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Running Head: Seed Invigoration In Relation To Drought

Effect of Seed Invigoration with Nitrate Salt on Chlorophyll Stability Index, RWC and Proline in Relation to Drought in Maize

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ABSTRACT

The present piece of work focus on the effect of seed Invigoration treatment with salt of nitrate on chlorophyll stability index (CSI), RWC % and proline content (µg g^{-1}) under short term moisture stress induced by PEG-6000 under laboratory condition.A wide range of PEG-6000 was used to produce elevated levels of moisture stress i.e. 1.5, 2.5, 3.5 and 4.5%. Increased level of moisture stress from 1.5 to 4.5%, causedreduction in CSI and relative water content RWC % significantly as compare to control set while the content of proline was recorded in reverse proportion as compare to CSI and RWC % in first set of treatment i.e. PEG-6000 + none prime seed at both the time of observations i.e. 120 and 240 hours. However, in the second set of treatment, i.e. PEG-6000 + prime seed, the trend of the results was same as mentioned above for set first. Among all the treatments, T5 was best in respect to CSI and RWC% followed by T6. While the proline content were recorded highest in T8 followed by T4. A negative relationship was recorded between proline content and RWC % for both the sets of treatment. Among the sets of treatment, set second was observed better in respect of all the parameters as compare to set first.

Key words: PEG-6000, Priming, Relative water content, Chlorophyll stability index

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SEED INVIGORATION IN RELATION TO DROUGHT

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INTRODUCTION

Maize crop (*Zea mays* L.) is one of the most important leading cereal crops in word. It is recognized as classical crop for food, feed and forage around the world.Being an important cereal crop, it has very high value for mankind. Abiotic stress covers wide range of negative effect in plant life in which moisture stress or drought is one of them that reflect most detrimental effect at every stages of plant life from seed germination to harvesting of crop (Bulgari*et al.*, 2019; Khayatnezhad *et al.*, 2010 and Anaytullah *et al.*, 2007). Moisture stress not only affects the morphological changes like seed germination seedling emergence and healthy seedling establishment but also affectat biochemical and molecular level. During the moisture stress,availability of water content decrease that adversely affect the water uptake and other metabolic process like deactivation of necessary enzymes and conversion of endospermic food material into usable form that results inferior quality of seed germination hencereduction in photosynthetic efficiency, relative water content, membrane stability index etc. are a sequential event. (Anaytullah *et al.*, 2007; Tian*et al.*, 2014; Muscolo*et al.*, 2014 andAli and Elozeiri, 2017).Seed Invigorationis a process by which seed accelerate their internal metabolic process

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like imbibition, activation of enzymes, break down of endospermic substrate but not allow to the emergence of radicle and plumule through seed coat (Ali and Elozeiri, 2017 andAnaytullah and Bose, 2007). Therefore, seeds may be able to avoid initial discrepant condition caused by drought or moisture which further helpful for vigorous vegetative and reproductive phases growth (Chen and Arora, 2013; Pant and Bose, 2016)

MATERIALS AND METHOD

A variety of maize (*Zea mays* L.) SUNNY-NMH-777 was selected to conduct an experiment to find out the negative effect of PEG-6000 and their overcome effect by seed Invigorationtechnique under lab condition.The experiment was arranged in completely randomized design with three replications and nine treatments including control. The whole experiment was groped into two sets for better understanding, in which first set was different concentrations of PEG-6000 + none prime seed and another set of trial was different concentrations of PEG-6000 + prime seed with $Mg(NO_3)_2$ while one petridish was kept without any treatment neither PEG-6000 nor primed seed treatment. Elevated levels of moisture i.e. 1.5, 2.5, 3.5 and 4.5 % were created by the use of polyethylene glycol-6000 by dissolving 1.5, 2.5, 3.5 and 4.5 g of PEG-6000 within 100 ml of distilled water. While the disinfected seeds with 0.1% $HgCl_2$were used for seed Invigorationtreatment. The duration and concentrations of Invigorationtreatment were decided by a screening test i.e. 7.5 mM for 15 hours.Total fifty primed /unprimed seeds were used in every petridish as per the requirement of treatment and control sets. Growth chamber (Model number NU-151) were used to conduct the trial under controlled conditions (Temperature andRelative humidity 20±1 ^{0}C and 80 %.). The observation regarding the chlorophyll stability index (CSI %) and relative water content were recorded and calculated according to the methods given by Arnon *et al.*,1949 and Weatherley and Barrs, 1962.

