ISSN: 2287-688X

Annals of
Plant Sciences

Volume 11, Issue 06 (2022) pp. 5167-5175

Research Article

Seed Priming with Proline and Glycine Betaine Enhances Germination and Seedling Growth of Maize (*Zea mays*) Under Chilling Stress

Shaila Shermin Tania[1], Mohammad Saidur Rhaman[1]*, Md. Moklasur Rahaman[1] and Md Anamul Hoque[2]

[1]*Department of Seed Science and Technology, Bangladesh Agricultural University, Mymensingh-2202*

[2]*Department of Soil Science, Bangladesh Agricultural University, Mymensingh-2202*

Abstract

Abiotic stresses like drought, salinity, cold are not desirable for successful crop production. However, successful crop production faces abiotic stresses due to changing environment. Among the abiotic stresses, chilling is a common and destructive stress to crop production. Maize is one of the most demanding cereal crop and germination of maize seeds and their growth is severely interrupted by extreme low temperature or chilling stress (CS). Seed priming with different signaling molecules confer abiotic stress like chilling. The present research was carried out to ameliorate the chilling-inhibited germination and seedling growth of maize by seed priming with proline (Pro) and glycine betaine (GB). Results showed that CS significantly reduced maize seed germination and survival rate, shoot and root length, shoot and root weight, and photosynthetic pigments, chlorophyll (Chl) a, Chl b, and total Chl contents. Seed priming with Pro and GB has a positive impact on germination and survival rate, shoot and root length, shoot and root weight and photosynthetic pigments of leaf under CS. Among the treatments, Pro and GB at 20 mM and warm water performed better; while priming with tap water and Pro and GB at 10 mM showed insignificant performance over control but significant over stress in most of the parameters. Thus, priming may be an effective tool to increase seed germination, better maize seedling growth under cold chilling stress, and proline or betaine (at 20 mM) or warm water priming (at 45 ^{0}C for 5 min) can be considered as an effective priming agent.

Keywords: *Abiotic stress, germination, seedling growth, photosynthetic pigments, vigour, chilling stress.*

Introduction

Abiotic stresses, salinity, drought, extreme temperature (heat or cold) are the barrier for crop production (Rhaman. *et al*., 2022). Maize is one of the most important cereal crops and a C_4 tropical plant grown with a temperature range of 30–35 degrees Celsius and extremely susceptible to low temperatures, especially in the early phases of development (Presterl,T. *et al*., 2007). Despite adjusting some hybrids to temperatures that are lower than the ideal range, but long-term exposure to freezing temperatures can disrupt various developmental and physiological processes and the effect is most visible in the early stages of growth (Marocco, A. *et al*., 2005). Low temperatures, along with a lack of water, are one of the greatest barriers to plant productivity and geographic distribution over the world (Fenza, M. D. 2013). During the seed germination and seedling growth stages, plants are the most vulnerable to stresses (Almansouri, M. *et al*., 2001). During early growth phase, chilling stress inhibits seed germination and disrupts plant growth and developmental processes (Allen, D. J. *et al*., 2001). Chilling has the detrimental effects on the growth and development of rice (*Oryza sativa*) (Wang, W. *et al*., 2016), and cotton (*Gossypium hirsutum* L.) (Cheng, C. *et al*., 2018).

