



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 $Mg(NO_3)_2$ 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 T_1 to T_4 (i.e. 1.5 to 4.5 %, Set-I) as compared to control set (T_0 , i.e. without treated set), while the least values of phytotoxicity were recorded in T_5 and onwards increased slowly up to T_8 (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 T_1 to T_4 in treatment set-I as compared to T_0 , i.e. control. While the highest amount of chlorophyll content was recorded in T_5 followed by T_6 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 losses 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 $Mg(NO_3)_2$, KNO_3 and $Ca(NO_3)_2$ 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 $Mg(NO_3)_2$ @ 7.5mM up to

15 hours. 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 $20 \pm 1^\circ\text{C}$ 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.

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

Statistical Analysis

The data obtained from the present work were analyzed using SPSS software version 23 and compared the results by DMRT at ≤ 0.05 .

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 $\text{Mg}(\text{NO}_3)_2$ 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

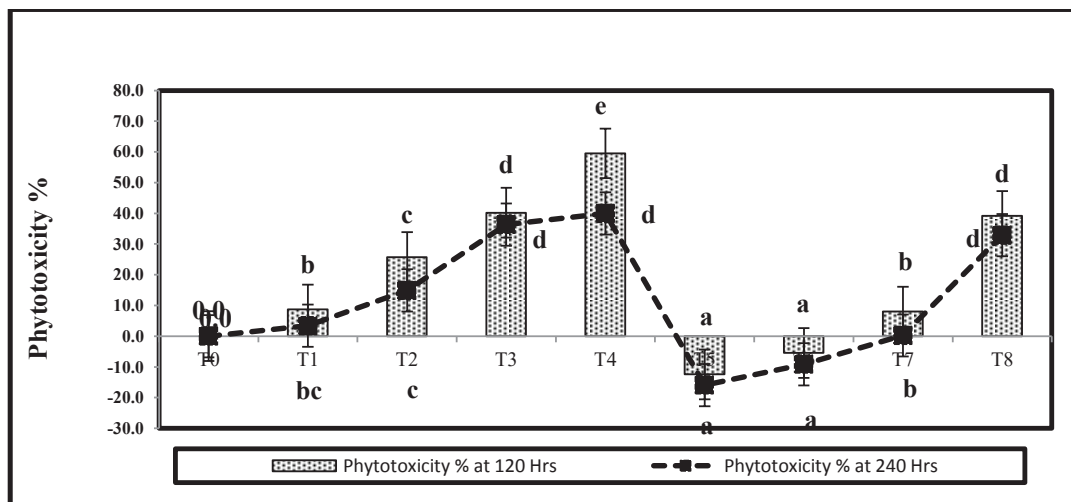


Fig. 1a: Effect of seed priming treatment with $\text{Mg}(\text{NO}_3)_2$ on phytotoxicity (%) in shoot under PEG-6000 induced moisture stress in maize.

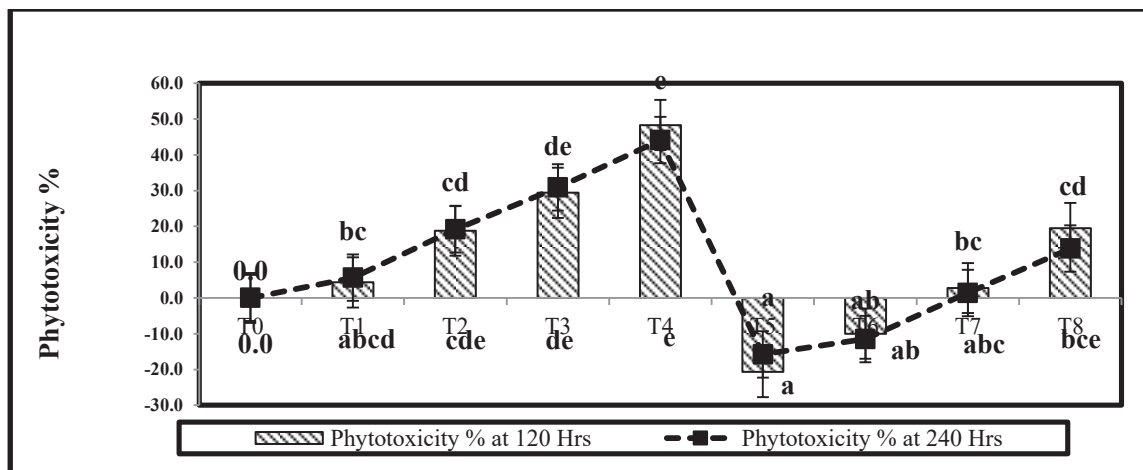


Fig. 1b: Effect of seed priming treatment with $\text{Mg}(\text{NO}_3)_2$ on phytotoxicity (%) in root under PEG-6000 induced moisture stress in maize.

was decreased as the concentration of PEG-6000 was increased from 1.5 % to 4.5% either alone or in combination with $\text{Mg}(\text{NO}_3)_2$ 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

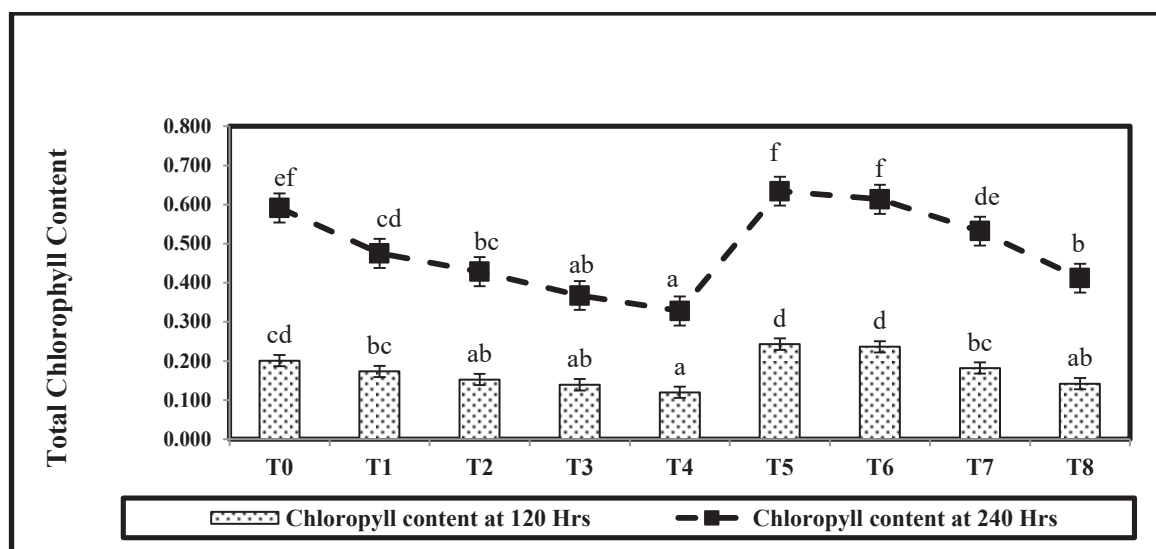


Fig. 2: Effect of seed priming treatment with $\text{Mg}(\text{NO}_3)_2$ on chlorophyll content (mg.g^{-1}) in root under PEG-6000 induced moisture stress in maize.

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^- , OH^* and H_2O_2 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).

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