Seed Priming Improves Seed Germination and Seedling Growth of *Isatis indigotica* Fort. under Salt Stress

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Abstract. The effects of CaCl₂, GA₃, and H₂O₂ priming on *Isatis indigotica* Fort. seed germination characteristics, seedling growth parameters, and antioxidant enzyme activities under salt stress were investigated. NaCl had an adverse effect on the germination and seedling performance of *I. indigotica*. However, these three priming agents alleviated salt stress by increasing the germination percentage, improving seed vigor, accelerating germination velocity, and establishing strong seedlings. The optimal concentrations were 15 g/L for CaCl₂, 0.2 g/L for GA₃, and 40 mM for H₂O₂. Seed priming treatments enhanced the activities of antioxidant enzymes in seedlings, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), under a salt environment, which reduced the oxidative injury caused by salt. Seed priming is a promising technique that can enhance the ability of *I. indigotica* seed germination when salt is present.

Isatis indigotica Fort, a member of the Brassicaceae family, is widely distributed in China. *I. indigotica* is a biennial herbaceous plant. In the first year, the root and leaf are harvested to be processed into indigowoad root and indigowoad leaf that are believed to have antibacterial, antiviral, and immunomodulating effects and used as a traditional Chinese medicine (TCM) in China. In the second year, seeds are collected to be used for the cultivation of the plant. Currently, propagation by seeds is considered the most efficient method of *I. indigotica* production to fulfill an increasing demand.

Salinity and abiotic stress are limiting factors for plant growth. At least 20% of irrigated fields across the world are affected by salt (Pitman and Läuchli, 2002) due to improper watering and drainage systems, little rain, and evaporation (Munns and Tester, 2008). In China, according to the second national soil survey, the total area of saline soil is 9.913×10^7 ha. Although salinity stress appears to be an inhibitory factor at every stage of plant growth, the most sensitive

phase comprises the germination and seedling periods (Patade et al., 2011), which are essential for plant growth (Hubbard et al., 2012). Therefore, effective and suitable measures should be developed to enhance seed germination in saline soils and facilitate plant establishment.

Seed priming is considered a critical and vital technique for enhancing stress tolerance through pretreatment with stimulating factors to adapt seeds more resistant to subsequent adverse factors (Tanou et al., 2012). Priming causes some metabolic changes to prepare seeds for plumule emergence (Farooq et al., 2007). It has been well-documented that priming treatment has enhanced salinity resistance in *Cassia obtusifolia* L. (Zhang et al., 2012, 2013, 2016), *Glycyrrhizauralensis* (Zhang et al., 2015), *Cynanchum bungei* Decne (Zhang, 2015), maize (Panuccio et al., 2018), wheat (Ali et al., 2017), broccoli and cauliflower (Wu et al., 2019), and lettuce (Ouhibi et al., 2014). The catalytic role of seed priming is related to a series of physiological, biochemical, and molecular changes. Among them, inducing the antioxidant system is a typical stress-avoidance response. Priming treatments increases the expression of antioxidant enzymes (Panuccio et al., 2018), which can optimize host defense mechanisms and reduce the oxidative damage caused by the accumulation of reactive oxygen species (ROS).

Although there have been many studies regarding the impact of seed priming on alleviating salt stress in a variety of plants, no investigation of the roles of priming in seed germination and seedling growth of *I. indigotica* under salt stress has been reported. Therefore, this study was designed to investigate the roles of three priming agents at different concentrations in seed germination,

early seedling growth, and antioxidant enzyme activities of *I. indigotica* under salinity conditions.

Materials and Methods

Seed materials and priming. Seeds of *I. indigotica* Fort. were provided by Mashan TCM base in Jinan City, Shandong Province, China. Seeds were soaked in CaCl₂ (5, 10, 15 g/L), GA₃ (0.2, 0.4, 0.6 g/L), and H₂O₂ (20, 40, 60 mM) for 12 h using a 1:5 ratio of seed weight (g) to solution volume (mL). Then, seeds were rinsed with distilled water and air-dried to the original weight.