***Corresponding Author:**
Mohammad Saidur Rhaman;

DOI: http://dx.doi.org/10.21746/aps.2022.11.6.3

Seed priming has appeared as an effective, easy and adoptive approach to improve the seedling emergence, vigour, and stress tolerance capability of many horticultural and field crops (Hussain, H. A. *et al*., 2018; Rhaman. *et al*. 2021a; Tania, S. S. *et al*., 2021). It is a pre-sowing treatment which encompasses the control hydration of seed for commencement and regulation of pre-germinative metabolic processes (Hussain, S. *et al*., 2015, Tania. *et al*. 2020). Seed priming could be a crucial and facile technique in increasing seed germination, enhancing vigour, and improving stand establishment of crop plants under various abiotic stresses (Zheng, M. *et al*., 2016; Li, Z. *et al*., 2017, Rhaman. *et al*. 2020). A plenty of studies have advocated the beneficial effects of various seed priming methods such as hydro-priming, osmo-priming, hormonal-priming, chemical-priming, nutri-priming, and redox-priming under chilling stress in different field crops (Jisha, K. C. *et al*., 2013; Paparella, S. *et al*., 2015; Hussain, S. *et al*., 2016). Although, seed priming has been extensively studied as a tool for boosting up seed germination and seedling growth of different crop species, but this issue has not been properly addressed so far especially for sweet corn under cold stress conditions. The present study was therefore undertaken to evaluate the efficacy of different seed priming agents in increasing seed emergence, seedling growth, vigour and survivability under cold stress.

Materials and Methods

Plant Materials

The experiment was carried out at the laboratory of the Department of Seed Science and Technology of Bangladesh Agricultural University, Mymensingh. Seeds of well-known cultivated maize variety named "sweet corn" were used as plant materials in this experiment. To reduce contamination during priming, seeds surface was washed with sterile distilled water in three times.

Seed Priming and Experiment Conduction

To accomplish the role of seed priming in alleviating the unwanted effects of chilling stress, seed priming approaches like, hydro-priming (normal tap water and warm water), and chemical priming (proline and glycine betaine) were examined in this experiment. In the experiment, the following treatments were maintained: Control, C; Chilling stress, CS; Hydro-priming with warm water, WW; Hydro-priming with warm water under chilling stress, WwCS; Hydro-priming with room temperature tap water, TW; Hydro-priming with room temperature water under chilling stress, TwCS; Priming with 10 mM Proline, Pro_{10}; Priming with 10 mM Proline under chilling stress, $Pro_{10}CS$; Priming with 20 mM Proline, Pro_{20}; Priming with 20 mM Proline under chilling stress, $Pro_{20}CS$; Priming with 10 mM Glycine betaine, GB_{10}; Priming with 10 mM Glycine betaine under chilling stress, $GB_{10}CS$; Priming with 20 mM Glycine betaine, GB_{20}; and Priming with 20 mM Glycine betaine under chilling stress, $GB_{20}CS$. In case of hydro-priming tap water around 25^0C and warm water about 45^0C was used for priming or soaking of the seeds. In all treating agent's seeds were primed for 30 min except warm water. Priming with warm water time duration was only 5 minutes. After the completion of soaking, the primed seeds were washed with distilled water for 2 min, surface-dried using blotting paper. Subsequently washing and drying, the seeds (twenty seeds per dish) were positioned on Petri dishes (150×20 cm^2 diameter) having three layers of Whatman filter papers, covered with lid and kept in normal laboratory (the room temperature was 25±1^{o}C and relative humidity was 95%) conditions while, the primed seeds dishes were exposed to cold stress (under 5^0C) in the refrigerator. Distilled water was sprayed to seeds to avoid desiccation. After 7 days of seed placement, when the germination just started the stressed seeds were placed in the growth chamber for 23 days to record the data. The chilling stress was imposed in growth chamber by maintaining the day and night temperatures at 15 ^{0}C, while temperature for control treatment was set at 25 ^{0}C in a separate growth chamber. ; The experiment was laid out in a completely randomized design replicated in three times.

Data Collection

Data were collected on germination (%), survival rate (%), seed vigour index (SVI), shoot and root length, shoot and root weight (both fresh and dry) and chlorophyll content. The final germination percentage was calculated at 10 DAS. At 20 DAS representative seedlings (10) from each replicate were randomly selected to get the data of percent survival, shoot and root length and the fresh weight of shoot and root. The survival percentage is the ratio of the number of survived seedlings (at 20 DAS) to the number of seedlings that emerged (at 10 DAS) and expressed as a percent. Root length was measured from the base of the plant up to the end of the longest root and expressed in cm. Shoot length was measured from the base of the plant up to the tip of the longest leaf and expressed in cm. After taking the fresh weight, those were kept in an oven at 60°C for 72 hrs. Both the weight (fresh and dry) of shoot and root was expressed in mg. The dry weight of the seedling was calculated as the sum of root and shoot dry weight and expressed in mg. Germination, survival percentage and seed vigour index were calculated by the following formula:

