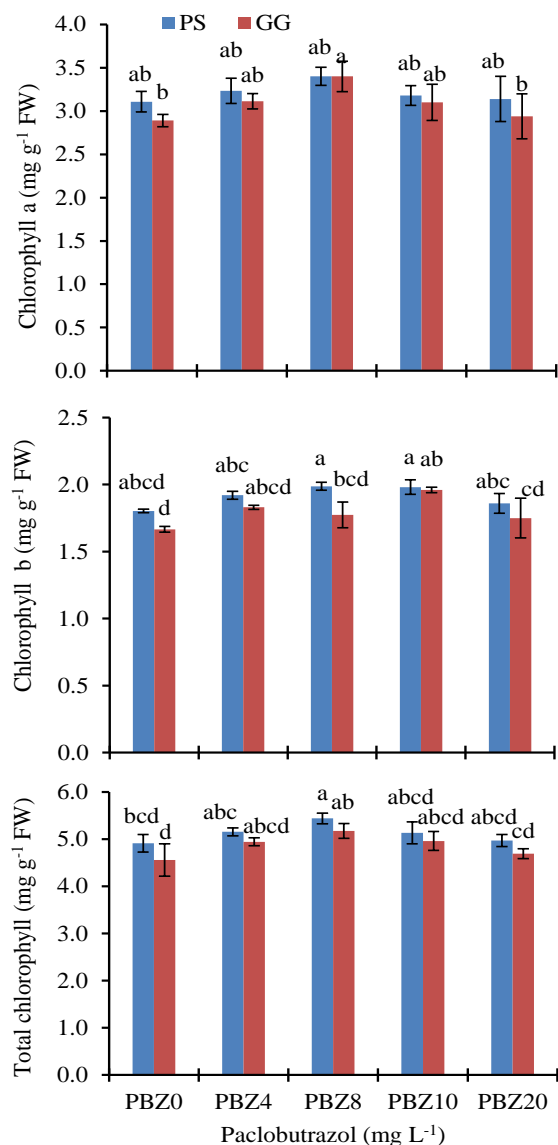
Varieties	Treatments	Number of pods plant <sup>-1</sup>	Pod length (cm)	Number of seeds pod <sup>-1</sup>	100 Seed weight (g)	Grain yield/plant (g)
PS	PBZ0	7.40 ± 0.58 <sup>b</sup>	10.56 ± 0.79 <sup>b</sup>	40.01 ± 1.15 <sup>a</sup>	8.5 ± 0.29 <sup>a</sup>	34.33 ± 2.03
	PBZ4	9.50 ± 1.73 <sup>ab</sup>	12.44 ± 1.60 <sup>ab</sup>	38.11 ± 1.15 <sup>ab</sup>	6.5 ± 0.94 <sup>abc</sup>	38.33 ± 1.85
	PBZ8	12.5 ± 1.30 <sup>a</sup>	14.3 ± 0.72 <sup>a</sup>	35.03 ± 1.73 <sup>bcd</sup>	6.17 ± 0.44 <sup>bcd</sup>	50.5 ± 1.25
	PBZ10	8.17 ± 1.01 <sup>b</sup>	11.0 ± 0.57 <sup>b</sup>	30.50 ± 2.47 <sup>de</sup>	5.03 ± 0.72 <sup>cde</sup>	32.82 ± 2.33
	PBZ20	2.61 ± 0.45 <sup>c</sup>	5.76 ± 0.91 <sup>c</sup>	27.38 ± 1.44 <sup>e</sup>	4.33 ± 0.36 <sup>de</sup>	27.33 ± 1.30
GG	PBZ0	7.07 ± 0.52 <sup>b</sup>	10.67 ± 0.92 <sup>b</sup>	38.13 ± 1.45 <sup>ab</sup>	7.14 ± 0.64 <sup>ab</sup>	33.5 ± 2.18
	PBZ4	8.0 ± 1.15 <sup>b</sup>	12 ± 1.15 <sup>ab</sup>	36.33 ± 1.16 <sup>abc</sup>	6.25 ± 0.43 <sup>abc</sup>	36.15 ± 1.85
	PBZ8	11.50 ± 1.51 <sup>a</sup>	13.01 ± 1.44 <sup>ab</sup>	32.17 ± 1.59 <sup>cd</sup>	5.67 ± 0.72 <sup>bcd</sup>	40.33 ± 0.88
	PBZ10	7.37 ± 0.68	10 ± 1.15 <sup>b</sup>	26.17 ± 3.32 <sup>ef</sup>	5.33 ± 0.88 <sup>bcd</sup>	24.33 ± 1.76
	PBZ20	2.27 ± 0.67 <sup>c</sup>	4.37 ± 1.01 <sup>c</sup>	21.67 ± 2.33 <sup>f</sup>	3.5 ± 0.57 <sup>e</sup>	22.00 ± 1.52
ANOVA	Varieties ( $F_v$ )	1.26 <sup>ns</sup>	1.37 <sup>ns</sup>	10.92 <sup>**</sup>	1.67 <sup>ns</sup>	22.94 <sup>***</sup>
	PBZ ( $F_p$ )	106.24 <sup>***</sup>	18.18 <sup>***</sup>	29.12 <sup>***</sup>	9.43 <sup>***</sup>	40.47 <sup>***</sup>
	$F_v \times F_p$	16.35 <sup>***</sup>	0.17 <sup>ns</sup>	0.57 <sup>ns</sup>	0.43 <sup>ns</sup>	2.50 <sup>ns</sup>