Germination tests. Our preliminary experiments demonstrated that 100 mM NaCl (abbreviated as NaCl) decreased germination of *I. indigotica* seeds by approximately 40%. Therefore, the concentration of NaCl was used to induce salinity stress in this study. The treatments and their concentrations were as follows: distilled water [control (CK)]; NaCl; 5 g/L CaCl₂ + NaCl; 10 g/L CaCl₂ + NaCl; 15 g/L CaCl₂ + NaCl; 0.2 g/L GA₃ + NaCl; 0.4 g/L GA₃ + NaCl; 0.6 g/L GA₃ + NaCl; 20 mM H₂O₂ + NaCl; 40 mM H₂O₂ + NaCl; and 60 mM H₂O₂ + NaCl. Thirty-six seeds were sown in plastic containers (length, 19 cm; width, 13 cm; height, 12 cm) filled with 1.2 kg sand containing a ratio of 1:6 of either distilled water or NaCl solution to sand (Zhang, 2012). Six replications were arranged for each treatment. The plastic containers were placed in an incubator under alternating cycles of 25 °C for 8 h in the light and 15 °C for 16 h in the dark for 20 d.

The germinated seeds were checked and counted every day. Seed was considered to germinate when cotyledon emerged above the sand. The final germination percentage was determined on day 14. Germination index, vigor index, germination velocity, and mean germination time were calculated according to the reported method (Hu et al., 2005; Timson, 1965; Zhang, 2015). On day 20, shoot height, root length, and seedling fresh weight were measured. Seedling was dried at 60 °C until constant weight to quantify the seedling as dry weight.

Antioxidant enzyme assays. The most effective concentrations of three priming agents to alleviate salt detriment on germination and seedling performance were 15 g/L CaCl₂, 0.2 g/L GA₃, and 40 mM H₂O₂, respectively. Five treatments (i.e., CK, NaCl, 15 g/L CaCl₂ + NaCl, 0.2 g/L GA₃ + NaCl, 40 mM H₂O₂ + NaCl) were chosen for testing the ROS activity. Leaves on day 20 were sampled for enzyme assays.

Leaves were homogenized in a prechilled mortar and pestle with ice-cold 0.05 M phosphate buffer consisting of 1% polyvinylpyrrolidone. The subsequent mixture was centrifuged and the supernatant fraction was used to determine enzyme activity. The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were determined according to Zhang and Li (2011). SOD activity was detected by the prevention of the photochemical reduction of

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Table 1. Germination percentage of *I. indigotica* seeds according to different priming treatments under 100 mM NaCl stress.

Treatment	Germination percentage (%)												
	2 d	3 d	4 d	5 d	6 d	7 d	8 d	9 d	10 d	11 d	12 d	13 d	14 d
Control	0	48.0 a ^z	75.3 a	81.3 a	86.0 a	92.0 a	92.0 a	92.0 a	92.0 a	92.0 a	92.0 a	92.0 a	92.0 a
NaCl	0	0.0 f	13.9 g	21.3 g	35.2 e	39.8 d	40.7 d	50.9 e	54.6 e	54.6 d	56.5 d	58.3 d	58.3 d
5 g/L CaCl ₂ + NaCl	0	3.7 e	22.2 f	35.2 f	51.9 d	62.0 c	63.9 c	63.9 d	64.8 d	66.7 c	68.5 c	69.4 bc	70.4 bc
10 g/L CaCl ₂ + NaCl	0	8.3 d	38.9 d	50.0 cd	56.9 c	63.9 bc	65.3 c	68.1 bc	69.4 b	69.4 bc	69.4 bc	69.4 bc	69.4 bc
15 g/L CaCl ₂ + NaCl	0	15.7 c	38.0 d	49.1 cd	62.0 b	66.7 b	70.4 b	71.3 b	71.3 b	71.3 b	71.3 b	71.3 b	71.3 b
0.2 g/L GA ₃ + NaCl	0	22.2 b	50.0 b	59.3 b	63.9 b	65.7 b	68.5 b	68.5 bc	69.4 b	71.3 b	72.2 b	72.2 b	72.2 b
0.4 g/L GA ₃ + NaCl	0	19.4 bc	50.0 b	55.6 bc	62.5 b	62.5 c	65.3 c	66.7 c	68.1 bc	68.1 c	69.4 bc	69.4 bc	69.4 bc
0.6 g/L GA ₃ + NaCl	0	15.3 c	43.1 c	51.4 cd	59.7 c	62.5 c	65.3 c	65.3 d	66.7 c	68.1 c	69.4 bc	69.4 bc	69.4 bc
20 mM H ₂ O ₂ + NaCl	0	15.7 c	39.8 d	48.1 cd	59.3 c	65.7 b	68.5 b	68.5 bc	68.5 bc	68.5 c	68.5 c	68.5 c	68.5 c
40 mM H ₂ O ₂ + NaCl	0	18.1 bc	43.1 c	48.6 cd	62.5 b	63.9 bc	68.1 b	69.4 b	70.8 b	72.2 b	72.2 b	72.2 b	72.2 b
60 mM H ₂ O ₂ + NaCl	0	8.3 d	31.5 e	45.4 e	58.3 c	63.9 bc	64.8 c	66.7 c	66.7 c	68.5 c	68.5 c	68.5 c	68.5 c

