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Effect of Seed Priming and Molybdenum Foliar Application in some Physiological and Anatomical Traits in Wheat Crop (*Triticum aestivum*)

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ABSTRACT

A laboratory and field experiments subsequently were conducted in order to investigate the effects of molybdenum in some physiological and anatomical traits in wheat. Four treatments of seed priming were used in lab experiment (0, 20, 40 mg Mo L^{-1} and hydro-priming with H_2O) and laid out in CRD with four replications, whereas in the field experiment, three treatments of molybdenum were used as seed priming (0, 20, 40 mg Mo L^{-1}) and as foliar application (20, 40 mg Mo L^{-1} and spraying with water) and laid out as factorial in RCBD with three replications. Results showed that the concentration 40 mg Mo L^{-1} was significantly superior as seed primer in length and fresh and dry weight of radical and coleoptile, coefficient of germination velocity and number of spikes in square meter, as well as in number of grain per spike and fertility ratio as foliar application. Anatomically the treatments were significantly differed in leaf anatomy. The concentration 40 mg Mo L^{-1} showed that the leaf was slightly wrapped and the upper and lower epidermis cell were larger and not organized while chlorenchyma cells were smaller and also not organized. It can be concluded that the use of molybdenum is very useful in wheat as seed primer or foliar application and it can be recommended to use it in higher concentration in different growth stages in wheat.

تأثير النقع والرش الورقي بالمولبدنيوم على بعض الصفات الفسلجية والتشريحية لنبات الحنطة (*Triticum aestivum* L.)

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الخلاصــة

الكلمات المفتاحية:
نقع البذور، الرش الورقي، المولبدنيوم ، الصفات الفسلجية ، الصفات التشريحية، الحنطة.

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اجريت تجربة مختبرية وحقيلة بالتتابع لمعرفة تأثير المولبدنيوم في بعض الصفات الفسلجية والتشريحية لمحصول الحنطة. استخدمت اربعة معاملات نقع للبذور في التجربة المختبرية (0، 20 و 40 ملغم.لتر $^{-1}$ Mo) اضافة الى معاملة النقع بالماء، طبقت التجربة بتصميم العشوائي التام بأربعة مكررات. بينما في التجربة الحقلية استخدمت ثلاث معاملات من المولبدنيوم (0، 20 و 40 ملغم Mo لتر $^{-1}$ كمعاملات نقع للبذور قبل الزراعة ومعاملة رش (20 و 40 ملغم Mo لتر $^{-1}$) اضافة للرش بالماء، طبقت كتجربة عاملية بتصميم القطاعات الكاملة المعشاة بثلاث مكررات. اظهرت النتائج ان التركيز 40 ملغم.لتر $^{-1}$ المضاف كمعاملة رش ورقي قد تفوق معنويا في الطول والوزن الجاف والرطب للجذير والرويشة، معامل سرعة الانبات و عدد السنابل بالمتر المربع، اضافة الى عدد الحبوب بالسنبلة ونسبة الاخصاب. تشريحيا اختلفت المعاملات معنويا في تشريح الورقة. التركيز 40 ملغم.لتر $^{-1}$ اظهرت ان الورقة ملفوفة نوعا ما وان الطبقة السفلى والعليا لخلايا البشرة كانت اكبر وغير منتظمة في حين خلايا الكلورنكيما كانت اصغر وكذلك غير منتظمة. يمكن الاستنتاج ان استخدام المولبدنيوم ذو فائدة كبيرة للحنطة كمعاملة نقع البذور او رش ورقي ويمكن التوصية باستخدام تركيز عالي في مراحل نمو مختلفة للحنطة.

INTRODUCTION:

Wheat (*Triticum aestivum* L.) is the most important and cultivated crops due to its usage in human nutrition in more than two thirds of the world. Wheat crop has exceeded all plant species in cultivated areas and it can be harvested every month of the year somewhere in the world. However, wheat breeders and producers are facing bigger challenges as the population is increasing beside the risks of the climate change (Tahir, 2008), therefore the increase in wheat production is the goal of wheat producers. Increasing the productivity of wheat mainly in areas that subjected to abiotic stresses is significant challenge for plant researchers because of the abiotic stress are variable in their timing and unpredictable in their intensity (Dolferus et al., 2011). Recent studies have pointed the great importance of molybdenum (Mo) to wheat at physiological and molecular levels (Al-Issawi et al., 2016, Al-Issawi et al., 2013, Babenko and Alikulov, 2014). Mo is inactive in biological system if not combined with special cofactors (Rihan et al., 2014, Sun et al., 2009). It found to be co-factor in more than forty molybdoenzymes in all organisms, however it found only in four enzymes in plants namely nitrate reductase (NR), xanthin dehydrogenase (XDH), sulphate oxide (SO) and aldehyde oxidase (AO) (Mendel and Hänsch, 2002) which participate in many metabolic reactions; mainly assimilation of nitrate, phytohormone synthesis, purine catabolism and sulphate detoxification in plants (Mendel and Hänsch, 2002, Mendel and Schwarz, 1999). Contradictionlly, the toxicity of Mo in plants under all agricultural condition is very rare. Therefore, shedding more lights on this rare, transient essential element is required especially on its effect on the molecular and anatomy level. However, its availability in soil is reasonably less than zinc, manganese, iron, and cupper. More recently Al-Issawi et al (2016) has highlighted the role of Mo in abiotic stress tolerance in wheat as it upregulate the expression of gene responsible for cold tolerance as well as it increased the expression of COR15a protein without environmental stimuli. Due to the importance of wheat crop and essential element Mo the present study came to investigate its role on some physiological and anatomical traits in wheat.

