Effect of Seed Priming Treatment with Nitrate Salt on Phytotoxicity and Chlorophyll Content Under Short Term Moisture Stress in Maize (Zea mays L.)

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ABSTRACT
An experiment was carried out to appraise the effect of seed priming treatment with Mg(NO₃)₂ against various levels of externally imposed moisture stress by polyethylene glycol-6000 on phytotoxicity in shoot and root and chlorophyll content in maize plant under laboratory conditions. The phytotoxicity of shoot and root was increased as the elevated levels of PEG-6000 towards T₁ to T₄ (i.e. 1.5 to 4.5 %, Set-I) as compared to control set (T₀, i.e. without treated set), while the least values of phytotoxicity were recorded in T₅ and onwards increased slowly up to T₈ (i.e. 1.5 to 4.5 % of PEG-6000 + primed seed, Set-II). The same trend of phytotoxicity was recorded for both the plant parts at both the times of observations, i.e. shoot and root 120 and 240 hours. The chlorophyll content of shoot was recorded in decreasing trend onwards from T₁ to T₄ in treatment set-I as compared to T₀, i.e. control. While the highest amount of chlorophyll content was recorded in T₅ followed by T₆ as compared to the rest of the treatments.

INTRODUCTION
Maize (Zea mays L.) is one of the valuable crops for human beings in respect to food, feed and as a raw material for many food industries. As the production of maize is concerned, it has the third rank after wheat and rice in the world (Tian et al. 2014). Rapid and uniform seed germination, emergence and healthy seedlings establishment are essential for keeping the good foundation for future vegetative as well as reproductive growth of maize crop to have optimum crop production. Drought is one of the very devastating conditions that reflect their presence very fast on every stage of growth. Due to the change of internal levels of water status, various physiological, biochemical and molecular changes appear within the plant especially seed germination, seedling growth, chlorophyll, sugar and proline content etc. Deviation in chlorophyll content leads to disturbing photosynthetic process results adversely in production of photosynthate (Chutia & Borah 2012, Wang et al. 2018). In general, plant loses their turgor pressure because of drought stress, but up to a certain extent, plant try to make a balance between soil and plant by accumulating solutes by producing osmoregulatory compounds like proline, glycine and betaine (Anaytullah et al. 2007, Siddique et al. 2018, Reddy et al. 2004). Seed priming technique is now one of the options that show positive results in the various crops to improve not only seed germination and early seedling emergence but also for long term effect during plant life up to grain yield under various kinds of environmental stresses like heat stress, drought stress, salinity stress, etc. (Zhou et al. 2009, Tian et al. 2014, Yari et al. 2010). Priming treatment with nitrate salts like Mg(NO₃)₂, KNO₃ and Ca(NO₃)₂ are one of the most beneficial compounds that help to improve all the stages of growth especially beginning stage of the plant under normal as well as overcome the effect of temperature, heat, salinity and drought stress (Anaytullah & Bose 2007, Anaytullah et al. 2012, Siddique & Bose 2015, Srivastava et al. 2017, Mahmoudi et al. 2012).

MATERIALS AND METHODS
Genetically pure and fresh seeds of maize variety SUN-NY-NMH-777 were collected and the effect of seed priming treatment with nitrate salt against externally imposed moisture stress under laboratory condition was appraised. The trial was split into two sets for better understanding, i.e. elevated concentrations of polyethylene glycol-6000 + without treated seed (Set-I) and elevated concentrations of polyethylene glycol-6000 + primed seed (Set-II). The experiment was conducted in CRD along with nine treatments and three replications. The various concentrations of externally imposed moisture stress were created by the use of polyethylene glycol-6000 (i.e. 1.5, 2.5, 3.5 and 4.5 %). The priming treatment was applied through Mg(NO₃)₂ @ 7.5mM up to...
After completion of priming duration, seeds were washed properly with distilled water and dried up to original weight under room temperature. Both the sets of treatment arranged systematically by transferring the fifty seeds in each Petri dish according to their sequence. The lab trial was conducted under growth chamber (Model No-NU-151) at 20±1°C temperature and 80 % RH. The phytotoxicity of shoot and root was derived from the formula given by Chou & Lin (1976) at both the times of observations, i.e. 120 and 240 hours intervals.

\[
\text{Phytotoxicity (\%)} = \frac{\text{Length of shoot or root (Control plant)} - \text{Length of shoot or root (Treated plant)}}{\text{Length of shoot or root (Control plant)}} \times 100
\]

The extraction and determination of chlorophyll content in shoots of maize were made according to the formula given by (Witham et al. 1971). As per the protocol, one gram shoot sample was homogenized by pestle and mortar with 80% acetone. The resulting green liquid was transferred to a 100 mL volumetric flask and the final volume was made to 100 mL by adding 80% acetone. The O.D. of chlorophyll extract was recorded at 645 and 630 nm by a spectrophotometer. The amount of total chlorophyll was calculated by the formula given below.

\[
\text{Total chlorophyll (mg/g)} = \frac{20.2(645) + 8.02(663)}{V} \times 1000 \times W
\]