^zMeans of treatments designated with the same letter in a given column were not significantly different ($P \leq 0.05$).

nitro blue tetrazolium determined at 560 nm. POD activity was measured in terms of the guaiacol oxidation through H₂O₂ at 470 nm. CAT activity was quantified spectrophotometrically in accordance with the reduction in absorbance at 240 nm.

Data analysis. Data were conducted using an analysis of variance and least significant difference test at a 5% probability level using SPSS 11.5 software.

Results

Effects of seed priming on seed germination of *I. indigotica* under salt stress. *I. indigotica* seeds began to germinate on the third day and had a final germination percentage of 92.0% under the control condition (Table 1). NaCl treatment significantly inhibited seed germination. Only a few seeds began to germinate on the fourth day, and the final germination percentage decreased to 58.3% (Table 1). All priming treatments improved the germination percentage during the entire germination period under salt stress. In addition, treatments with 15 g/L CaCl₂, 0.2 g/L GA₃, and 40 mM H₂O₂ were found to result in the most promising improvements according to the data displayed in Table 1.

The germination index, vigor index, and germination velocity of *I. indigotica* seeds under salt stress were significantly lower than those of the CK, whereas the mean germination time was significantly longer than that of the CK (Table 2). Priming treatments alleviated the adverse impacts caused by salinity. Compared with the NaCl treatment, treatments with 5, 10, and 15 g/L CaCl₂ increased the germination index by 37.0%, 56.7%, and 66.9%, the vigor index by 38.4%, 58.0%, and 76.1%, and the germination velocity by 39.0%, 59.6%, and 69.0%, respectively. These same treatments decreased the mean germination time by 12.9%, 26.5%, and 29.1%, respectively. Treatments with 0.2, 0.4, and 0.6 g/L GA₃ increased the germination index by 82.3%, 74.4%, and 64.9%, the vigor index by 122.1%, 109.4%, and 95.3%, the germination velocity by 81.7%, 74.2%, and 65.5%, respectively; however, they decreased the mean germination time by 33.2%, 32.8%, and 28.4%, respectively. Treatments with 20, 40, and 60 mM H₂O₂ increased the germination index by 63.3%, 71.1%, and 50.4%, the vigor index by 75.1%, 89.3%, and 57.5%, and the

Table 2. Germination parameters of *I. indigotica* seeds at different priming treatments under 100 mM NaCl stress.

