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Phytohormonal priming improves germination and antioxidant enzymes of soybean (*Glycine max*) seeds under lead (Pb) stress

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Increasing heavy metals in agricultural soils has deleterious effects on germination and growth of many crops. The present study investigated whether priming with phytomormones could alters the physiological responses of soybean seeds germinated under water and lead (Pb) stress. Seeds were soaked for solutions containing 10 or 100 µM of indolebutyric acid (IBA), gibberellin (GA_3), cytokinin (CK), ethylene (ET), abscisic acid (ABA) and distilled water (hydro-priming). Non-priming seeds (NP) were taken as control. Daily germinated seeds (Gd%), final germination (fG%), germination speed index (GSI), average germination time (AGT) and the time taken to reach 50% germination ($T_{50\%}$) as affected by the phytohormone treatments were studied. Data showed that germination of Pb-stressed seeds improved with hormone treatments. High levels of the hormones gave higher Gd values than low levels. Moreover, the highest Gf values were recorded with Ck treatments of 10 µm and 100 µm at which Gf increased by about 77.5% and 73.8%, respectively. Treatments significantly affected the activity of anti-oxidant enzymes Catalase (CAT) and Peroxidase (POX). Water stress (NP) and ABA caused a significant increase in CAT and POX activities. Phytohormones can be used as an efficient priming method to enhance soybean seed germination under Pb stress.

Keywords: Lead, seed priming, soybean, germination, antioxidants.

INTRODUCTION

Seed germination is known to be controlled by a number of physiological mechanisms. These mechanisms are necessary for the growth and development of the embryo. Seed germination is also the most sensitive stage to abiotic stress, thus under environmental stress seed of many species cannot germinate.

Elevated concentrations of heavy metals (HM) in plant growth media often cause inhibition of growth and toxicity in many plant species. These metals usually affect seed germination and growth of plants via the interfering with metabolism processes within plants (Rahimi et al. 2012). There are many ways for HMs, at high concentrations, to cause embryo and seedling toxicity such as binding to the SH groups in protein molecules, which could reduce or inhibit the activity of some physiological key enzymes; HM can also disrupt the structure of proteins in cell membranes, leading to restriction of vital physiological processes in plant tissues (Oves et al. 2016). Moreover, high concentrations of heavy metals encourage the formation of reactive oxygen species (ROS) and free radicals, leading to an oxidative stress toxic to the embryo (Abdul-

Qados 2015). These ROS could be determined through the antioxidant system (Arya and Roy 2011).

Lead (Pb) is one of the toxic HM that increased in the agricultural soils and became a significant pollutant because of improper use of agricultural practices and industrial activities (Rakesh Sharma and Raju 2013). Even relatively low concentrations of Pb were found to disturb many fundamental processes during seed germination and seedling growth (Silva et al. 2016; Hadi and Aziz 2015).

There are a number of factors and aspects controlling seed dormancy and germination, including phytohormones, which are produced by both plant and soil organisms. These hormones can be used as seed priming agents.

Seed priming method is a technique by which seed germination and seedling growth can be improved under different adverse conditions including heavy metal stress. With priming, the process of germination is encouraged by soaking seeds in solutions containing of different compounds such as salts, metals, growth regulators or phytohormones (Hussain *et al*. 2016a) for certain time and then seeds are air-dried. Same authors found that, primed-seeds gave better germination and high growth rate even under stress conditions. In this regard, priming was found to be effective for the production and substantially increased the yield of many crop species (Nawaz et al. 2013).

Even though the mechanisms of how priming enhances seed germination and growth are still unknown, it was suggested that priming activates a series of physiologically vital processes that improve plant growth under stress conditions (Abdel Latef and Tran 2016), including the induction of antioxidant systems (Hussain et al. 2016b).