$$\text{Germination percentage (GP\%)} = \frac{Number\ of\ seeds\ germinated\ at\ 10\ DAS}{Number\ of\ seeds\ placed\ in\ the\ Petridish} \times 100$$

$$\text{Survival rate (\%)} = \frac{Number\ of\ survived\ seedlings}{Number\ of\ emerged\ seedlings} \times 100$$

$$\text{Seed vigour index (SVI)} = \frac{Germination\ percentage \times Seedling\ length(cm)}{100}$$

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

$$\text{Chlorophyll a} = 0.999 \times A663 - 0.0989 \times A645$$

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

$$\text{Total chlorophyll} = \text{chlorophyll a} + \text{chlorophyll b}$$

Statistical Analysis

Collected data were subjected to a one-way analysis of variance (ANOVA) using Minitab 17.0 statistical software (Minitab Inc., State College, PA, USA) and Tukey's pair wise comparisons were used to compare statistical differences among the mean values of different treatments and cold stress at 5% significance level.

Results

Seed Priming Enhances Germination and Survival Rate of Maize under CS

Data revealed that the percentage of germination and survival of maize seedlings were significantly enhanced by seed priming for both the normal and stressed condition. Seed priming with 20 mM Pro, 10 mM Pro, 20 mM GB, and 10 mM GB exhibited the significant increment in germination percentage by 67.01, 53.03, 54.37 and 47.36%, respectively (Table 1). In case of survival the findings were 86.36, 82.3, 83.27 and 82.13%, respectively. Results obtained for hydro-primed seeds also found significant over the stressed condition and the results were 66.53% (warm water priming) and 45.03% (tap water priming) for germination. The survival percent was 87.13% and 80.0% in respect of warm water and tap water primed seeds. The highest germination percentage was recorded for 20 mM Pro (67.01%) compared with control (42.40%) and stressed (34.0%) respectively (Table 1). On the other hand, warm water primed seeds gave the

highest percent survival (87.13%) in compared to un-primed (81.0%) and stressed (66.0%).

Table 1: Effects of different priming agents on germination percentage and survival rate of maize under chilling stress. Values are means of 3 replicates and means with different letters in a column indicating significant differences ($p \leq 0.05$)

Treatments	Germination Percentage (%)	Survival rate (%)
C	42.4 de	81 cde
CS	34 f	66 h
TW	45.03 cd	80 de
TwCS	40.4 e	69 gh
WW	66.53 a	87.13 a
WwCS	42.3 de	74 f
Pro_{10}	53.03 b	82.3 cd
$Pro_{10}CS$	41.07 e	71 fg
Pro_{20}	67.01 a	86.36 ab
$Pro_{20}CS$	42.63 de	78 e
GB_{10}	47.36 e	82.13 cd
$GB_{10}CS$	41 e	71 fg
GB_{20}	54.37 b	83.27 bc
$GB_{20}CS$	41.07 e	74 f
SE	2.62	1.78

Seed Priming Enhances Seedling Growth and Vigour of Maize under CS

The results showed that CS significantly reduced shoot and root biomass and seed priming had a significant effect on shoot and root length of maize seedling. Primed seeds (with 20 mM Pro) increased the shoot of maize seedling by 30.95% and 12.50% in compared to cold stressed and control seedlings (Table 2). Root length also found in increasing rate by 38.88% and 17.0% due to priming of the seeds with 20 mM Pro in comparison with chilling and control condition. Moreover, seedlings grown from other treated seeds showed better growth than the control and stressed. Shoot and root length due to hydro-priming (priming with warm water) and 20 mM GB priming was observed as 16.2 cm, 10.6 cm, 15.9 cm and 10.73 cm respectively. In this study, seed vigour index (SVI) was measured from the plant height and percent germination. Proline treatment increased vigour index of maize by 66.30% and 44.51% in compared to chilling stress and un-soaked control treatment. Other treatments of priming and priming with cold stress combinations also performed better than only stressed treatment (Table 2).