### RESULTS

#### Phytotoxicity to Shoot and Root

Data in Fig-1a and 1b show the effect of different concentrations of PEG-6000 and the overcome effect of seed priming treatment on phytotoxicity in shoot and root of 5 and 10 days old maize seedlings. Phytotoxicity in shoot and root was increased as the concentrations of PEG-6000 was increased from 1.5 % to 4.5% either alone or in combination with Mg(NO$_3$)$_2$ treated sets. But the level of phytotoxicity was increased maximum in PEG-6000 treated set in comparison to PEG-6000 + primed seed sets in both shoot and root. The observations were recorded at two different intervals, i.e. 120 hrs and 240 hrs for phytotoxicity of root and shoot. The minimum phytotoxicity in shoot and root was recorded in T$_5$ (1.5 % of PEG-6000 + primed seed) which was followed by T$_6$ and T$_7$ at both the time of observations (120 hrs and 240 hrs) in comparison to control set. Data in Figs. 1a and 1b show that T-5 and T$_6$ have a non-significant difference for each other but have a significant difference with rest of the treatments at both the times of intervals for phytotoxicity in shoot and root. Data also reveal that T$_6$ and T$_7$ overcome the effect of PEG-6000 induced moisture stress and recorded lower phytotoxicity of shoot and root in comparison to T$_1$ and T$_2$. It was realized from Figs. 1a and 1b that the phytotoxicity of shoot was greater than the phytotoxicity of root at both the times of observations including all the treatments.

#### Chlorophyll Content

Data presented in Fig. 2 reflect the effect of different concentrations of PEG-6000 and overcome the effect of primed seed treatment on chlorophyll content. Chlorophyll content

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**Fig. 1a:** Effect of seed priming treatment with Mg(NO$_3$)$_2$ on phytotoxicity (%) in shoot under PEG-6000 induced moisture stress in maize.
was decreased as the concentration of PEG-6000 was increased from 1.5% to 4.5% either alone or in combination with Mg(NO₃)₂ treatments. But the maximum reduction was recorded in PEG-6000 treated set in comparison to PEG-6000 + primed seed. Observations were recorded at two different intervals of 120 hrs and 240 hrs for chlorophyll content. Among the treatments (i.e. different concentrations of PEG-6000 alone and along with magnesium nitrate treated sets), the chlorophyll content was recorded better in PEG-6000 + primed seed in comparison to control. The maximum chlorophyll content was recorded in T₅ (1.5% of PEG-6000 + primed seed) followed by T₆ in comparison to control, i.e. 0.201 and 0.591 mg·g⁻¹ at both the times of observations. Data presented in Fig. 2 show that T-5 and T-6 have non-significant differences between them but have a significant difference with most of the treatments at both the times of intervals. The least value of chlorophyll content was recorded by T₄ and T₃. When data were compared between 120 hrs and 240 hrs of intervals, it was found that chlorophyll content records greater values at 240 hrs intervals than 120 hrs of intervals including all the treatments.

The statistical analysis of chlorophyll content was carried
out through SPSS and found that the data is overall significant at p 0.05 % and have a significant difference between the treatments.

DISCUSSION

Elevated levels of PEG-6000 from lower to higher concentration, create the phytotoxicity in the seedlings by suppressing the water uptake as per the intensity of external moisture stress by the seed (Siddique & Dubey 2017, Anaytullah et al. 2007). Synthesis of photosynthetic pigment within seedling is very essential to become independent or maintaining continuous growth of seedlings or plants. In our study, it is observed that the reduction of photosynthetic pigment depends on the intensity of moisture stress or drought while the similar results were also reported by Mohammadkhani & Heidari (2007) and Rahbarian et al. (2011). It is well known that under such drought stress, the plant generates reactive oxygen species like $O_2^{-}$, $H_2O_2$ and $OH^*$ and every ROS act adversely on different molecules like singlet oxygen dominantly damage lipid and protein molecules while $H_2O_2$ react with cellular components (Das & Roychoudhury 2014, Yadav & Sharma 2016). These ROS groups of compounds are produced mainly in chloroplasts and mitochondria within the plant cells under different types of stresses, hence the probability is always very high to get damaged their skeleton due to increased levels of phytotoxicity, therefore, inhibiting or suppressing the biosynthesis of chlorophyll molecules in the chloroplast as per the severity of stress (Noctor et al. 2017, Aswani et al. 2019). The similar result in respect to overcoming the effect of moisture stress by various types of seed priming treatments was also reported by Pant & Bose (2016) and Murungu (2011).

REFERENCES

Anaytullah and Bose, B. 2007. Nitrate hardened seeds increase germination, amylase activity and proline content in wheat seedlings at low temperature. Physiology Molecular Biology of Plants, 13 (3&4): 199-207.

Anaytullah, Bose, B. and Yadav, R.S. 2007. PEG-induced moisture stress: Screening for drought tolerance in rice. Indian J. Plant Physiology, 12(1): 88-90.