Phytohormones are commonly used for seed priming approach to enhance the germination of different seeds under stress conditions (Hu *et al*. 2013; Jisha *et al*. 2013). In earlier studies, Ansari *et al*. (2013) found that germination of rye (*Secale montanum*) seeds, treated with gibberellic acid (GA_3), increased under water stress conditions. Also Khan et al. (2009) found that germination of pepper (*Capscum annum* L.) seeds pretreatment with salicylic acid improved significantly under high salinity levels. In addition, Nascimento (2004) used ethylene to minimize the effect of high temperatures on lettuce (*Lactuca sativa* L.) seed germination.

Because of the insufficient evidence about the effect of hormonal priming on seed germination of soybean under heavy metal stress conditions, the present study attempt to investigate the effect of phytohormonal priming on the physiological responses of the economically important soybean (*Glycine max* L. Mill) seeds when planted under lead (Pb) stress.

MATERIALS AND METHODS

Seed priming treatments

Soybean (*Glycine max* (L.) Mill) seeds were brought from authorized agency and soaked in 250 mL solutions containing 10 or 100 µM of the following phytohormones: gibberellic acid (GA_3), indole-butyric acid (IBA), cytokinin (CK, benzyl adenine), abscisic acid (ABA), ethylene (ET, chloro-ethyl-phosphonate) and distilled water (hydro-priming). Soaking was performed in dark condition for 16 hours at 25 °C. Non-priming (NP) treated-seeds were used as a control treatment. After priming, seeds were washed with distilled water and placed on laboratory tables and dried using filter papers for 24 hours at room temperature according to Nawaz et al. (2013).

Stress initiation

After drying, primed-seeds were sterilized using solutions of 5% sodium hypochlorite for five minutes and then allowed to germinate in 10 cm plastic containers containing two-three layers of filter papers moistened with distilled water. After five days of germination, stress conditions were imposed. Germinated seeds were organized in sets with 6 replicate each and were treated with a solution of 500 µM lead chloride ($PbCl_2$) added to the containers. Another set of seeds was left in water as a control treatment. The experiment was carried out in a growth chamber at 25°C and 16 hours light (long-day photoperiod).

Germination test

Germination is defined as the visible emergence of the radicle through the seed coat. Six replicates from each treatment were used to calculate the following parameters according to the methods of Galmés *et al*. (2006) and Farooq *et al*. (2005a):

(a) percentage of daily germinated seeds following the equation [(Gd% = (*nd* ÷ *N*) × 100)]; (b) final germination percentage as [(Gf% = (*nf* ÷ N) × 100)], where *nd* is the number of seeds germinated every day, *nf* is the total number of seeds germinated at the end of the whole

experiment (one week), and *N* is the number of seeds used in the test; (c) germination speed index calculated as [GSI = *Σ*(*nd/td*)], where *td* is a specific day *d*; (d) average germination time as [AGT = *Σ* (*td.nd*) / *Σn*] and; (e) the time taken to reach 50% germination [T50=*td*+[(*N/2*)– *nd*](*tj* – *td*)/*nj* – *nd*], where *N* is the final number of seeds that germinated and *nd* and *nj* were the cumulative number of seeds germinated by adjacent counts at times *td* and *tj* when *nd* < *N/2* < *nj* (Farooq et al. 2005a).

Enzyme activities

Catalase activity (CAT)

Catalase activity was determined by monitoring the decrease in the absorbance of hydrogen peroxide (H_2O_2) at 240 nm according to the method of Noctor and Foyer (1998). Phosphate buffer solution (pH=7) was used in the extraction of the enzyme. While the assay solution was phosphate buffer and hydrogen peroxide. Unite of activity was µmol of CAT used to decompose one mole of hydrogen peroxide (H_2O_2) in one min (µmol H_2O_2 disappeared/ min/ gm fwt).

Peroxidase activity (POX)

The activity of peroxidase was determined in a reaction mixture containing phosphate buffer (pH 7.0), EDTA, guaiacol, H_2O_2 and enzyme extracts as explained by Urbanek et al*.* (1991). Just at the addition of enzyme, the increase in absorbance at 470 nm was recorded for 1 min. Unit of activity was assigned as the amount of tetra-guaiacol formed (µmol tetra-guaiacol formed/ min/ gm fwt) .