MATERIALS AND METHODS :

Laboratory experiment:

An experiment was conducted in laboratory of field crops department/ Agriculture college- University of Anbar in 15-10-2015 in order to investigate the effect of seed priming with Molybdenum as Molybdate: $[(NH_4)6Mo_7O_{24}.4H_2O]$ in radical and plumule length, fresh and dry weight of radical and plumule, germination percentage, coefficient of germination velocity and anatomy of grainling of wheat (local cultivar). Four treatments were used; 0, 20 and 40 mg Mo L^{-1} and hydro-priming with H_2O were laid in CRD with four replication of each. Wheat seeds were soaked in required solution in a plastic containers with covers in order to prevent the evaporation for 8 hours at room temperature (Wang et al., 1999, Al-Issawi et al., 2013). Seeds were then washed with distilled water and left to air drying until their moisture approximately get to original moisture (≈14%). Then 50 seed were placed to petri dishes (20*10 cm) on two layers of moist tissue paper. The data were recorded after 24 hours until there was no more germination observed.

Anatomy of plumule under study treatments:

Five plumules were taken from each treatment (Dried seed, H_2O, 20 and 40 mg Mo L^{-1}) and all prepared in the central service Laboratory in College of Education for pure science college (Ibn Al-Haitham)/ University of Baghdad. Plumules were put in fixation solution of Formalin Acetic Acid glacial (A.F.A) for 24 hours. Then a hydrogenation has been done with series of alcohols starting with concentration of 70%, 80%, 90% then with 95% withhold one hour for each concentration then all were placed in absolute alcohol for one day. Samples after then were put in Xylene and 100% alcohol for 30 minutes in order to softening the samples then were put only in Xylene for also 30 minutes. Samples were then put in paraffin wax with xylene (1:1) in small tubes for 30 minutes then in only paraffin wax for also 30 minutes at 58 C°. Samples after that were poured in frames and left to air dry for 24 hours. Sectioning was made after samples were dried by Microtome (10-14 mm) as stripes. Then stripes containing samples were put on clean slides with some drops of distilled water and then left to dry for one day. Next day coloring process has been made by outing the slides in specific tubes called Coplin jar. The samples were put in Xylene for 5 minutes in order to solute paraffin wax then were put in Xylene with alcohol (1:1) for 5 minutes. Samples were put in ether (100%) then absolute alcohol (100%) then in 95% alcohol, then 70% alcohol for 5 minutes for each concentration. Samples were left in Safranin color for whole day then washed with distilled water for 1-2 seconds. After that samples were put in 95% alcohol for 10 seconds; then colored with Fast Green FCF for 3 seconds then were put in Xylene and alcohol (1:1) after 5 minutes from coloring then were put in Xylene for 10 minutes. Then a preserve substance should be added (DPX) and covers were placed on slides in order to make ready for picturing under microscope (Bancroft et al., 2013).

Field experiment:

A field experiment was carried out in winter season of 2015-2016 in field of department of field Crops-Agriculture College/ University of Anbar (Abu-Ghariab) in order to study the effect of Mo in some wheat traits. The experiment included two factors, the first is seed priming with Mo in concentration of 0, 20, 40 mg Mo L^{-1} and the second is foliar application with Mo in same concentrations. The experiment was laid out as split-plots arrangement in RCBD with three replications. Primed seeds were sown in well prepared field and divided to experimental units (2.5*3 m) each unit contained 11 lines and 20 cm left between each two lines. The field was fertilized with 160 kg N ha^{-1} as urea and 96 Kg P ha^{-1} as tri-super-phosphate (20%). Plants were sprayed with Mo concentrations at two growth stages, the first at tiller stage and the second at flowering stage. Plants were well sprayed with Mo solutions and the control treatment was sprayed with water only. Data were recorded for plant height, number of spikes in square meter, spike length, number of grain per spike and fertility ratio (%). All data from the two experiments were subjected to the statistical analysis according to the used arrangement and ANOVA table and the significant differences between means were picked according to L.D.D test at 0.05 probability.

RESULTS :

Laboratory experiment :

The statistical analysis (Figure 1) showed that there were significant differences between all treatments in length of either radical or plumule. Generally, plumule was taller than radical under all treatments. It is clear that the concentration of 40 mg Mo L^{-1} was superior along with hydro-priming with H_2O by giving vigor seedling in comparison with the other two treatments. Mo in high concentration has the positive effect compared to the low concentration which might not be reached threshold of effectiveness. This result confirms the role of seed priming in good field establishment.

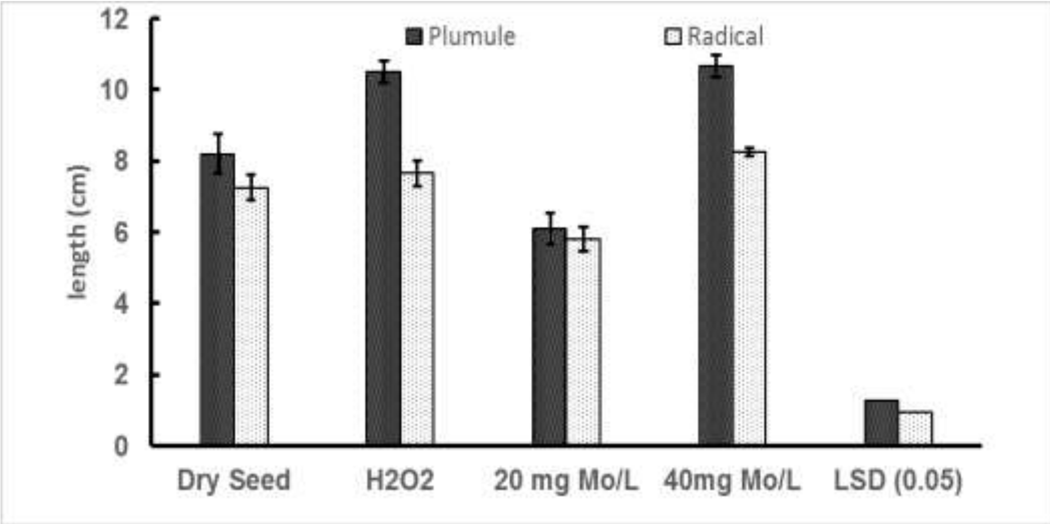


Figure 1 . Effect of seed priming treatments in length of wheat Plumule and Radical (cm).