Anaytullah, Srivastava, A.K. and Bose, B. 2012. Impact of seed hardening treatment with nitrate salts on nitrogen and anti-oxidant defense metabolisms in wheat (Triticum aestivum L.) under timely, late and very late sown conditions. Vegetos, 25(1): 292-299.

Aswani, V., Rajasheel, P., Bapatla, R.B., Sunil, B. and Raghavendra, A.S. 2019. Oxidative stress induced in chloroplasts or mitochondria promotes proline accumulation in leaves of pea (Pisum sativum): Another example of chloroplast-mitochondria interactions. Protoplasma, 256(2): 449-457.

Chou, C.H. and Lin, H.Z. 1976. Autoinoculation mechanisms of Oryza sativa. I. Phytotoxic effects of decomposing rice residues in soil. J. Chemical Ecology, 2(3): 353-367.

Chutia, J. and Borah, S.P. 2012. Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice (Oryza sativa Linn.) genotypes of Assam, India. Protein and proline status in seedlings under PEG induced water stress. American Journal of Plant Sciences, 3(7): 971-980.

Das, K. and Roychoudhury, A. 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science, 2: 1-13, https://doi.org/10.3389/fenvs.2014.00053.

Mahmoudi, H., Massoud, R.B., Baatour, O., Tarchouve, I., Salah, I.B., Nasri, N., Abidi, W., Kaddour, R., Hannoufa, A.A., Lachaal, M. and Ouergi, Z. 2012. Influence of different priming methods for improving salt stress tolerance in lettuce plants. Journal of Plant Nutrition, 35(12): 1910-1922.

Mohammadkhani, N. and Heidari, R. 2007. Effects of water stress on respiration, photosynthetic pigments and water content in two maize cultivars. Pakistan Journal of Biological Science, 10(22): 4022-4028.

Murungu, F. S. 2011. Effect of seed priming and water potential on seed germination and emergence of wheat (Triticum aestivum L.) varieties in laboratory assay and in the field. African Journal of Biotechnology, 10(21): 4365-4371.

Noctor, G. Reichheld, J.P. and Foyer, C.H. (ed.) 2017. ROS-related redox regulation and signaling in plants. Seminar in Cell and Developmental Biology. Elsevier Ltd., 80, pp. 3-12.

Pant, B. and Bose, B. 2016. Mitigation of the influence of PEG-6000 imposed water stress on germination of halo primed rice seeds. International Journal of Agriculture, Environment and Biotechnology, 9(2): 275-281.

Rahbarian, R., Khavari-Nezad, R., Ganjali, A., Bagheri, A. and Najafi, F. 2011. Drought stress effects on photosynthesis, chlorophyll fluorescence and water relations in tolerant and susceptible chickpea (Cicer arietinum L.) genotypes. Acta Biologica Cracoviensia Series Botanica, 53(1): 47-56.

Reddy, A.R., Chaitanya, K.V. and Vivekanandan, M. 2004. Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology, 161(11): 1189-1202.

Siddique, A. and Bose, B. 2015. Effect of seed invigoration with nitrate salts on morpho-physiological and growth parameters of wheat crop sown in different dates in its cropping season. Vegetos, 28(1): 76-85.

Siddique, A., Kandpal, G. and Kumar, P. 2018. Proline accumulation and its defensive role under diverse stress condition in plants: an overview. Journal of Pure and Applied Microbiology, 12(3): 1655-1659.

Siddique, A. and Kumar, P. 2018. Physiological and biochemical basis of pre-sowing soaking seed treatment—an overview. Plant Archives, 18 (2): 1933-1937.

Srivastava, A.K., Siddique, A., Sharma, M.K. and Bose, B. 2017. Seed priming with salts of nitrate enhances nitrogen use efficiency in rice. Vegetos, 30(4): 99-104.

Tian, Y., Guan, B., Zhou, D., Yu, J., Li, G. and Lou, Y. 2014. Responses of seed germination, seedling growth, and seed yield traits to seed pretreatment in maize (Zea mays L.). Scientific World Journal, 1-8.

Wang, Z., Li, G., Sun, H., Ma, L., Guo, Y., Zhao, Z., Gao, H. and Mei, L. 2018. Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. Biol. Open., 7(11): 1-9.

Witham, F.M., Devid, F.B. and Roberts, M.D. (ed.) 1971. Experimental Plant Physiology, Van Nostrand Reinhold, New York, pp. 55-56.

Yadav, N. and Sharma, S. 2016. Reactive oxygen species, oxidative stress and ROS scavenging system in plants. Journal of Chemical and Pharmaceutical Research, 8(5): 595-604.
Yari, L., Aghaalikani, M. and Khazaei, F. 2010. Effect of seed priming duration and temperature on seed germination behavior of bread wheat (Triticum aestivum L.). ARPN Journal of Agricultural and Biological Science, 5(1): 1-6.

Zhou, Z.S., Guo, K., Abdelrahman, A.E. and Yang, Z.M. 2009. Salicylic acid alleviates mercury toxicity by preventing oxidative stress in roots of Medicago sativa. Environmental and Experimental Botany, 65(1): 27-34.