Values (mean ± standard errors) followed by same letters within a column indicate non-significant difference at  $P = 0.05$  based on least significant difference (LSD) test.  $F_v$ ,  $F_p$ ,  $F_v \times F_p$  mean  $F$ -values of varieties, PBZ and their interactions in variance of analysis. \*, \*\* and \*\*\* indicate significant difference at the  $P < 0.05, 0.01$  and  $0.001$ , respectively



**Fig. 1:** Changes in growth characteristics in two okra varieties by exogenous application of PBZ ( $n = 3 \pm SE$ ). Based on LSD test, means shown by same letters are not significantly different at  $P = 0.05$





**Fig. 2:** Changes in chlorophyll contents in two okra varieties by exogenous application of PBZ ( $n = 3 \pm SE$ ). Based on least significant difference (LSD) test, means shown by same letters are not significantly different at  $P = 0.05$

### Yield components

Seed soaking with PBZ significantly affected the number of pods, number of seeds, pod length and 100-seed weight of okra (Table 1). Number of pods and pod length were increased at lower concentrations of PBZ with maximum at PBZ8 and then decreased by further increasing the concentration of PBZ. Number of pods showed a maximum increase of 69% in PS and 63% in GG at PBZ8 as compared to control. The maximum increase in pod length was 35% at PBZ8 in PS and 22% in GG. PBZ priming did not increase the number of seeds per pod and 100 seed weight. Number of seeds and 100 seed weight were gradually reduced in

PBZ seed priming. Maximum reduction was observed at PBZ20 in GG. The 100 seeds weight was minimum at PBZ20, which was reduced to 49% in PS and 51% in GG as compared to control.

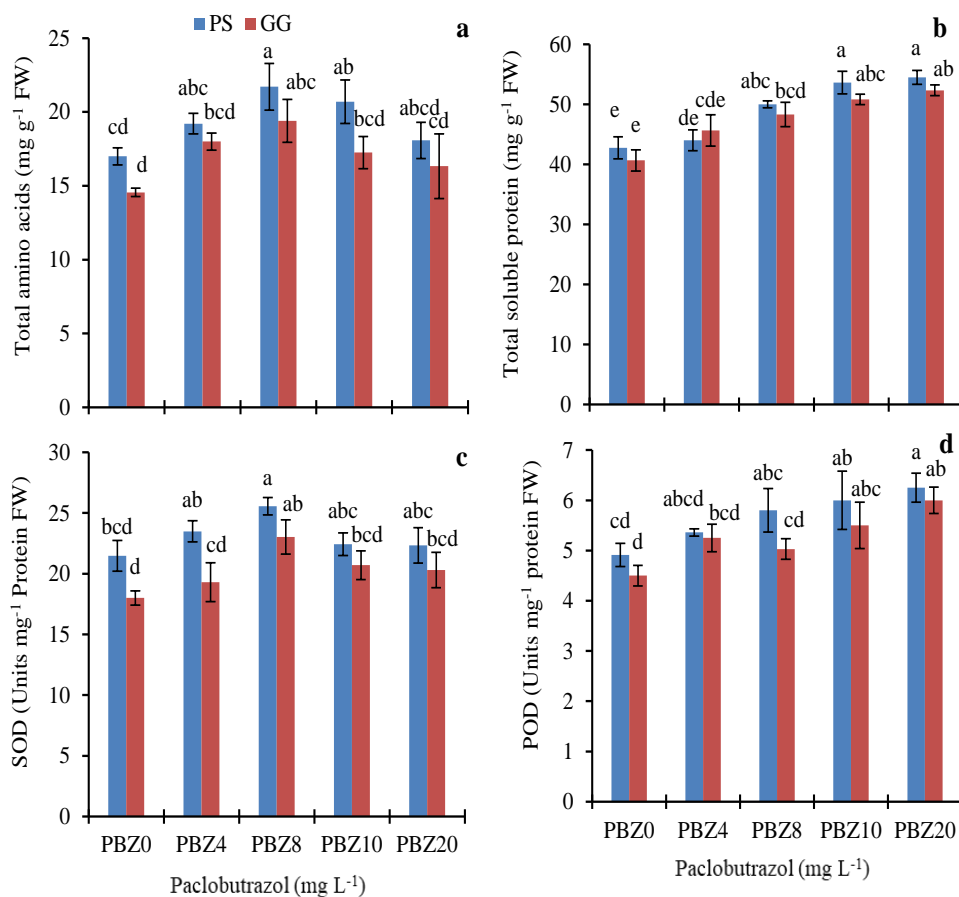
### Correlation of yield attributes with growth and physiological parameters