As for the weight of plumule and radical in fresh and dry status, it was slightly different from their length. However, the statistical analysis showed no significant differences between treatments in dry weight but there was significance in fresh weight of seedlings. The concentration of 20 mg Mo L^{-1} gave the lowest fresh weight compared to the rest of treatments which were similar in their effect of the weight (Figure 2). Maintaining a certain level of moisture in seedling is useful in giving them a tolerance against environmental stress such as temperature, drought and/or salinity.

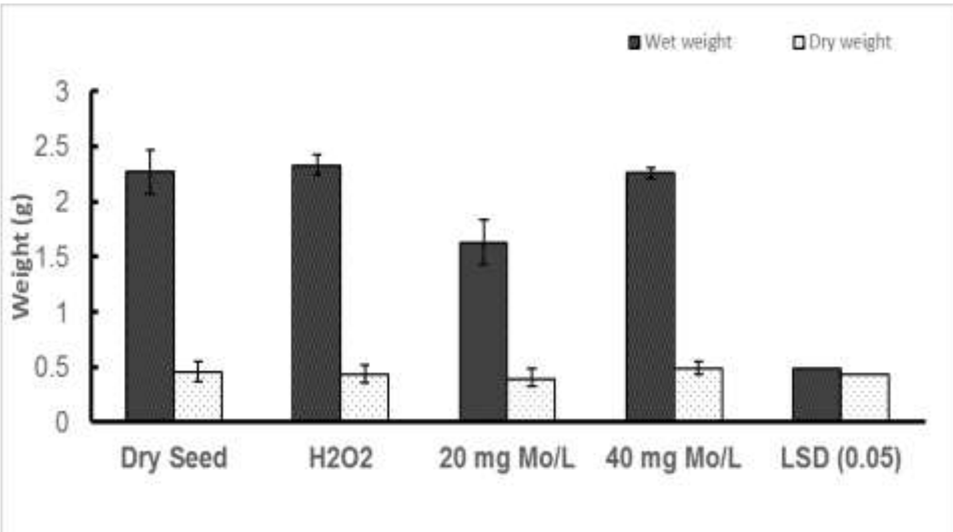


Figure 2 . Effect of seed priming treatments in fresh and dry weight of wheat Plumule and Radical.

As for germination percentage, it seems that the treatments of the study did not get to the threshold of affecting in the germination percentage; although there was a slight superiority of the priming treatments e.g. adding 40 mg MoL^{-1} (Figure 3). The germination percentage got to 90.1% when seeds were soaked in solution of 40 mg Mo L^{-1} while the obtained percentage of germination was only 80.5% when dried seeds were used and this difference did not get to the limit of significance. The other two treatments were laid in between those values (Figure 3).

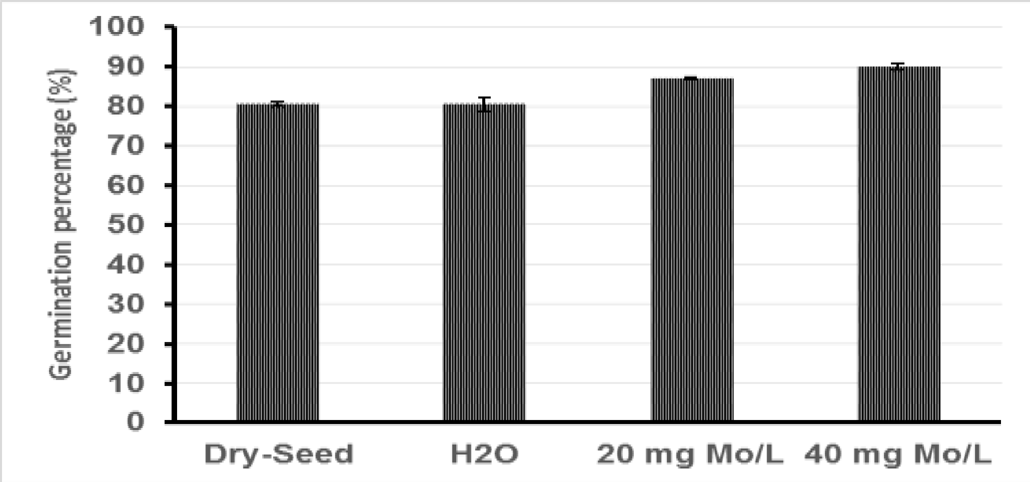


Figure 3. Effect of seed priming treatments in germination percentage (%) of wheat seeds.

The statistical analysis in figure 4 showed that the concentration of 40 mg Mo L^{-1} gave the highest average of coefficient of germination velocity (CVG) which gave 83.39% and was significantly different from 20 mg Mo L^{-1}, hydro-priming and dry grain which gave 67.58, 64.81, and 62.06% respectively. This spot more light on the role of seed priming with high concentration of Mo in enhancing germination speed in wheat.