Statistical analyses

All data were statistically analyzed using the analysis of variance (ANOVA) following the methods of Snedecor and Cochran (1980). Mean values were compared between all treatments using LSD at 0.05 confidence level.

RESULTS

Percentage of daily germination

Data recorded in Fig (1) showed clearly that daily germination (Gd) of soybean seeds improved 2 d after the application of IBA, CK, GA_3, ABA and H_2O priming either in the presence or absence of Pb stress, as compared with NP control treatment. As compared with other treatments, it was clear that H_2O and the low concentrations of hormones (10 µm) recorded the highest Gd values all over the experimental period, either in the presence or absence of Pb, however, the obtained values were relatively higher in Pb-untreated than those recorded in Pb-treated seeds.

Data obtained in Fig.1 showed also that both the concentrations (10 and 100 µm) of the auxin IBA priming enhanced the Gd either under control conditions, in the absence of Pb (Fig. 1a) or in the presence of the pollutant (Fig. 1b), however, Gd values at the high IBA level were higher than those recorded at the low level. In contrast Gf values at the low IBA concentration were slightly higher than those recorded at 100 µm (Fig. 2) under control or Pb polluted medium.

Effect of auxin (IBA)

Data recorded in Fig. 1 showed that IBA priming enhanced seed germination as compared to unprimed seeds. Collected results showed clearly that IBA priming enhanced daily germination percentage (Gd%) after 2 days of sowing to reach its maximum value after 4 days of germination under control condition (Fig. 1a), while at Pb stress condition Gd% increased after the first day of sowing and reached its maximum value after 5 days of germination (Fig. 1b).

The results indicated also that IBA was effective to improve final germination percentages (Gf%) of soybean seeds in the absence or the presence of Pb stress. At 10 and 100 µm of IBA, Gf% values were about 70.6% and 768.2%, respectively. While unprimed seeds recorded Gf% values of about 48% and 28%, respectively (Fig. 2).

Moreover, IBA priming speeded the germination time therefore GSI values were higher than those of unprimed seeds (NP) either under control or Pb stress condition (Fig. 3). Thus, AGT values of IBA primed seeds were significantly higher than those recorded at NP treatment (Fig. 4).

The stimulating effect of IBA priming on germination process apparently decreased the time for seeds to reach 50% germination, therefore, T_{50} was reached after about 93 h or 81 h with 10 or 100 µm of IBA, respectively, under control condition; while under Pb stress condition, the corresponding values were about 99 h or 78 h (Fig. 5). Comparable values of T_{50} of unprimed seeds were 120 h or 160 h, in the absence or the presence of Pb stress condition, respectively.

Effect of cytokinin (CK)

It was clear that cytokinin (CK) priming caused a substantial increase in the Gd% after