$$\text{Chlorophyll Stability Index \%} = \frac{\text{Total chlorophyll content of plant sample in treated set}}{\text{Total chlorophyll content of plant sample in control set}} \times 100$$

$$\text{Relative Water Content \%} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

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RESULTS

Chlorophyll stability index (%):

Data of (Fig-1) shows the effect of different concentrations of PEG-6000 and their overcome effect of primed seed treatment on chlorophyll stability index (%). Chlorophyll stability index (%) was decreased as the concentrations of PEG-6000 were increased from 1.5 % to 4.5% either alone or in combination with primed seed. But the reduction was maximum recorded in PEG-6000 treated set in comparison PEG-6000 + primed seed. Observations were recorded at two different intervals i.e. 120 Hrs and 240 Hrs for chlorophyll stability index (%). Among the treatments (i.e. different concentrations of PEG-6000 alone and along with Magnesium nitrate treated set), the chlorophyll stability index (%) was recorded better in PEG-6000 + primed seed in comparison to control. The maximum chlorophyll stability index (%) was recorded in T_5 (i.e. 1.5 % of PEG-6000 + primed seed) 121.30 and 107.3 % which was followed by T6 (i.e. 2.5 % of PEG-6000 + primed seed) 117.83 and 103.7 % in comparison to control one (without treated set) at both the time of observations (i.e. 120 Hrs and 240 Hrs). Data presented in (Fig-1) shows that T-5 and 6 both are showing non-significant in between but having significant difference with most of the rest treatments at both the times of intervals. The least value of chlorophyll stability index (%) was recorded by T_4 and T_3 (i.e. 3.5 and 4.5 % PEG-6000 treatment) 59.58 and 55.60 respectively. When data compared between 120 Hrs and 240 Hrs of intervals, it was realized from (Fig-1) that chlorophyll stability index (%) was decreased at 240 Hrs intervals as compare to 120 Hrs of intervals including all the treatments.

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The statistical analysis of chlorophyll stability index (%) was carried out through SPSS and found that the data is over all significant at $p>0.05$ % and having significant difference between the treatments.

Relative water content (%):

Data depicted from fig-2 shows the effect of different concentrations of PEG-6000 and their overcome effect of primed seed treatment on relative water content % (RWC %). Relative water content was decreased as the concentrations of PEG-6000 was increased from 1.5 % to 4.5% either alone or in combination with Mg $(NO_3)_2$ treatment. But the reduction of RWC % was recorded maximum in PEG-6000 treated set in comparison to PEG-6000 + primed seed. The observation was recorded at two different intervals i.e. 120 Hrs and 240 Hrs for the same. Among the entire sets of treatments (i.e. different concentrations of PEG-6000 alone and along with Magnesium nitrate treated set), the maximum RWC % was recorded in T_5 (i.e. PEG-6000 + primed seed) 90.1 and 89.9% which was followed by T_6, T_1 and T_2 i.e. 89.5, 87.7, 85.5, 88.1, 86.8 and 85.4 % while control was recorded overall highest % of RWC (i.e. 90.3 and 90.9%) at both the time of observations (i.e. 120 Hrs and 240 Hrs).

The statistical analysis of relative water content % was carried out through SPSS and found that the data is over all significant at $p>0.05$ % and having significant difference between the treatments. Data presented in (Fig-2) shows that T-5 have significant relationship with T_3, T_4, T_7 and T_8 while T6 shows significant relationship with T_3, T_4 and T_7 for RWC % at 120 Hrs of intervals. However, T_6 shows significant difference with T_2.

Proline content (µg g^{-1}):

Data depicted from fig-3 shows the effect of different concentrations of PEG-6000 and their overcome effect of primed seed treatment on Proline content (µg g^{-1}). Proline content (µg g^{-}

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^{1})was increased as the concentrations of PEG-6000 was increased from 1.5 % to 4.5% either alone or in combination with Mg $(NO_3)_2$ treatments. But the enhancement of proline content (µg g^{-1}) was recorded maximum in PEG-6000 treated set in comparison to PEG-6000 + primed seed. The observation was recorded at two different intervals i.e. 120 Hrs and 240 Hrs for the same. Among the entire sets of treatments (i.e. different concentrations of PEG-6000 alone and along with Magnesium nitrate treated set), the maximum RWC % proline content (µg g^{-1})was recorded in T_8 (i.e. PEG-6000 + primed seed) 19.10 and 32.60µg g^{-1} which was followed by T_4 and T_7 i.e. 17.47, 30.84 and 16.71, 30.07 µg g^{-1} while control was recorded overall minimum amount of proline contenti.e. 10.20 and 16.98µg g^{-1} at both the time of observations (i.e. 120 Hrs and 240 Hrs).