Treatment	Germination index	Vigor index	Germination velocity	Mean germination time (d)
Control	9.24 a ^z	74.82 a	1.39 a	4.09 g
NaCl	3.54 h	20.98 i	0.54 h	6.76 a
5 g/L CaCl ₂ + NaCl	4.85 g	29.04 h	0.75 g	5.89 b
10 g/L CaCl ₂ + NaCl	5.55 f	33.15 g	0.86 ef	4.97 cd
15 g/L CaCl ₂ + NaCl	5.91 de	36.95 f	0.91 cd	4.79 de
0.2 g/L GA ₃ + NaCl	6.45 b	46.59 b	0.98 b	4.52 f
0.4 g/L GA ₃ + NaCl	6.17 c	43.93 c	0.94 c	4.55 f
0.6 g/L GA ₃ + NaCl	5.84 e	40.97 d	0.89 de	4.84 cde
20 mM H ₂ O ₂ + NaCl	5.78 e	36.74 f	0.89 de	4.87 cde
40 mM H ₂ O ₂ + NaCl	6.06 cd	39.72 e	0.93 cd	4.66 ef
60 mM H ₂ O ₂ + NaCl	5.32 f	33.05 g	0.83 f	5.06 c

^zMeans of treatments designated with the same letter in a given column were not significantly different ($P \leq 0.05$).

Table 3. Seedling growth parameters of *I. indigotica* with different priming treatments under 100 mM NaCl stress.

Treatment	Shoot ht (cm)	Root length (cm)	Seedling fresh wt (mg/plant)	Seedling dry wt (mg/plant)
Control	10.10 a ^z	10.26 a	55.47 a	5.26 a
NaCl	6.93 f	3.60 e	31.98 f	3.33 e
5 g/L CaCl ₂ + NaCl	7.98 e	4.62 d	34.23 e	3.81 d
10 g/L CaCl ₂ + NaCl	7.98 e	4.67 d	35.95 e	3.81 d
15 g/L CaCl ₂ + NaCl	8.25 d	5.19 c	38.86 d	4.30 c
0.2 g/L GA ₃ + NaCl	9.22 b	6.23 b	47.93 b	4.84 b
0.4 g/L GA ₃ + NaCl	9.12 bc	5.20 c	44.27 c	4.36 c
0.6 g/L GA ₃ + NaCl	8.72 c	5.13 c	43.23 c	3.92 d
20 mM H ₂ O ₂ + NaCl	8.36 d	4.64 d	39.98 d	4.05 d
40 mM H ₂ O ₂ + NaCl	8.96 bc	5.18 c	43.16 c	4.45 bc
60 mM H ₂ O ₂ + NaCl	7.91 e	4.77 cd	37.15 de	4.02 d

^zMeans of treatments designated with the same letter in a given column were not significantly different ($P \leq 0.05$).

germination velocity by 65.2%, 71.3%, and 53.4%, respectively; however, they decreased the mean germination time by 31.0%, 28.0%, and 25.1%, respectively (Table 2). Nevertheless, it was found that the most effective concentrations for priming treatments were 15 g/L CaCl₂, 0.2 g/L GA₃, and 40 mM H₂O₂.

Effects of seed priming on seedling growth of *I. indigotica* under salt stress. Compared with the CK, shoot height, root length, seedling fresh weight, and dry weight in the NaCl treatment group were decreased by 31.4%, 64.9%, 42.4%, and 36.7%, respectively, suggesting that salt inhibited early seedling growth of *I. indigotica* and that root growth was more sensitive to salt than shoot growth (Table 3). Similar to the findings for the germination parameters described, seed priming treatments (compared with the NaCl

treatment) accelerated the seedling performance. Additionally, different priming agents and concentrations had different effects. Generally, early seedling growth was the best for the GA₃ priming treatment, followed by the H₂O₂ treatment and the CaCl₂ treatment.

Effects of seed priming on antioxidant enzymes activity of *I. indigotica* under salt stress. Antioxidant enzymes activities following the NaCl treatment were increased compared with the control. In general, antioxidant enzymes activities were higher among priming treatment groups than among the NaCl treatment group (Fig. 1). Compared with NaCl treatment, CaCl₂ priming increased SOD, POD, and CAT activities by 37.9%, 25.6%, and 7.3%, GA₃ priming increased the activities of the three enzymes by 75.0%, 47.2%, and 26.9%, and H₂O₂ priming increased

Fig. 1. Activities of SOD, POD, and CAT on *I. indigotica* at different priming treatments under 100 mM NaCl stress. Data are presented as mean $\pm$ SD. Bars sharing the same letter for a parameter indicated no significant ($P \leq 0.05$) between the groups. CK = control; CaCl₂ = 15 g/L CaCl₂; GA₃ = 0.2 g/L GA₃; H₂O₂ = 40 mM H₂O₂; SOD = superoxide dismutase; POD = peroxidase; and CAT = catalase.

the activities of the three enzymes by 48.3%, 32.8%, and 13.8% (Fig. 1), respectively.