Table 2: Effects of different priming agents on seedling attributes of maize under chilling stress. Values are means of 3 replicates and means with different letters in a column indicating significant differences ($p \leq 0.05$).

Treatments	Shoot length (SL) cm	Root length (RL) cm	Seedling length (cm)	Seed vigour index (SVI)	Shoot fresh wt (gm)	Shoot dry wt (gm)	Root fresh wt (gm)	Root dry wt (gm)
C	14.7 efg	9.23 c	23.93 ef	10.16 de	1.87 c	0.69 de	0.55 bc	0.07 bcde
CS	11.6 i	6.6 g	18.2 j	6.17 i	1.5 e	0.67 f	0.48 d	0.03 f
TW	14.97 def	9.5 bc	24.47 de	10.85 cd	1.87 c	0.81 f	0.56 bc	0.07 bcde
TwCS	12.2 h	8.57 d	20.77 i	8.18 h	1.66 de	0.7 ef	0.52 cd	0.04 ef
WW	16.2 b	10.6 a	26.8 b	17.7 a	2.45 a	1.25 a	0.66 a	0.09 abc
WwCS	14.2 g	7.4 f	21.6 h	8.9 fgh	1.78 cd	0.78 de	0.55 bc	0.07 bcde
Pro_{10}	15.4 cd	9.9 b	25.3 c	13.25 b	2.1 b	0.9 c	0.58 b	0.08 abcd
$Pro_{10}CS$	12.5 h	7.9 e	20.4 i	8.2 h	1.67 de	0.72 def	0.52 cd	0.05 def
Pro_{20}	16.8 a	10.8 a	27.6 a	18.31 a	2.4 a	1.1 b	0.67 a	0.1 ab
$Pro_{20}CS$	14.8 ef	8.7 d	23.5 fg	9.73 ef	1.84 c	0.79 de	0.56 bc	0.06 cdef
GB_{10}	15.1 de	9.8 b	24.9 cd	11.04 c	1.9 c	0.81 cd	0.56 bc	0.08 abcd
$GB_{10}CS$	12.2 h	7.8 ef	20.33 i	8.33 gh	1.67 d	0.76 def	0.55 bc	0.04 ef
GB_{20}	15.9 bc	10.73 a	26.63 b	14.21 b	2.3 a	1.14 b	0.66 c	0.11 a
$GB_{20}CS$	14. 5 fg	8.5 d	23 g	9.29 efg	1.86 c	0.78 de	0.55 bc	0.06 cdef
SE	0.43	0.34	0.97	0.96	0.07	0.04	0.01	0.006

Priming Effect on Shoot and Root Weight

Data revealed that the weight of shoot and root significantly varied with priming treatments under chilling stress. Shoot fresh weight was found 37.50% higher in plants grown from primed (20 mM Pro) seeds than the weight of plants derived from stressed seeds. Similarly, shoot dry weight also significantly affected by seed priming and chilling stress. Shoot dry weight was significantly reduced by 17.91% and 86.56% by CS compared to control and hydro-priming with warm water (Table 2). On the other hand, similar pattern of changes on root weight of maize seedling due to priming and CS were also observed. Table 2 shows that CS reduced the root fresh and dry weight by 14.58% and 133% compared to the seedlings from unstressed control condition.

Seed Priming Enhances Chlorophyll Content of Maize Leaf under CS

Different priming treatments and CS significantly affected the chlorophyll content of leaf of maize seedling. Seedlings derived from the primed seeds (20 mM Pro) accumulated 7.4% and 16.04% higher content of Chl a compared with control and stressed seedlings (Fig. 1a). This increment was also recorded for the content of Chl b. About 27.1% and 6.54% higher accumulation of Chl b was found than the leaf from stressed and non-primed seedlings (Fig 1b). In Figure 1c showed the highest content of total Chl was recorded in response to 20 mM Pro primed seeded leaves. GB in 20 mM and warm water priming also produced higher the seedlings

with higher amount of Chl in compared to un-primed and stressed seedlings.