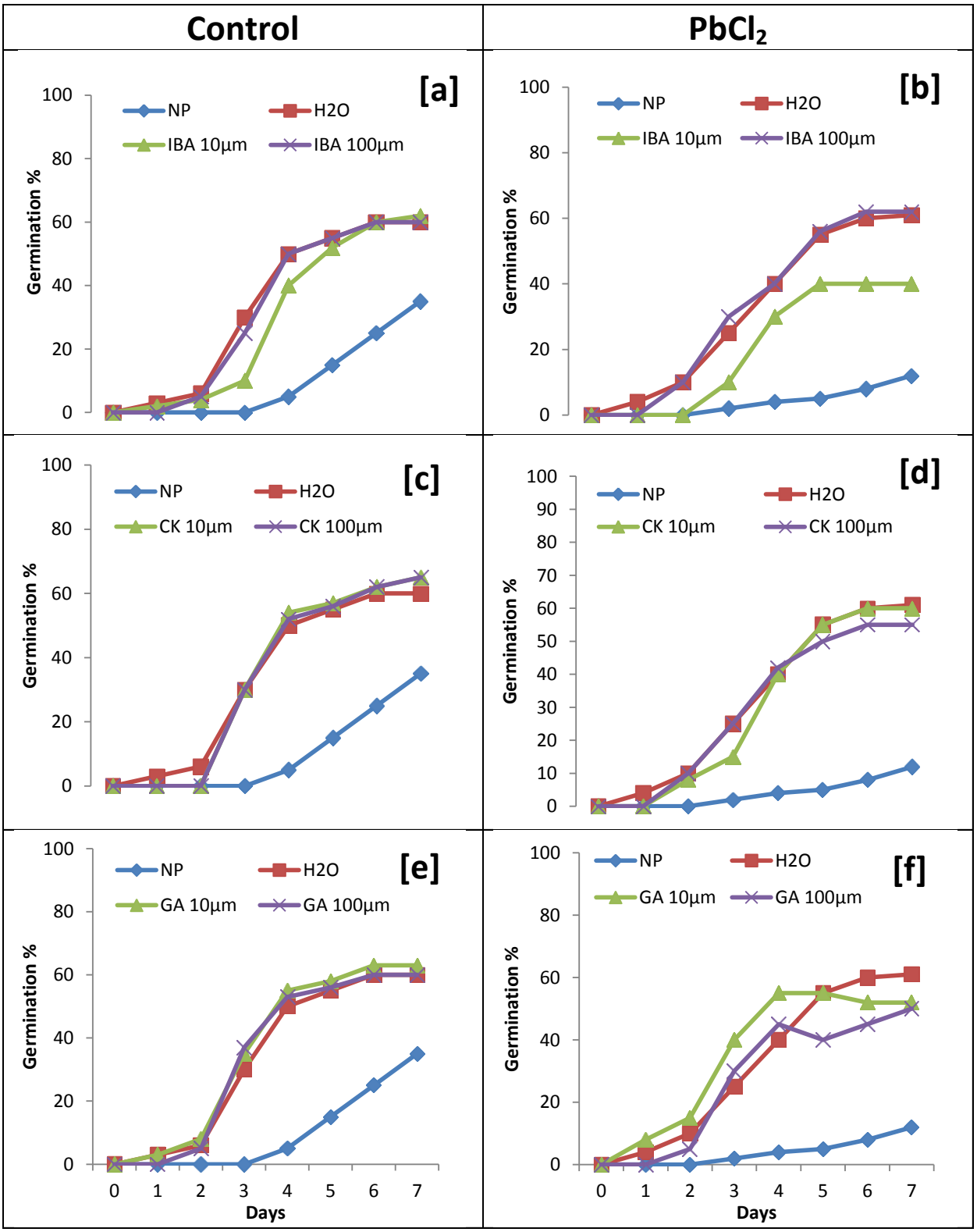

Figure 1: Daily seed germination percentage (at 24h intervals) of soybean seeds pretreated with auxin (IBA) (a & b), cytokinin (CK) (c & d) and gibberellins (GA_3) (e & f), in control conditions or under lead stress ($PbCl_2$) during 7 days.