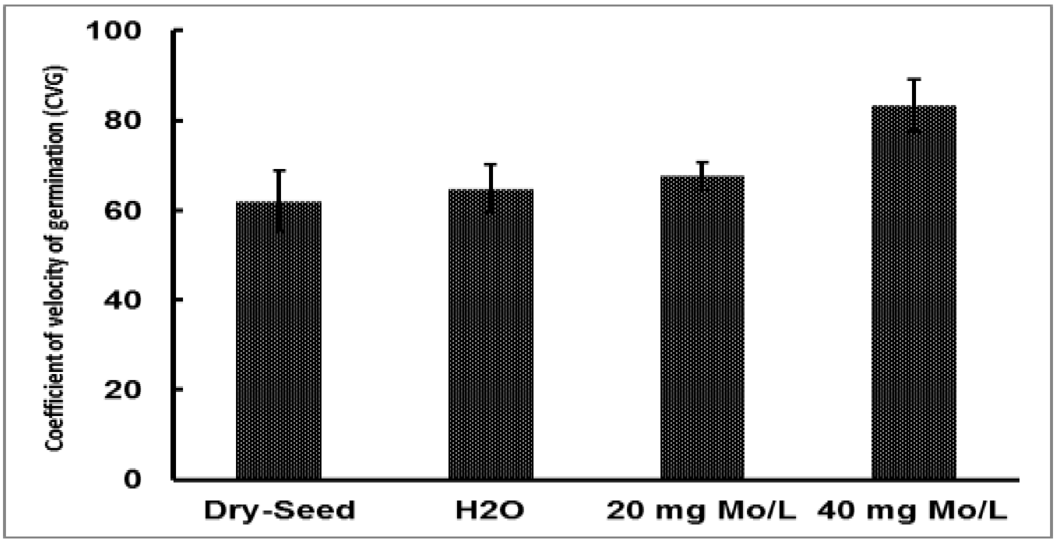
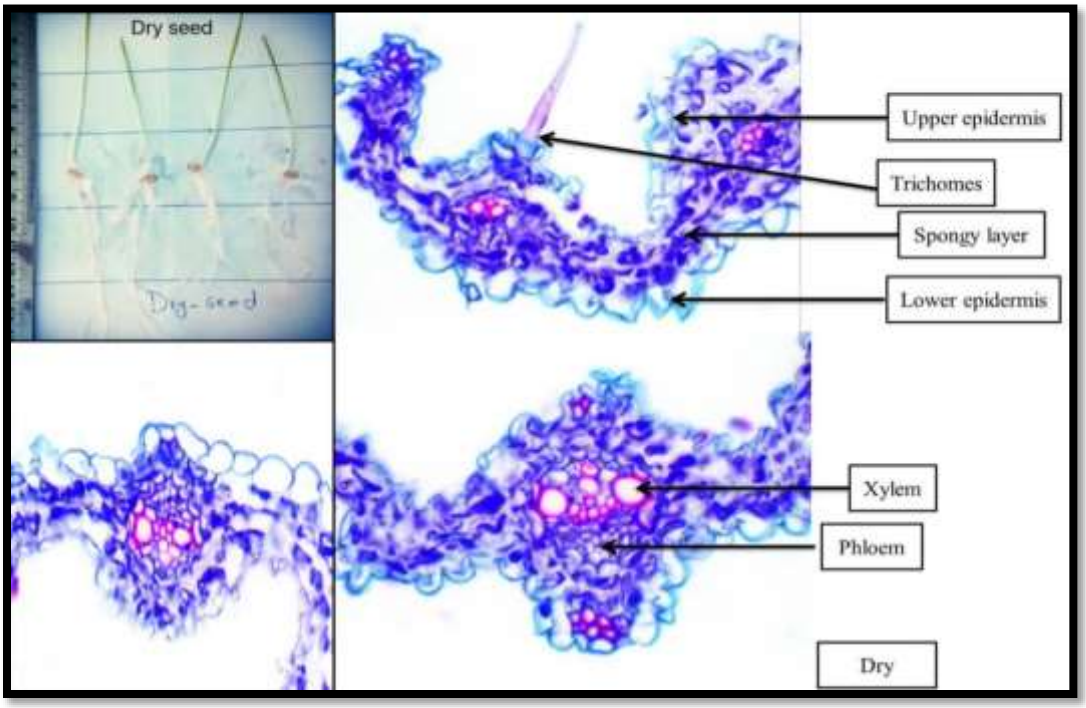


Figure4 . Effect of seed priming in Coefficient of velocity of germination (CVG) of wheat grains.

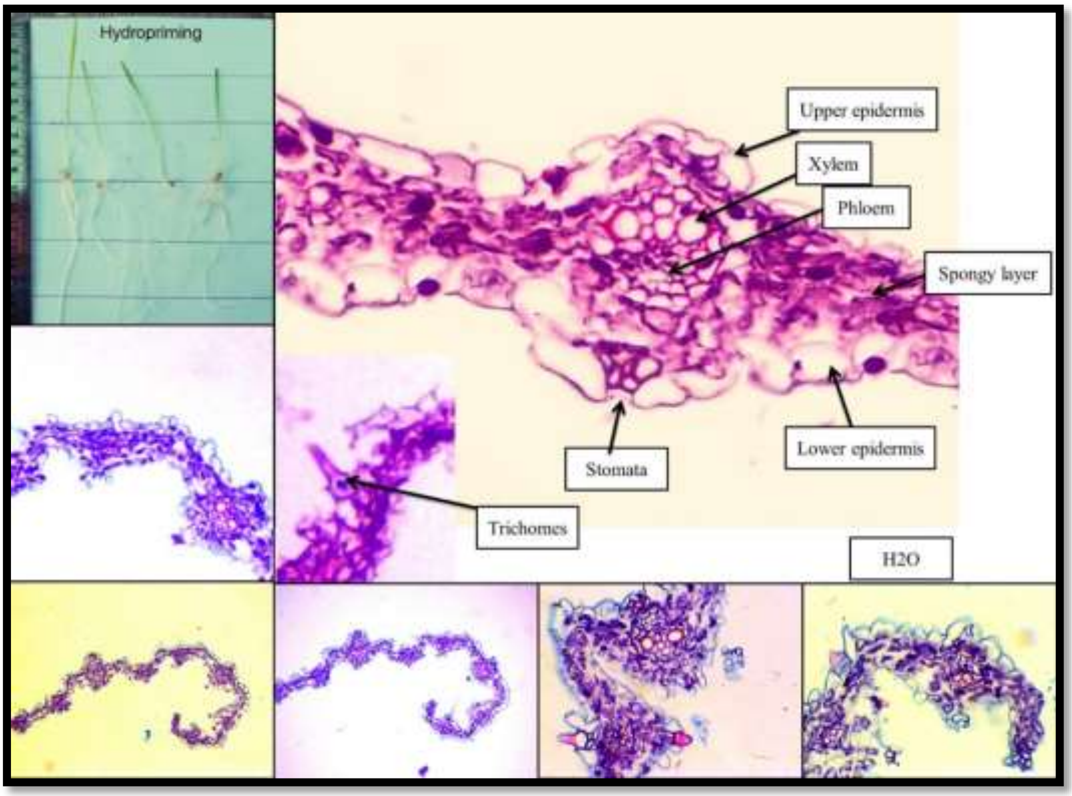
Plumule histological study under different treatment of seed priming:

The dry seed treatment in picture 1 showed that the morphology of plumule appears to be completely wrapped. The epidermis layer cells became shrinkage, small in size and unorganized, followed by foundation tissue of chlorenchyma cells also were shrinkage and small in size. The bundle sheath cells became longitudinal and less in size as well xylem diameter became less. Lower epidermis is similar to upper epidermis but the stomata became less in size and approximately closed. However, the not glandular trichomes became longer under this treatment (Picture 1).



Picture1 . Anatomy of wheat Plumule under dry seed treatment.

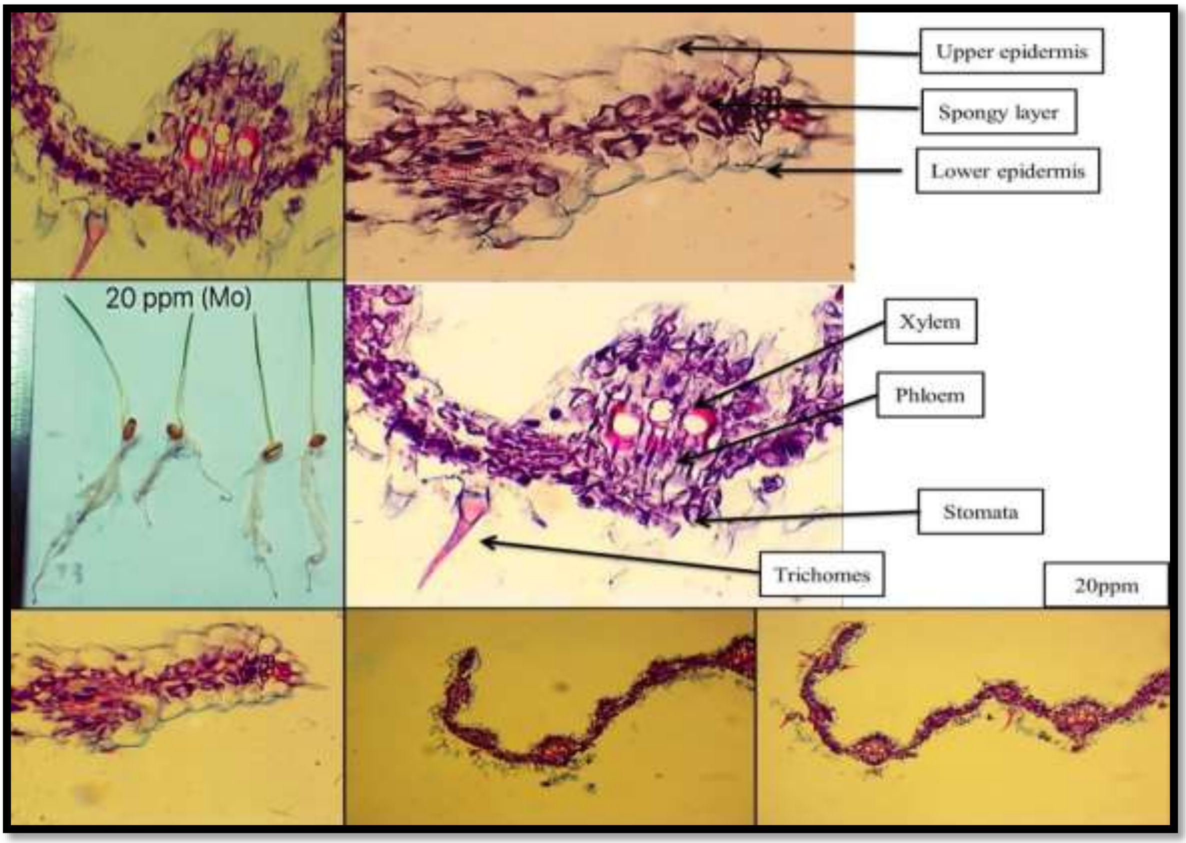
In hydro-priming treatment (Picture 2), plumule tissue morphology was unwrapped and anatomical the epidermal cells are normal, bigger and their shape was between oval and longitudinal followed by foundation tissue that consist of chlorenchyma where the photosynthesis occurs. This tissue can't be distinguished by vertical and spongy cells in monocotyledon plants e.g. wheat.



Picture2 . Anatomy of wheat Plumule under hydro–priming treatment.