Discussion

In this study we investigated the effect of seed priming on the germination and other morphological attributes of sweet corn variety of maize under both stressed (cold) and non-stressed conditions. The findings showed that cold stress had significantly affected germination and growth. Our study also demonstrated that germination recorded from primed seeds were significantly higher than non-primed treatment when exposed to stressed environment. Seed priming enhanced seed germination, growth and leaf pigment contents compared with the non-primed and stressed seed. However, this study shows the potentiality of seed priming in terminating cold stress during seed germination and seedling establishment of maize. Seed priming with higher dose (20 mM) Pro increased germination, survival and growth of maize than the using of lower (10 mM Pro) concentration (Table 1 & 2). Such variation of the findings indicates that seed priming with proline in stress mitigation is concentration dependent which is similar with the findings of previous studies by Ali. *et al*. 2007 and Ami. *et al*. 2020. These variations might also be explained in view of proline role in activation of seed metabolism for mobilization of food reserves toward growing plumule and radical during seed germination,

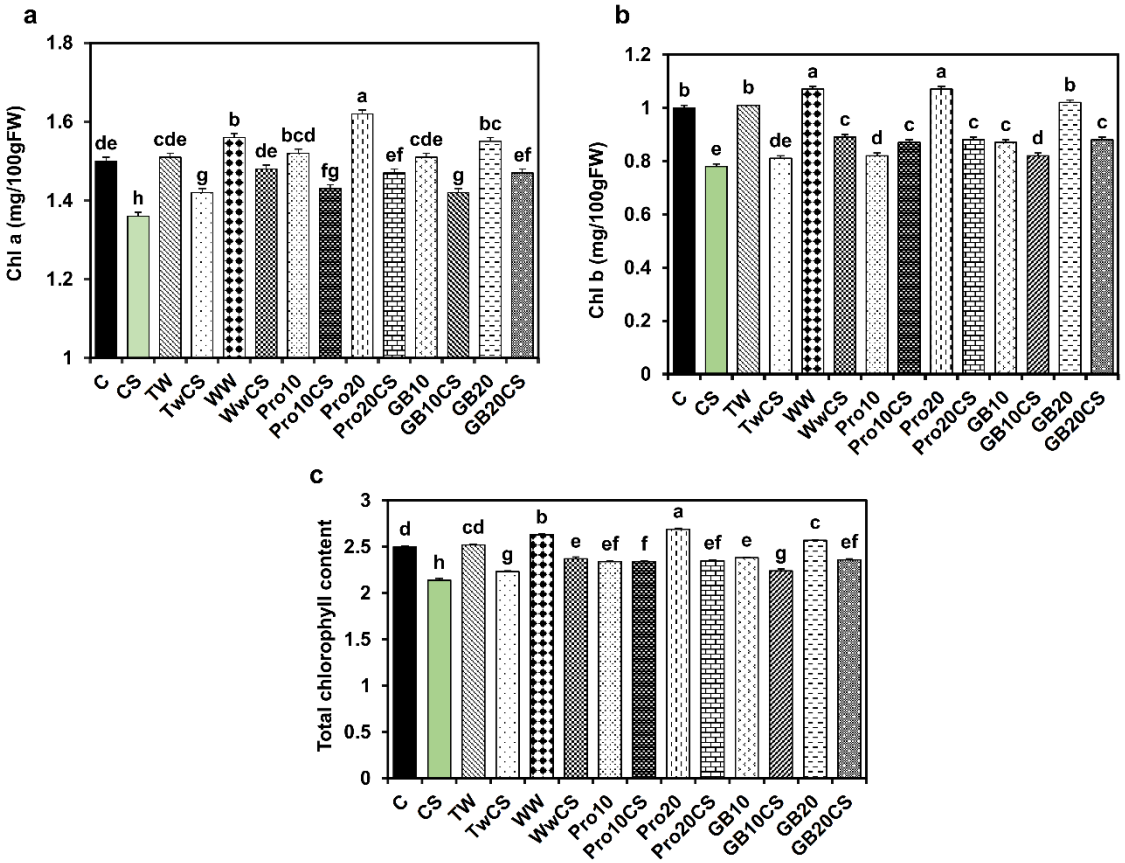

Figure 1: Effects of different priming agents on photosynthetic pigments of maize under chilling stress