The correlation matrix analysis was performed to study the correlation between studied parameters. Among yield attributes, yield per plant showed positive correlation with other yield attributes and growth parameters, while negative correlation was observed with some parameters like proteins and POD. 100 seed weight depicted positive correlation with all parameters except Chl. *b*, total free amino acids, proteins and antioxidants. Number of seeds per pod exhibited negative correlation with Chl. *b*, protein and POD. Pod length and number of pods per plant also showed positive correlation with all parameters except protein and POD (Fig. 4).

### Discussion

It is known that PBZ acts as plant growth regulator and at appropriate level makes a remarkable effect on crop growth, physiology and productivity by modifying photosynthetic rate and phytohormone levels (Kim *et al.* 2012). PBZ application has been known to reduce plant height, growth, internodal length and leaf area. Reduction in plant height is associated with inhibition of gibberellin synthesis, which reduced internodal length (Fletcher *et al.* 2000). The potential role of PBZ on growth, physiology and yield of tomato with  $1 \text{ mg L}^{-1}$  treatment of PBZ as soil application and with  $25 \text{ mg L}^{-1}$  foliar application has already been reported (Berova and Zlatev 2000). PBZ application at  $1.25 \text{ g m}^{-1}$  of canopy diameter on *Jatropha* showed positive effect on vegetative growth and yield (Ghosh *et al.* 2010). In this study, we investigated the positive response after seed priming with PBZ on okra growth, physiology and yield attributes. Results indicated positive correlation between PBZ use and yield enhancement in okra.

Priming with plant growth retardants offer some common problems like reduction or delay in seed germination and emergence. Similarly, seed germination rate of okra imparted a noticeable sensitivity to PBZ priming in the present investigation. The germination rate was decreased with an increase in PBZ concentration. Highest PBZ concentration ( $20 \text{ mg L}^{-1}$ ) significantly reduced the germination rate in both okra varieties, while lower concentration ( $4 \text{ mg L}^{-1}$ ) has a little effect on germination as compared to control (Fig. 1). Seed priming with 1000 ppm PBZ decreased the germination percentage in *Cosmos bipinnatus*, marigold, tomato and geranium seeds with 500 or 1000 ppm PBZ (Pasian and Bennett 2001; Pill and Gunter 2001). Decline in seed germination may be related to the nature of the seed coat as seeds do not absorb