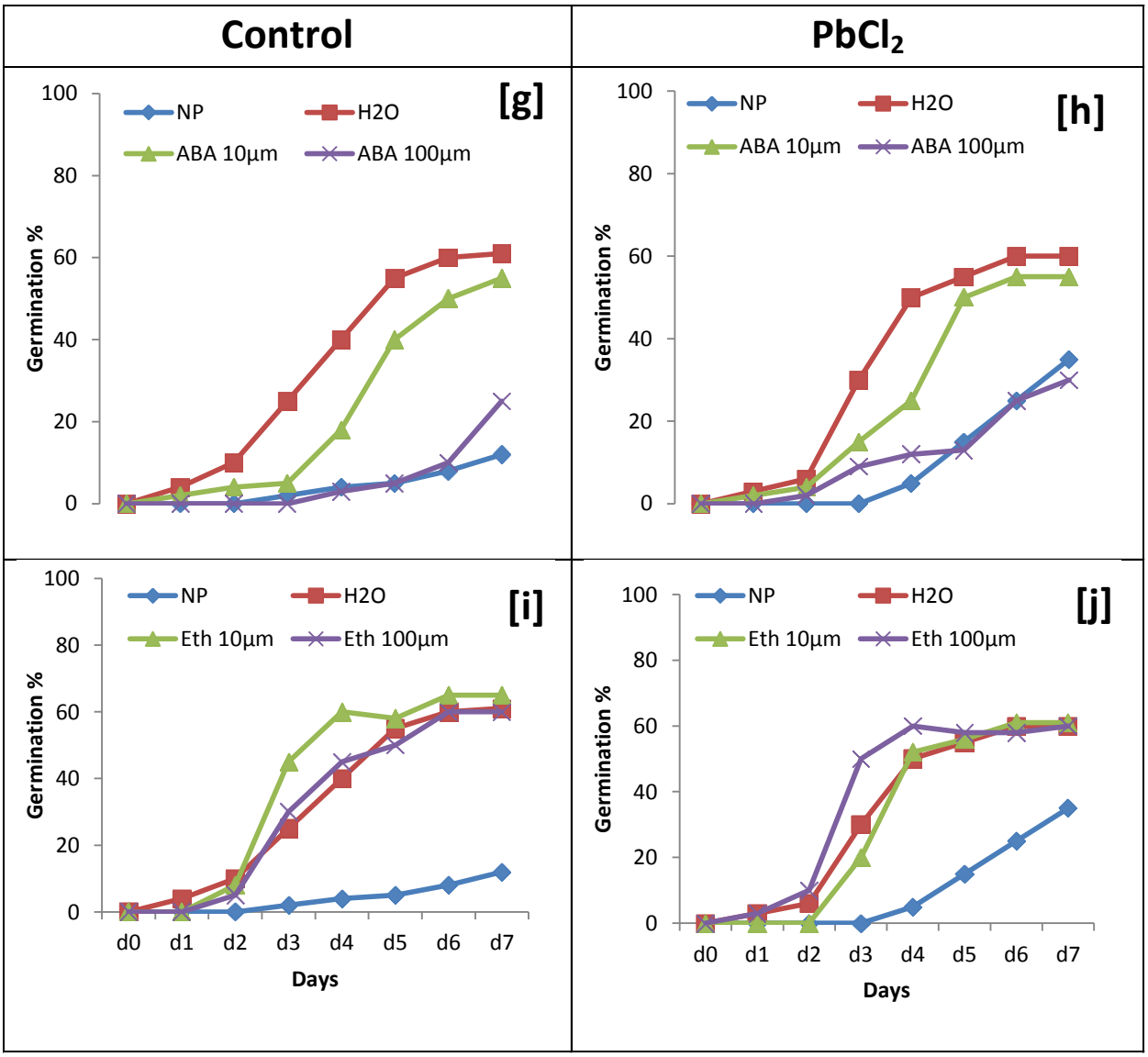

Figure 1 (continued): Daily seed germination percentage (at 24h intervals) of soybean seeds pretreated with abscisic acid (ABA) (g & h) and ethylene (Eth) (I & j) incubated in control conditions or under lead stress ($PbCl_2$) during 7 days.