which speed up seed germination and growth, particularly oxidative pentose phosphate pathway (OPPP) (El Mokhtari, A. *et al*., 2020). On the other hand, maize seeds primed with 10 and 20 mM GB speed up germination and growth with the better performance in higher dose in compared to cold stress which might have been due to differences in absorption of GB in seeds of different crops as well as genetic differences in seed metabolic machinery with the similar findings of Sadeghipour, O. 2020 and

Dikilitas, M. *et al*., 2020. Moreover, warm water priming of seeds significantly increased germination and also shoot and root length than priming of seeds with the water of room temperature. When the seeds are soaked in warm water rather than normal temperature water, it helps in the better activation of enzymes which are responsible for germination along with easier water imbibition and removing surface pathogens which might be explained with the findings of Tania, S. S. *et al*., 2019. In this study, similar responses to different priming both in normal and cold-stressed conditions also observed in case shoot-root weight and chlorophyll content of leaves (Fig.1). Low temperature may decrease the rate of both cell elongation and division; therefore, smaller leaf area under low temperature fails to support the requisite growth of plant (Ambreen, S. *et al*., 2021; Cao, Q. *et al*., 2019) as well as plant weight (khan, T. A. *et al*., 2017). Previous studies suggested that exogenous application of Pro and GB as seed priming or as foliar spray improved the growth by improving photosynthetic activity (Messedi, D. *et al*., 2016; Hmidi, D. *et al*., 2018; Pervaiz; A. *et al*., 2019; Kahlaoui, B. *et al*., 2018). After all, thorough observation from this study suggested that, each priming agents had a positive impact on seed germination, seedling emergence, seedling growth and leaf chlorophyll content under cold stress with the similar findings from whereas, seed priming with 20 mM proline or betaine, or warm water priming performed better. However, to obtain a better understanding of priming-induced mechanisms and for validation of this results larger field trials are needed.

Conclusion

Chilling stress led to a significant decrease in germination, survival, chlorophyll content and other growth parameters of maize seedlings. Higher dose of Pro and GB showed more effectiveness than the lower dose of these agents in reducing the detrimental effects of cold stress in maize plants. It is concluded that Pro was more effective than hydro-priming and GB in mitigating the detrimental effects of cold stress of maize.

References

1. Ali, Q., Muhammad, A. and Habib-Ur-Rehman, A. "Exogenously applied proline at diferent growth stages enhances growth of two maize cultivars grown under water defcit conditions." *Pakistan Journal of Botany* 39.4 (2007):1133–1144.
2. Allen, D. J. and Donald, R. O. "Impacts of chilling temperatures on photosynthesis in warm-climate plants." *Trends in Plant Science* 6.1 (2001):36–42.
3. Almansouri, M., Kinet, J. M. and Stanley, L. "Effect of salt and osmotic stresses on germination in durum wheat (Triticum durum Desf.)." *Plant Soil* 231.2 (2001): 243–254.
4. Ambreen, S., Habib-ur-Rehman, A., Ameer, K., Zafar, U. Z., Ahsan, A. and Hazem, M. K. "Seed priming with proline improved photosystem II efficiency and growth of wheat (Triticum aestivum L.)." *BMC Plant Biology* 21.1 (2021): 1-12.
5. Ami, K., Séverine, P., Cécile, C., Anne, G., Anne-Aliénor, V. and Majda, K. "Different proline responses of two Algerian durum wheat cultivars to in vitro salt stress." *Acta Physiologiae Plantarum* 42.2 (2020): 21.
6. Cao, Q., Gang, L., Zhengguo, C., Fentuan, Y., Xiaoli, J., Lamine, D. and Fanli, K. "Seed priming with melatonin improves the seed germination of waxy maize under chilling stress via promoting the antioxidant system and starch metabolism." *Scientific reports* 9.1(2019):1-12.
7. Cheng, C., Pei, L. M., Yin, T. T. and Zhang, K. W. "Seed treatment with glycine betaine enhances tolerance of cotton to chilling stress." *The Journal of Agricultural Science* 156.3 (2018): 323-332.
8. Dikilitas**,** M., Eray, S. and Aryadeep, R. "Role of proline and glycine betaine in overcoming abiotic stresses." *Protective chemical agents in the amelioration of plant abiotic stress: biochemical and molecular perspectives* (2020): 1-23.
9. El Moukhtari, A., Cecile, C. H., Mohamed, F. and Arnould, S. "How does proline treatment promote salt stress tolerance during crop plant devel- opment?" *Frontiers in Plant Science* 11(2020): 1127.