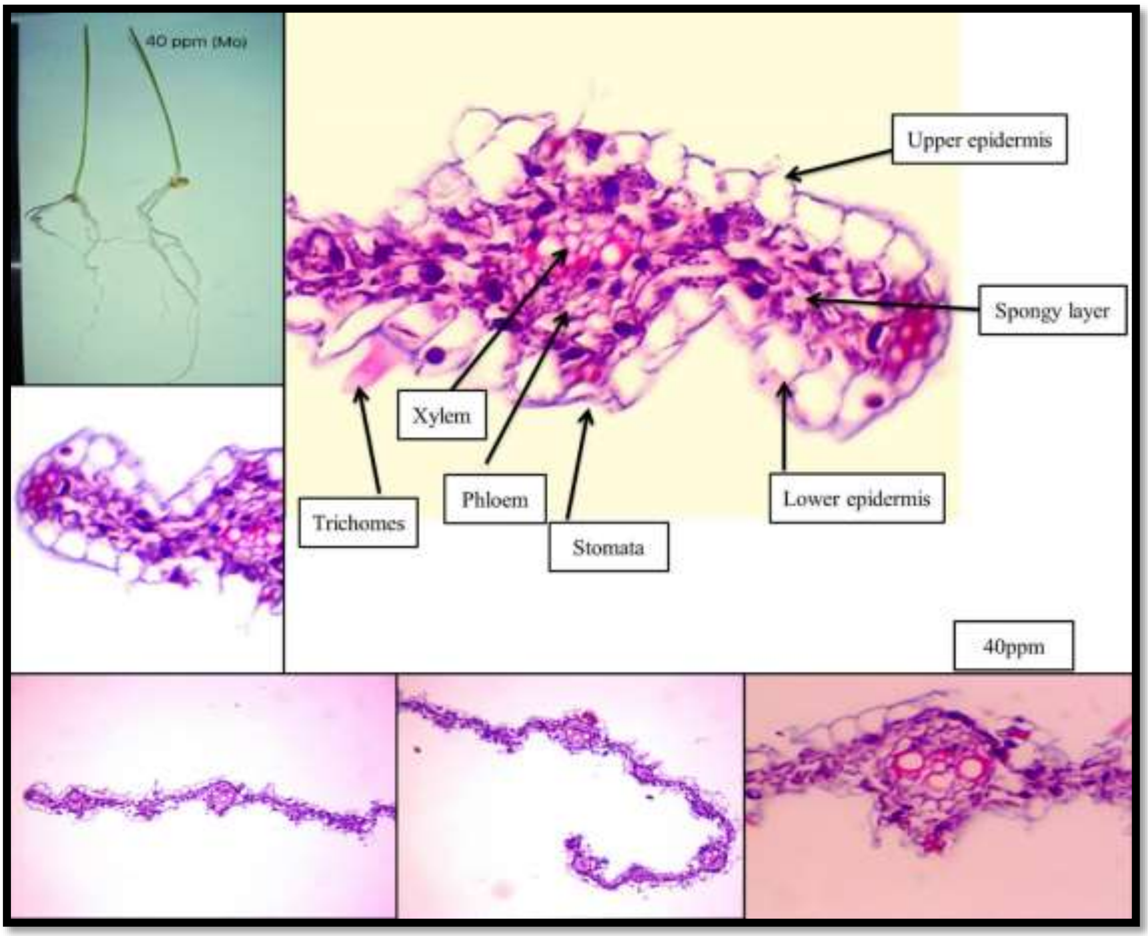
The bundle sheaths were with circle to oval shape. The bundle sheath tissues are normally parallel because of the anatomy of monocotyledon plants leaves therefore it appears slight bigger in across sections in middle and smaller at the edge. Lateral bundle is closed and form with Xylem from upper epidermis side Y or V shape while the phloem found from lower epidermis side. However lower epidermis is similar to upper epidermis and contains wide and clear stomata. Plumule contains not glandular trichomes, uniseriate and unicellular. It is clear that H_2O affected the anatomy of wheat plumule.

Under treatment of 20 mg Mo L^{-1} (Picture 3) the plumule was incompletely wrapped morphologically. Epidermis cells became bigger in size in comparison with normal cells and unorganized, followed by foundation tissue of chlorenchyma cells that became also bigger in size and unorganized. While the bundle sheath cells became shrinkage and xylem diameter became notably less. Lower epidermis is similar to upper epidermis but the stomata became bigger in size with wide aperture. Trichomes are not glandular and also became longer. Apparently, seedling under this treatment was less in length, weight and with dark green color, showing that this treatment was negatively affected the germination and its characteristics.



Picture3 . Anatomy of wheat Plumule under treatment of 20 mg Mo L^{-1}

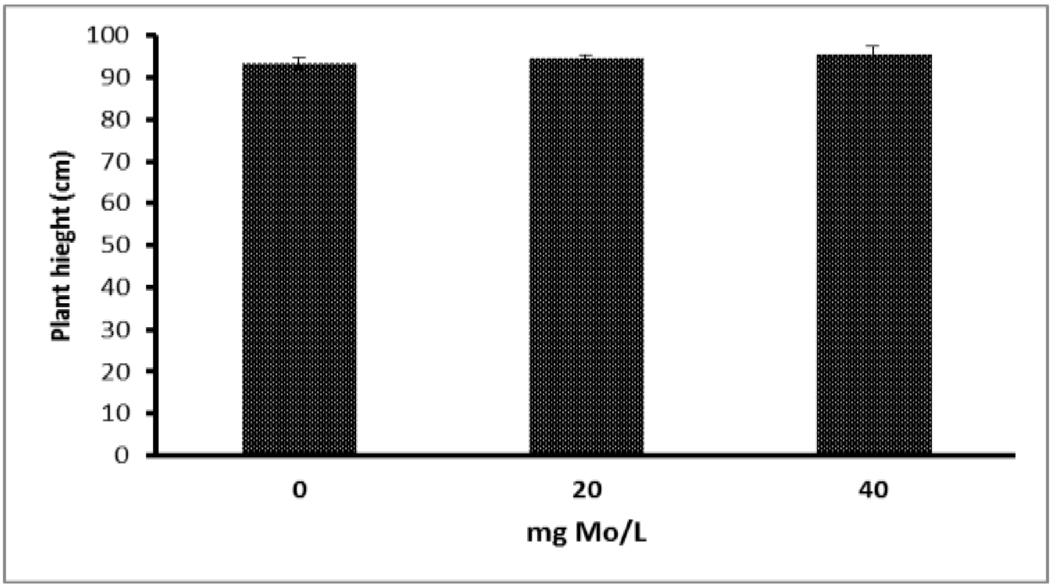
Apparently treating grain with 40 mg Mo L-1 (Picture 4) made plumule completely wrapped. Anatomically, epidermis layer cells became larger than normal size and not organized followed by foundation tissue of chlorenchyma cells that their cells became smaller than normal size and also not organized. Whereas the bundle sheath cells were shrinkage; however xylem diameter became much less. Lower epidermis is similar to upper epidermis but their stomata became larger than normal size with wide aperture. However, trichomes are not glandular and became longer than normal. It has been noticed that is this treatment is similar to hydro-priming treatment apart from plumule being wrapped evidencing that Mo is really effective and changed the anatomy of wheat plumules.