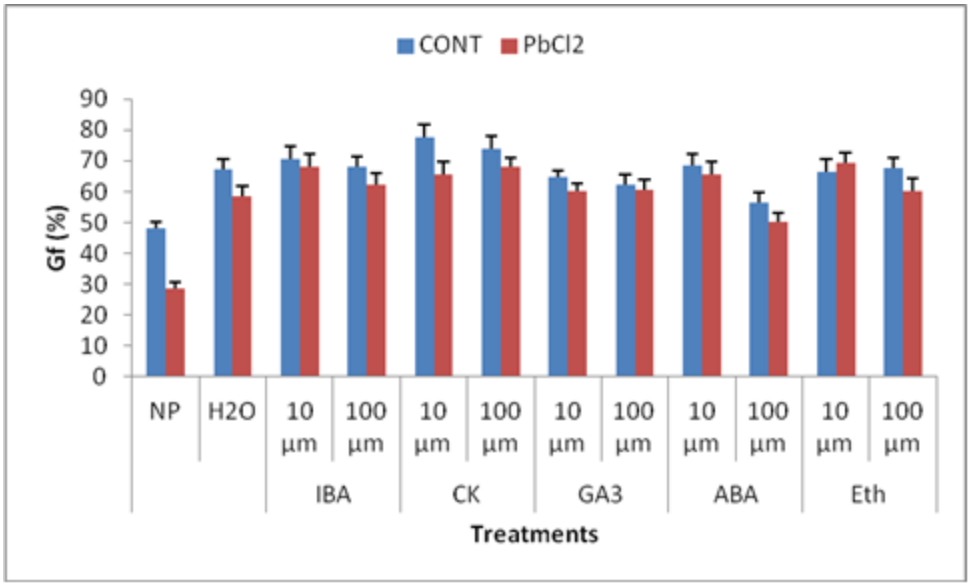

Figure 2: Final germination percentages of soybean seeds pretreated with auxin (IBA), cytokinin (CK), gibberellins (GA_3), abscisic acid (ABA) and ethylene (Eth) planted in control conditions (Cont) or under lead stress ($PbCl_2$). (vertical lines indicate SD values).

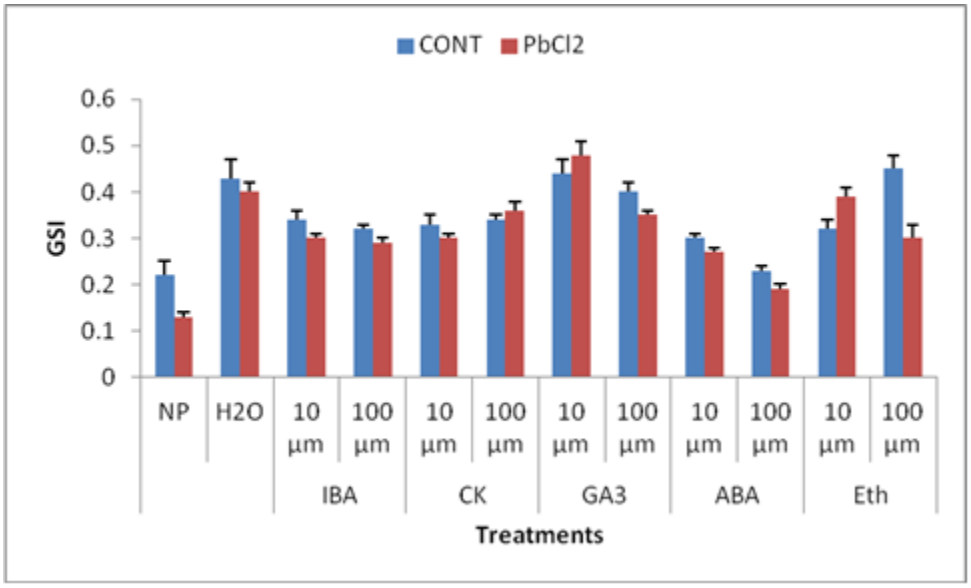

Figure 3: Germination speed index of soybean seeds pretreated with auxin (IBA), cytokinin (CK), gibberellins (GA_3), abscisic acid (ABA) and ethylene (Eth) planted in control conditions (Cont) or under lead stress ($PbCl_2$).(vertical lines indicate SD values).