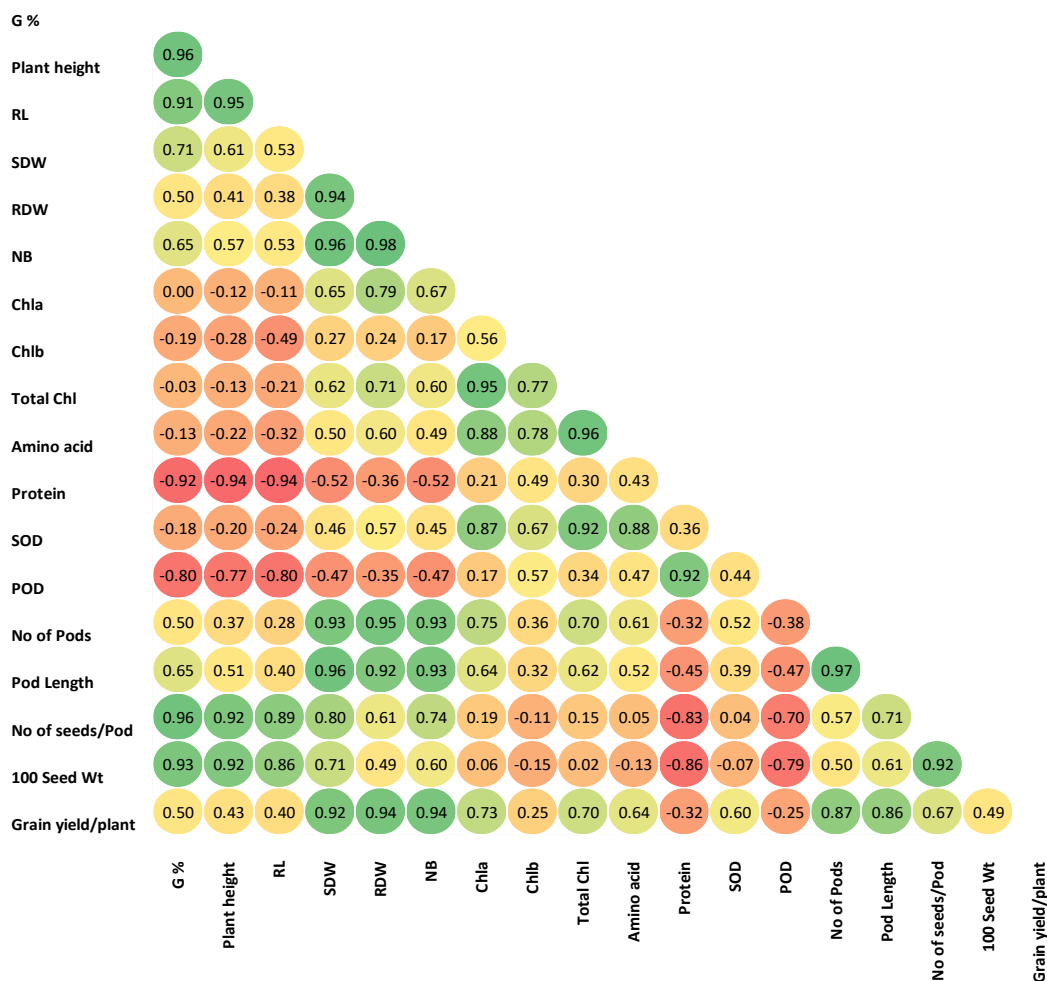
**Fig. 3:** Changes in (a) total amino acids content, (b) total soluble protein content, (c) superoxide dismutase (SOD) content and (d) peroxidase (POD) content in two okra varieties by exogenous application of PBZ ( $n = 3 \pm SE$ ). Based on least significant difference (LSD) test, means shown by same letters are not significantly different at  $P = 0.05$

PBZ; it only adheres to the seed coat. In general, the reduction in seed germination and emergence might depend on some factors like chemical nature, location and kind of seed coat properties. After sowing, it subsequently diffuses into the rooting media from where it is absorbed by roots (Pasian and Bennet 2001). PBZ repressed radical emergence by hindering gibberellin biosynthesis and its addition to soil medium reversed this process. Such results were demonstrated in proteome analysis of *Arabidopsis* (Gallardo *et al.* 2002).

In this study, plant height and root length of both okra varieties were reduced at all the concentrations, but maximum reduction was observed at 20 mg L<sup>-1</sup> PBZ (Fig. 1). These findings were in accordance with previously reported investigations by Kumar *et al.* (2012) in camellias (47.5% reduction), Syahputra *et al.* (2013) in rice plants, Koutroubas and DamLas (2015) in sunflower. Decrease in plant height was associated with the inhibition of gibberellin synthesis (Deneke and Keever 1992; Setia *et al.* 1995; Berova and Zlatev 2000) and decrease in internodal length (Tesfahun and Menzir 2018). Reduction in plant height also altered the branching pattern with respect to an increase in

the number of branches. These results corroborate with Kumar *et al.* (2012) in *Camelina*, Ghosh *et al.* (2010) in *Jatropha*, Bañón *et al.* (2002) in *Dianthus caryophyllus*, Yeshitela *et al.* (2004) in mango. The inhibition of gibberellin biosynthesis disturbed hormonal balance, which activates axillary bud initiation and branches (Woodward and Marshall 1988). It may be related to an increase in dry biomass and yield after PBZ application. In the present study, an increase in plant biomass was observed by PBZ application at 8 mg L<sup>-1</sup> but an increase in PBZ concentration declined plant biomass. Yan *et al.* (2013) reported an increase in root activity and growth in soybean by uniconazole application.