Picture4 . Anatomy of wheat Plumule under treatment of 40 mg Mo L^{-1}.

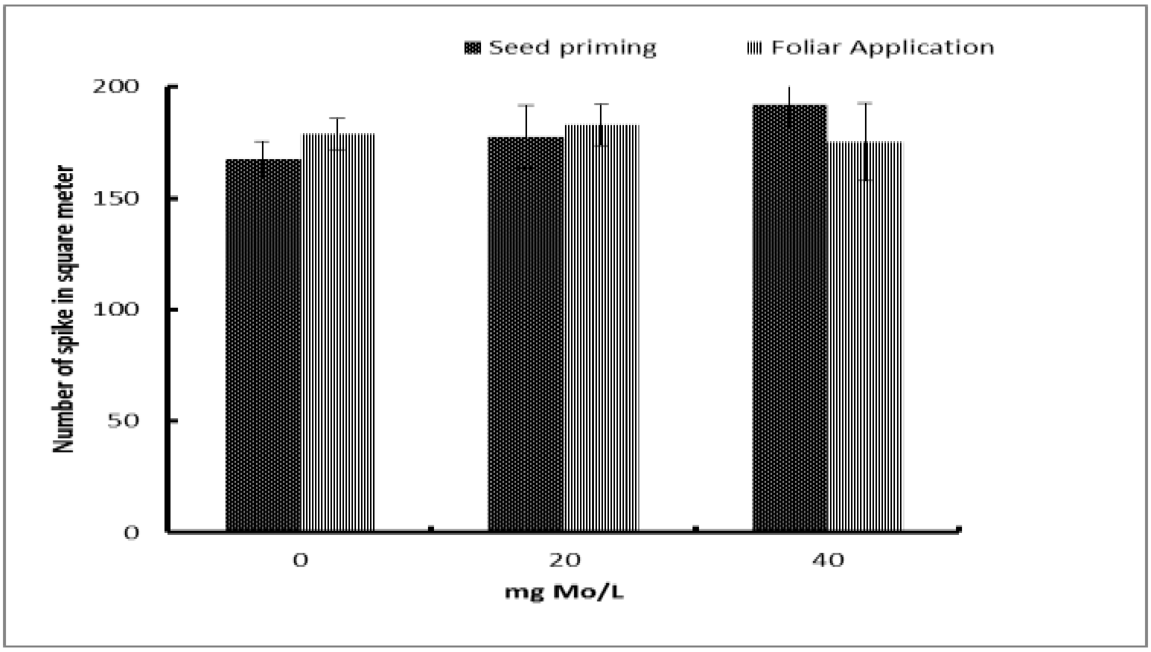
Field experiment:

Molybdenum was applied in two methods in the field. The first method, Mo was used as seed primer in the concentration of 0, 20 and 40 mg Mo L^{-1}, however seeds were soaked in the solutions of Mo for 8 hours then thoroughly washed with distilled water and left to air dried. Then treated seeds were sown in the field according to used experimental design. The second method of applying Mo was as foliar application at two growth stages, first at tiller stage and the second at flowering stage. The statistical analysis (Figure 5) showed that both methods did not affect plant height. It might be because the absorbed Mo by seeds was enough to affect plant height as well Mo as foliar was applied when plants were nearly in fixed length.



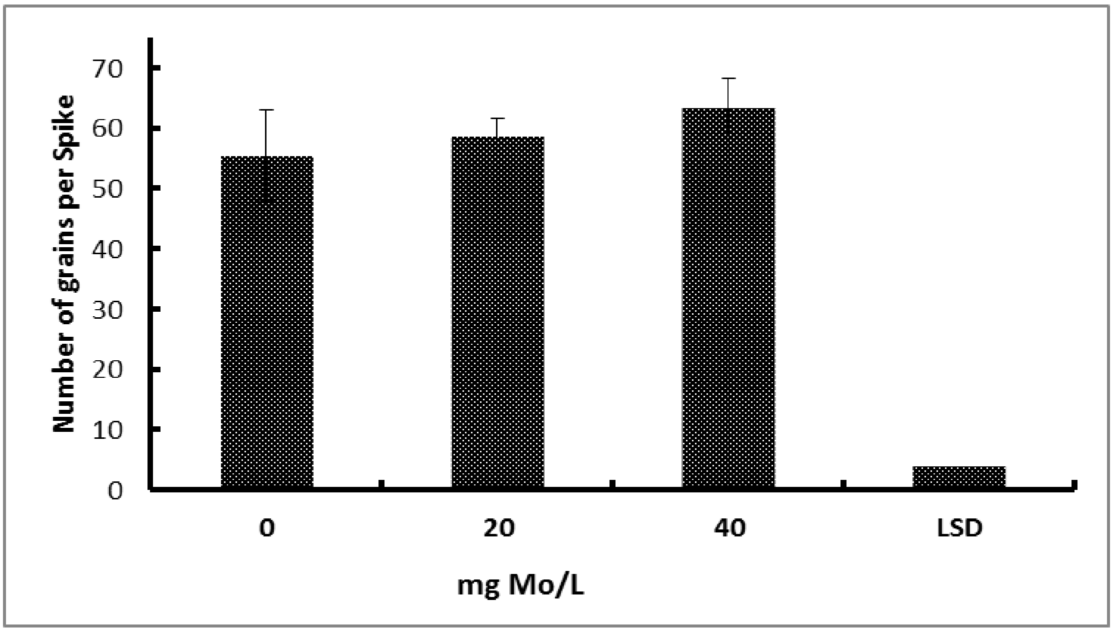
Picture 5 . The effect of Molybdenum treatments in wheat plant height (cm).

It is clear from figure 6 that the number of spikes in square meter was not affected by the application of Mo in either method. Although there was a slight increase at 40 mg Mo L^{-1} for the seed priming over foliar application and that might be because of the effect of this concentration on germination (Experiment 1).