The statistical analysis of proline content (µg g^{-1}) was carried out through SPSS and found that the data is over all significant at $p>0.05$ % and having significant difference between the treatments. Data presented in (Fig-3) shows that $T\text{-}_8$ and T_4 both have high significant relationship in between both the time of intervals. Results of the proline content also shows that it increases also at 240 Hrs of intervals as compare to 120 Hrs including entire treatments.

Discussion

Data depicted from (Fig-1) shows that the chlorophyll stability index % both are decreased with increased concentrations of moisture stress induced by PEG-6000 while trying to recover chlorophyll stability index % by sets of primed seed at both the times of observations i.e. 120 and 240 Hrs of intervals. Chlorophyll content is one of the most important pigment that adversely affect the rate of photosynthetic process under moisture stress condition result limited growth and development of plant ultimately grain yield (Fahad *et al.,* 2017 and Chutia and

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Borah, 2012). While chlorophyll stability index indicates the level of tolerance at particular concentration of PEG induced moisture stresses (Ananthi *et al.,* 2013) which ultimately shows the stability of photosynthetic apparatus i.e. chloroplast (Sivakumar *et al.,* 2017). It is also reported by various scientist that seed invigoration is a good techniques which help to improve the chlorophyll content as well as chlorophyll stability index % (Sade*et al.,* 2017 and Anaytullah *et al.*, 2012 and Moradi *et al.,* 2012).

Data pertaining in (Fig-3) shows that proline content was gradually increased under PEG induced moisture stress condition as compare to control one (Anaytullah *et al.,* 2007). It is also recorded from (Fig-4) that the sets of primed seed + PEG-6000 collectively lead to produce proline content in the seedling of maize plant that reflect a positive condition for survival of plant under moisture stress condition (Basha*et al.,* 2015 and Anaytullah *et al.,* 2012).

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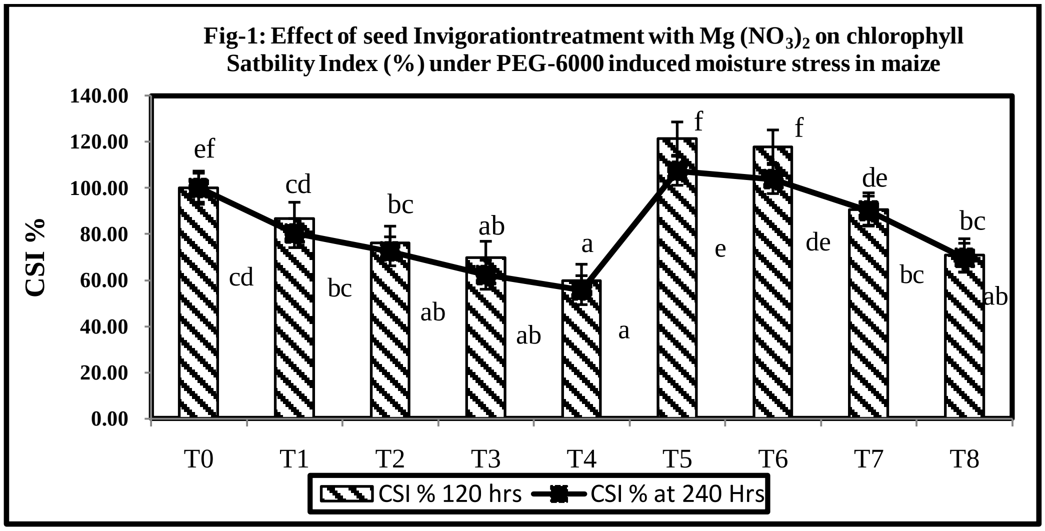