In the present study, after PBZ priming, the leaves were dark green in color and photosynthetic pigments were higher than the control. Maximum chlorophyll contents were observed at 8 mg L<sup>-1</sup> PBZ while in other treatments there was not much difference. An increase in the photosynthetic pigments has been positively associated with an increase in plant growth. Similar findings were reported by Kumar *et al.* (2012) in *Camelina*, Dalziel and Lawrence (1984) in sugar beet, Berova and Zlatev (2000) in tomato,



**Fig. 4:** Correlation among morphological, physiological, antioxidant and yield attributes of okra by PBZ seed priming. G%: germination percentage; RL: root length; SDW: shoot dry weight; RDW: root dry weight; NB: number of branches; SOD: superoxide dismutase; POD: peroxidase

Belakbir (1998) in pepper; and Sebastian *et al.* (2002) in *Dianthus caryophyllus*. Previous reports revealed that increased chlorophyll synthesis by PBZ application was due to an enhanced phytyl production, which is an essential part of chlorophyll molecule. Phytyl is synthesized by the same terpenoid pathway as do the gibberellins. As PBZ inhibits gibberellins biosynthesis, so the terpenoid pathway increased phytyl production by utilizing intermediates of gibberellins synthesis, which ultimately increased chlorophyll contents (Chaney 2003).

Okra seed priming with PBZ enhanced total protein contents in both cultivars as compared to control, but the difference between PBZ levels was not significant. PBZ application increased the cytokinins level, which in turn increased the protein content by stimulating its synthesis and preventing its degradation (Campbell *et al.* 2008). Priming of okra seeds with PBZ enhanced antioxidant enzyme activities in comparison with control. The increase in POD activity was observed at all PBZ concentrations, while SOD

activity was maximum at 8 mg L<sup>-1</sup> of PBZ in both varieties. Previously a positive role of PBZ in ameliorating the adverse effects of water stress by increasing the activity of antioxidants has been reported in many plants including tomatoes, mangoes, groundnuts and sesame seeds (Manivannan *et al.* 2008; Dahuja and Sharma 2010; Srivastav *et al.* 2010; Agamy and Rady 2011). An increase in photosynthetic pigments showed an improved photosynthesis and yield (Kumar *et al.* 2012; Jungklang *et al.* 2017).

PBZ priming increased the number of pods per plant and maximum number of pods was observed at 8 mg L<sup>-1</sup>. PBZ also exhibited an increase in the number of flowers, which showed its positive effect on flowering. There are reports that point to changes in phloem to xylem ratio of stem, phenolic contents of terminal buds and total non-structural carbohydrates in mango (Voon *et al.* 1991; Kurian and Iyer 1992). An increase in translocation of photoassimilates and nutrients to branches has been reported

in peanut (Setia et al. 1995) and *Brassica napus* (Addo-Quaye et al. 1985). In our study, PBZ concentration increased number of pods/plant and pod length while number of seeds and seed weight decreased when compared with control.

PBZ priming at 8 mg L<sup>-1</sup> followed by 4 mg L<sup>-1</sup> PBZ increased grain yield per plant maximally, but declined as compared to control. This increase may be correlated with an increase in morphological, physiological, and biochemical attributes after PBZ priming at these concentrations. Our studies demonstrated positive correlation of grain yield with germination percentage, plant height, root length, shoot and root dry weight, chlorophyll contents, amino acids and antioxidants (Fig. 4).

The PBZ priming of okra seeds had positive effects on seedling vigor and yield. Seedling vigor is associated with an increase in fresh and dry biomass, number of branches, photosynthetic pigments, antioxidant enzyme activities, protein contents, number of pods/plant and seed yield per plant. Likewise, seed yield per plant exhibited a positive correlation with different growth attributes and chlorophyll pigment in the present study, while negative correlation between yield attribute (100 seed weight) and biochemical attributes (total free amino acids, total soluble protein content, SOD and POD contents) were observed (Fig. 4). Therefore, incubation of okra seeds with PBZ improved seedling vigor by reducing plant height and improving morphological and physiological parameters.

## Conclusion

This is the first report on the use of PBZ as priming agent to minimize height and increase yield in okra. PBZ proved very effective in controlling plant height at lower concentration (8 mg L<sup>-1</sup>). It also improved all physiological parameters including chlorophyll, protein and total free amino acid contents together with antioxidant enzymes activities. The increase in physiological parameters has a positive influence on okra yield. Seed soaking with PBZ can be used as a potential strategy to improve growth and yield of crops.

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## Author Contributions

RB and IH supervised the whole research work; RR performed the research work; RB, IH, MA and SA interpreted results and wrote first draft. All authors read and approved the final script.

## Conflicts of Interest

No conflict of interest is declared by authors

## Data Availability

Data is available with the author and will be made available on a reasonable request.

## Ethics Approval

Not applicable

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