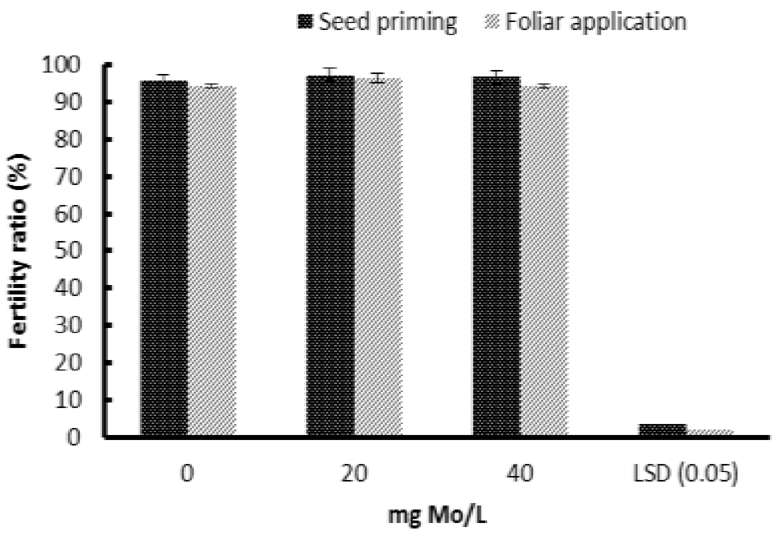
Picture 6 . Effect of Molybdenum as seed primer and foliar application in the average of number of spikes in square meter.

However, the number of grain per spike was significantly affected by the foliar application with Mo (Figure 7). However, the treatment of 40 mg Mo L^{-1} gave the highest number or grains in spike in comparison with the other two treatments. While the use Mo as seed primer did not make any difference in this trait. This highlight the importance of this element to wheat during different growth stages, from grain to grain.



Picture 7 . Effect of Molybdenum as foliar application in number of grains per spike.

According to figure 8, it is clear that the foliar application with Mo is more effective than using it as seed primer. However, spraying plant with 40 mg Mo L^{-1} at different growth stages gave the highest average of fertility ratio compared to either adding 20 mg Mo L^{-1} or control treatments. However, the use of Mo as seed primer had no significant effects in the number of grains in spike, eventually fertility ratio.



Picture 8 . Effect of Molybdenum in wheat fertility ratio (%)

Discussion:

The use of Molybdenum as seed primer or foliar application is useful technique that widely used in order to improve growth, tolerance and productivity of wide range of plant species (Basra et al., 2005, Williams et al., 2005). However, the availability of Mo for plant is dependent strongly on the soil PH as well as the organic matter or water drainage (Kaiser et al., 2005); therefore the use of exogenous application of Mo is very effective in providing plant with the required amount of it. It is approved that Mo deficiency causes number of negative symptoms on plant ending with hindering plant growth. Most of these causes are associated with the reduction of molybdoenzymes activity (e.g. NR, SO, XHD, OA) (Kaiser et al., 2005, Williams and Da Silva, 2002, Sun et al., 2006). The other use of Mo is to reduce the effects of Reactive Oxygen Species (ROS) the activation of many antioxidant enzymes e.g. SOD, CAT, GR…etc. (Al-Issawi et al., 2016, Al-Issawi et al., 2013, Mendel and Schwarz, 1999, Rihan et al., 2014, Sun et al., 2009). The use of Mo as seed primer was very useful especially in Plumule and Radical length (Figure 1), fresh weight of seedling (Figure 2) and velocity of germination (Figure 4) as the technique of seed priming is widely now used to enhance plant growth and productivity (Bodi et al., 2015, Fuller et al., 2012), however Mo as seed primer is very useful in increasing germination traits as well as growth and yield of wheat and these results are consistent with (Al-Issawi et al., 2013, Rihan et al., 2014, Sun et al., 2009) as they found that treating seed with Mo up-regulated the gene expression of genes that responsible for abiotic tolerance (Al-Issawi et al., 2013) and enhanced antioxidant enzymes (e.g. CAT, GR and SOD) (Al-Issawi et al., 2016). Molybdenum is the component of enzymes such as Nitrogenase and Nitrate reductase which are the main enzymatic system in plants (González et al., 2008); therefore the increase in growth traits observed in wheat seedlings is attributed to the application of Mo which might led to increase in N assimilation that eventually led to the biosynthesis of Chlorophyll. Molybdenum enhances plant growth at germination and growth levels (Zakikhani et al., 2014). From the observation of the section of wheat plumule, it has been noticed that Mo is a vital factor in changing the histology of wheat leaf and that might be reflected in its growth (Picture 1, 2, 3 and 4). It can be noticed that the bundle sheaths were shrinkage and lower epidermis has more stomata that upper epidermis and the apertures of the stomata were wide open and that might lead to smooth gas exchange; therefore photosynthesis will be more effective. Results from the field experiment supported that the use of 40 mg Mo L^{-1} is superior in number of spikes in square meter, number of grain in spike and fertility ratio. Molybdenum was applied to wheat as seed primer and that might be enhanced the field emergency based on the results of laboratory experiment. However yield components were significantly enhanced. However, molybdenum is very important element for nitrate reductase (NR) that catalyses the first step of nitrate assimilation which is very important pathway in plant nutrition (Hänsch and Mendel, 2009). The most important thing about applying molybdenum to plant is that the timing of application (Masi and Boselli, 2011). The growth stage of plant determining the efficiency of this element, there it has been found in this study that the application of Mo at two different growth stages was very efficient in increasing the fertility and of course eventually total yield. It can be concluded that the use of Mo is very important for plant growth, starting from germination to the final product. However, it has been proved in previous and current studies that Mo is important in plant physiology, anatomy, and biochemistry and molecular. Finally it can be recommended to use this essential element in different concentration and at different growth stages and for different plants.

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