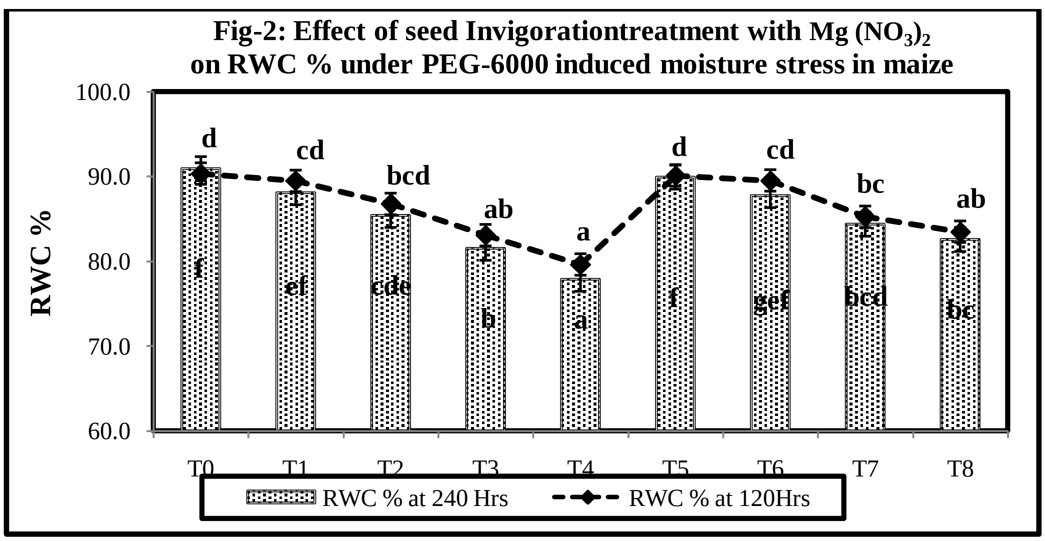

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Fig-3: Effect of seed Invigorationtreatment with Mg $(NO_3)_2$ on Proline content (μg g^{-1}) under PEG-6000 induced moisture stress in maize

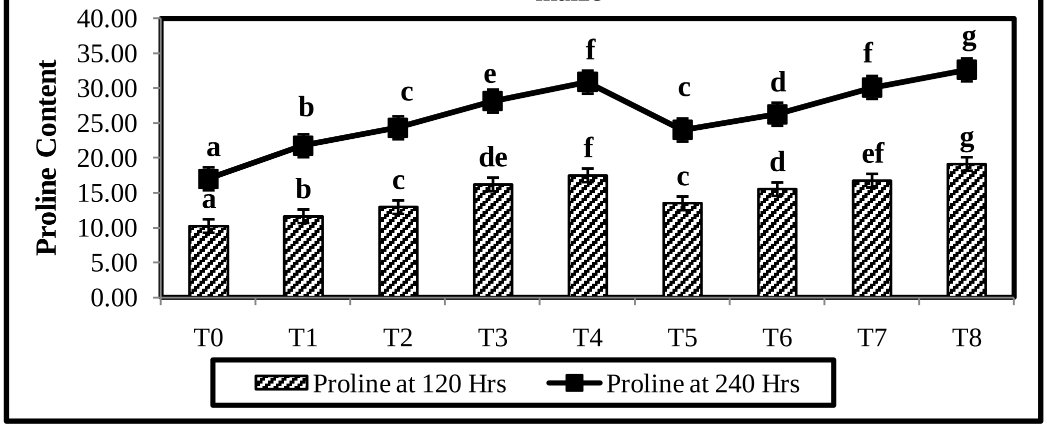

Fig-4a: Comparison between Proline and RWC % in maize seedling under moisture stress condition at 120 hours

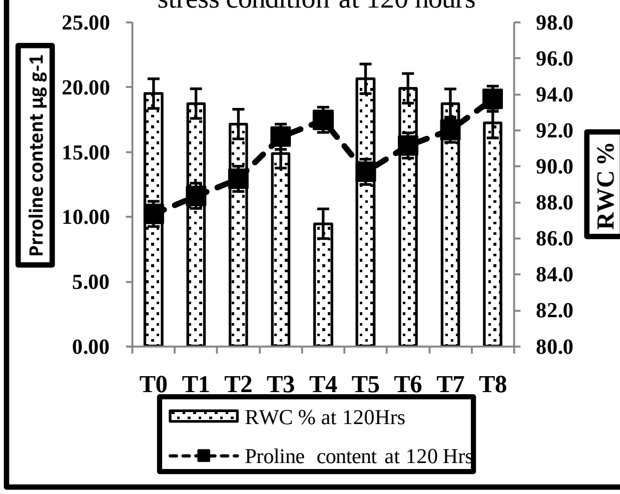

Fig-4b: Comparison between Proline and RWC % in maize seedling under moisture stress condition at 240 hours