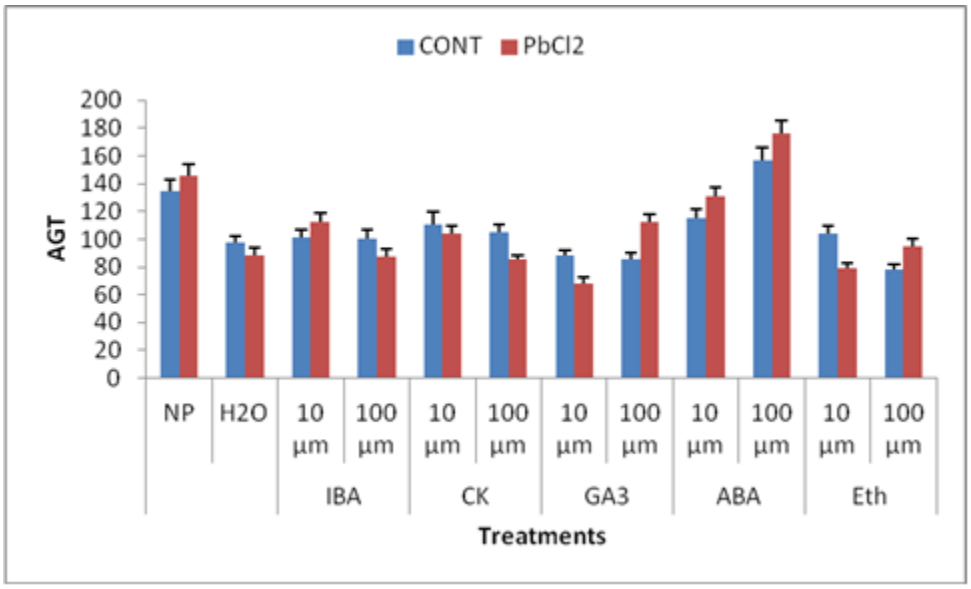

Figure 4: Average germination time of soybean seeds pretreated with auxin (IBA), cytokinin (CK), gibberellins (GA_3), abscisic acid (ABA) and ethylene (Eth) planted in control conditions (Cont) or under lead stress ($PbCl_2$). (vertical lines indicate SD values).

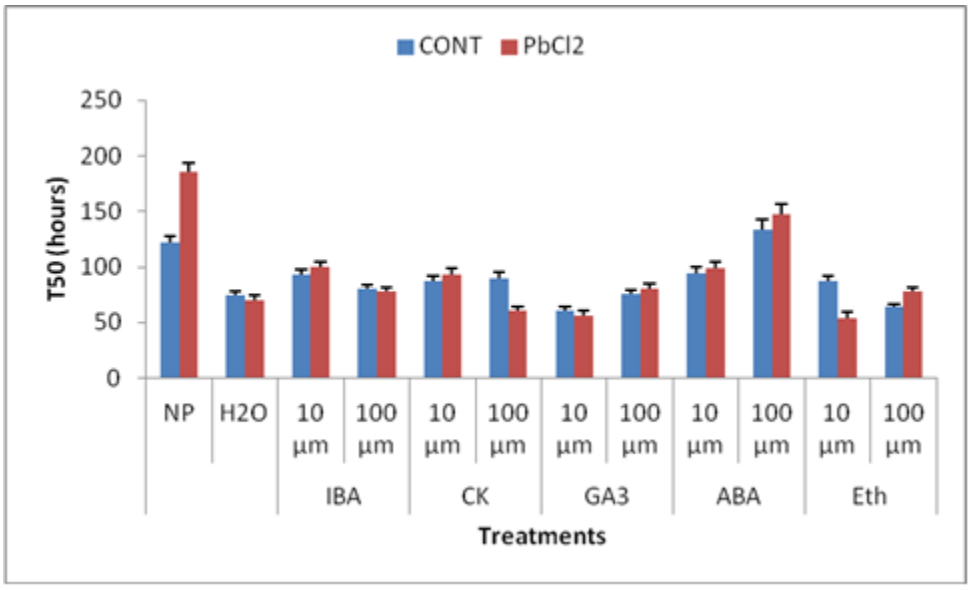

Figure 5: Time (hours) to reach 50% germination of soybean seeds pretreated with auxin (IBA), cytokinin (CK), gibberellins (GA_3), abscisic acid (ABA) and ethylene (Eth) planted in control conditions (Cont) or under lead stress ($PbCl_2$). (vertical lines indicate SD values).

2 days of germination either under control (Fig. 1c) or Pb contaminated (Fig. 1d) conditions. However, at control conditions the most increase in Gd% was recorded after 4 days of germination where T50 was reached, while under Pb stress the T50 value was recorded after 5 days of germination (Fig. 5). The results indicated also that CK priming was the most effective hormone improving final germination (Gf) of soybean seeds in the absence of Pb stress, at which Gf was about 77.5% and 73.8% at 10 µm and 100 µm of CK, respectively (Fig.2). The obtained results showed also that CK priming resulted in faster seed germination therefore GSI values were significantly higher than those of unprimed seeds (NP) as shown in (Fig. 3). As a consequence, AGT values of CK primed seeds were significantly less than those of NP treatment (Fig. 4).