Discussion

Salt stress affects plant growth through interrupting ionic equilibrium and imposing

osmotic stress (Zhu, 2003). In the current study, we found that salt resulted in a distinct decrease in the germination and seedling performance, indicating an unfavorable role of salt in *I. indigotica* growth. We also found that the inhibition was more prominent in the root than in the shoot, suggesting that salinity

has a more pronounced impact on the root. The results were consistent with previous studies of maize (Tang et al., 2019), *S. marianum* (Migahid et al., 2019), and *C. bungei* (Zhang, 2015).

Plants have sophisticated antioxidative defense mechanisms to clear ROS generated in the process of stress. Normally, plants produce some ROS and maintain a good balance between the generation and extinguish of these substances. Under stress conditions, such a balance may be broken because of a quick expansion in ROS level, leading to oxidative injury to lipids, proteins, and nucleic acids (Xie et al., 2019). Therefore, plants enhance the activities of their endogenous antioxidant enzymes against the damage under stress. Our study showed that the activities of SOD, POD, and CAT were increased upon NaCl treatment, suggesting that *I. indigotica* may have a protective response to salinity through an elevation of multiple antioxidant enzyme activities.

It was demonstrated that the priming agents tested in this study had a highly active role in the relief of the detrimental impact of salt stress. Germination and seedling improvements with CaCl₂ priming have also been reported for wheat (Khan et al., 2019), rice (Roy et al., 2019; Tahjib-Ul-Arif et al., 2019), and soybean (Dai et al., 2017), which were consistent with our findings. The CaCl₂-induced protective effects might be related to Ca²⁺ that helps to regulate the structural force of the cell wall, structural integrity, and penetrability of the cell membrane, as well as mitotic activation of cell (Hepler, 2005). Outside cells, Ca²⁺ also contributes to relieving salt damage due to the reduction in Na⁺ influx and K⁺ efflux through nonselective cation channel (Rathod and Anand, 2016).

In the case of salt, plant hormones have a vital role in modulating metabolic reactions that can adapt to adverse conditions. GA₃ priming could accelerate germination and seedling performance in a saline environment through improving antioxidant enzyme activities and alleviating membrane damage (Ghassemi-Golezani and Nikpour-Rashidabad, 2017; Younesi and Moradi, 2014). These were also verified in the current study. In addition, GA₃ activates the ABA-catabolizing enzymes, and then reduces ABA composition to promote seed germination (Miransari and Smith, 2014).

It is generally considered that H₂O₂ is a useful priming factor that can have a positive impact on the plant response to different kinds of stresses (Christou et al., 2014). The results of the current investigation indicated that H₂O₂-primed seeds exhibited an increase in the germination performance, seedling growth parameters, and increased SOD, POD, and CAT activities as compared with the seeds treated with NaCl. Therefore, H₂O₂ priming has an ameliorative effect on the growth of *I. indigotica* by preventing oxidative damage under salt stress. Furthermore, H₂O₂ pretreatment also alleviated the oxidative damage, maintained the membrane integrity, and promoted the expression of stress proteins in wheat when salt was present

(Wahid et al., 2007). Moreover, exogenous utilization of H₂O₂ stimulated some transcription factors and signal proteins that act together as resistance to various stresses (Hossain et al., 2015).

Conclusions

Seed priming with CaCl₂, GA₃, and H₂O₂ improved *I. indigotica* Fort. seed germination and seedling growth under salt stress. The optimal concentrations were 15 g/L for CaCl₂ priming, 0.2 g/L for GA₃ priming, and 40 mM for H₂O₂ priming. Seed priming treatments greatly promoted SOD, POD, and CAT activities and alleviated the oxidative damage induced by salt stress in *I. indigotica*. Therefore, it may be concluded that seed priming is a promising approach to accelerating *I. indigotica* growth under salt conditions.

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