10. Fenza, M. D. "Examining the physiological and genetic response of maize to lowtemperature conditions." *PhD thesis, University College Dublin* (2013).
11. Hmidi, D., Chedly, A., Habib-ur-Rehman, A., Muhammad, A. and Dorsaf, M. "Effect of salinity on osmotic adjustment, proline accumulation and possible role of ornithineδ-aminotransferase in proline biosynthesis in Cakile maritima." *Physiology and Molecular Biology of Plants* 24.6(2018):1017–33.
12. Hussain, H. A., Saddam, H., Abdul, K., Umair, A., Shakeel, A. A., Shengnan, M. and Longchang, W. "Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities." *Frontiers in Plant Science* 9 (2018): 393.
13. Hussain, S., Fahad, K., Hafiz, A. H. and Lixiao, N. "Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars." *Frontiers in Plant Science* 7 (2016): 116.
14. Hussain, S., Manman, Z., Fahad, K., Abdul, K., Shah, F. and Shaobing, P. "Benefits of rice seed priming are offset permanently by prolonged storage and the storage conditions." *Scientific Reports* 5.1 (2015):8101.
15. Jisha, K. C., K. Vijayakumari. and Jos, T. P. "Seed priming for abiotic stress tolerance: an overview." *Acta Physiologiae Plantarum* 35.5 (2013): 1381-1396.
16. Kahlaoui, B., M. Hachicha. and E. Misle. "Physiological and biochemical responses to the exogenous application of proline of tomato plants irrigated with saline water." *Journal of the Saudi Society of Agricultural Sciences* 17.1 (2018): 17–23.
17. Khan, T. A., Qazi, F. and Mohammad, Y. "Low-temperature stress: is phytohormones application a remedy?." *Environmental Science and Pollution Research* 24.27 (2017): 21574–21590.
18. Li, Z., Jungui, X., Yue, G., Chun, W., Genyuan, G., Ying, L., Yutao, H., W. Hu., Sheteiwy, M. S., Y. Guan. and J. Hu. "The synergistic priming effect of exogenous salicylic acid and H2O2 on chilling tolerance enhancement during maize (Zea mays L.) seed germination." *Frontiers in Plant Science* 8 (2017): 1153.
19. Lichtenthaler, H.K. "Chlorophylls and carotenoids: pigments of photosynthetic biomembranes." *Methods in Enzymology* 148 (1987): 350-382.
20. Marocco, A., Carlo, L. and Yves, F. "Chilling stress on maize." (2005): 571-578.
21. Messedi, D., Feten, F., Karim, B. H., Najla, T., Riadh, K., Habib-Ur-Rehman, A. and Chedly, A. "Highlighting the mechanisms by which proline can confer tolerance to salt stress in Cakile maritima." *Pakistan Journal of Botany* 48.2 (2016): 417–27.
22. Paparella, S., Araújo, S. S., G. Rossi., M. Wijayasinghe., D. Carbonera. and Alma, B. "Seed priming: state of the art and new perspectives." *Plant cell reports* 34.8 (2015): 1281-1293.
23. Pervaiz, A., Aisha, I., Ayesha, K., Azra, M., Sibgha, N. and Ahsan, A. "Proline induced modulation in physiological responses in wheat plants." *Journal of Agriculture and Environmental Sciences* 8.1 (2019): 112–9.
24. Presterl, T., Milena, O., Walter, S., Evelyn, M. M., Frank, K. R., Carsten, K., Karin, E., Peter, W. and Hartwig, H. G. "Quantitative trait loci for early plant vigour of maize grown in chilly environments." *Theoretical and Applied Genetics* 114.6 (2007): 1059-1070.
25. Rhaman, M.S., Shahin, I., Farjana, R., Mousumi, K., Carol, C. B., Yoshiyuki, M. and Mirza, H. "Seed priming with phytohormones: An effective approach for the mitigation of abiotic stress." *Plants* 10.1 (2020): 37.
26. Rhaman, M.S., Mohammad, G. K. and Anamul, H. "Climate Change and Its Adverse Impacts on Plant Growth in South Asia: Current Status and Upcoming Challenges." *Phyton*, 91.4 (2022): 695.
27. Rhaman, M.S., Farjana, R., Shaila, S. T., Masudul, M.K., Ashaduzzaman, S., Arif, H. K. R., Arafat, A. H. A. L., and Yoshiyuki, M. "Seed priming and exogenous application of salicylic acid enhance growth and productivity of okra (*Abelmoschus esculentus* l.) by regulating photosynthetic

attributes." *Journal of Experimental Biology and Agricultural Sciences* 9 (2021): 759-769
28. Sadeghipour, O. "Cadmium toxicity alleviates by seed priming with proline or glycine betaine in cowpea (Vigna unguiculata (L.) Walp.)." *Egyptian Journal of Agronomy* 42.2 (2020):163-170.
29. Tania, S. S., Hossain, M. M. and Hossain, M. A. "Effects of hydropriming on seed germination, seedling growth and yield of bitter gourd." *Journal of Bangladesh Agricultural University* 17.3(2019): 281-287.
30. Tania, S.S., Moklasur, R., Farjana, R., Mehera, A. S., Muhammad, H. K., Anamul, H. and Mohammad, S. R. "Seed priming with Salicylic Acid (SA) and Hydrogen Peroxide (H2O2) Improve Germination and Seedling Growth of Wheat (Triticum aestivum) under Salt Stress." *Asian Journal of Research in Crop Science* 6 (2021): 60-69.
31. Tania, S.S., Rhaman, M.S. and Hossain, M.M. "Hydro-priming and halo-priming improve seed germination, yield and yield contributing characters of okra (Abelmoschus esculentus L.)." *Tropical Plant Research* 7.1 (2020): 86-93.
32. Wang, W., Qian, C., Saddam, H., Junhao, M., Huanglin, D., Shaobing, P., Jianliang, H., Kehui, C. and Lixiao, N. "Pre-sowing seed treatments in direct-seeded early rice: consequences for emergence, seedling growth and associated metabolic events under chilling stress." *Scientific reports* 6.1 (2016): 1-10.
33. Zheng, M., Ye, T., Saddam, H., Qingwei, J., Shaobing, P., Jianliang, H., Kehui, C. and Lixiao, N. "Seed priming in dry direct-seeded rice: consequences for emergence, seedling growth and associated metabolic events under drought stress." *Plant growth regulation* 78.2 (2016): 167-178.

Source of support: Nil;
Conflict of interest: The authors declare no conflict of interests.

Cite this article as:
Shaila, S.T., Rhaman, M.S., Rahaman, M. and Hoque, A. "Seed Priming with Proline and Glycine Betaine Enhances Germination and Seedling Growth of Maize (Zea mays) Under Chilling Stress." *Annals of Plant Sciences*.11.06 (2022): pp. 5167-5175.

DOI: http://dx.doi.org/10.21746/aps.2022.11.6.3