Effect of gibberelline (GA_3)

Obtained results showed that GA_3 priming resulted in a considerable increase in Gd% in a very short time after sowing. In this concern, Gd% increased after nearly 30 h from germination either at control condition (Fig. 1e) of at Pb stress condition (Fig. 1f). It seemed that GA_3 priming was more effective in speeding seed germination under Pb stress than under control condition. Therefore, Gd% reached its maximum value after 6 days of germination under control condition and after only 4 days at Pb stress condition (Fig 1e&f). Moreover, Gf% values increased significantly with GA_3 priming as compared with unprimed seeds (NP), either in the presence or absence of Pb stress (Fig. 2). The stimulating effects of GA_3 priming on seed germination were comparable with high GSI values (Fig. 3) of primed seeds. In addition GA_3 priming shortened the time for seeds to germinate, therefore AGT values were significantly lower than those of unprimed seeds (Fig.4). Thus, T_{50} values at 100 µm of GA_3 were reached at about 75 h and 80 h under control and Pb tress conditions, respectively, while T_{50} values of unprimed seeds (NP) recorded about 120 h and 160 h, respectively (Fig. 5).

Effect of abscisic acid (ABA)

Interestingly, data showed that until day 5 (120 h) after induction of germination, the 100 µM ABA primed seeds showed germination rate nearly comparable to NP treated seeds (Fig. 1g and 1h). It was interesting to find that enhancing effect of ABA priming on seed germination under Pb stress condition was more pronounced than that under control condition, thus Gd% increased after about 48 h at control (Fig. 1g) and after only 24 h at Pb stress (Fig. 1h). However, the final germination percentage (Gf%) of ABA primed seeds under control condition was higher than that under Pb stress condition (Fig. 2). Comparing with AGT values obtained with other hormones, ABA priming resulted in higher values and it means that the average germination time with ABA priming was longer than that of other hormones (Fig. 4). Therefore, T_{50} values recorded for ABA primed seeds were significantly higher than those recorded for other hormones (Fig. 5). This means that the time required for ABA primed seeds to reach 50% germination is longer than that required for other hormonal primed seeds.

Effect of ethylene (Eth)

Data in Fig. 1i and 1j indicated that the percentage of daily germinated soybean seeds (Gd%) pre-treated with H_2O, or primed with 10 or 100 µm of ethylene (Eth) was higher than that of NP treatment in both control (without Pb) and Pb treated seeds, however, the Gd% values at the absence of Pb stress were much better than those recorded under Pb stress condition. In this regard, at day 7 the Eth 10 µm and 100 µm priming treatments significantly improved the Gf% by about 37.1% and 40.7%, respectively, than the NP seeds in the absence of Pb treatment (Fig. 2). At the Pb treatments, the enhancing effect of Eth priming was even better. It was interesting to find that, after 2 days germination rate increased substantially with the 10 µm of Eth priming in the presence of Pb (Fig. 1j). This finding suggests that Eth pretreatments might decrease the sensitivity of seeds toward Pb stress condition.

No significant differences were observed in GSI (Fig. 3), AGT (Fig. 4) or T_{50} (Fig. 5) values that obtained from Eth priming as compared with other phytohormonal priming on soybean seeds.

Effect of hydro-priming (H_2O)

Although, phytohormonal seed-priming produced relatively higher values of Gf% than those recorded for NP treatment, these values did not significantly different from the values obtained in case of hydro-priming treated-seeds. This findings indicates that H_2O itself may act as a germination stimulating primer and in the absence of plant hormones it might lead to the improvement of seed germination as indicated in Fig. 1 and Fig. 2. Results of the present study indicated that hydro- priming, with no hormones, could improve significantly the germination of soybean seeds (Fig. 3).

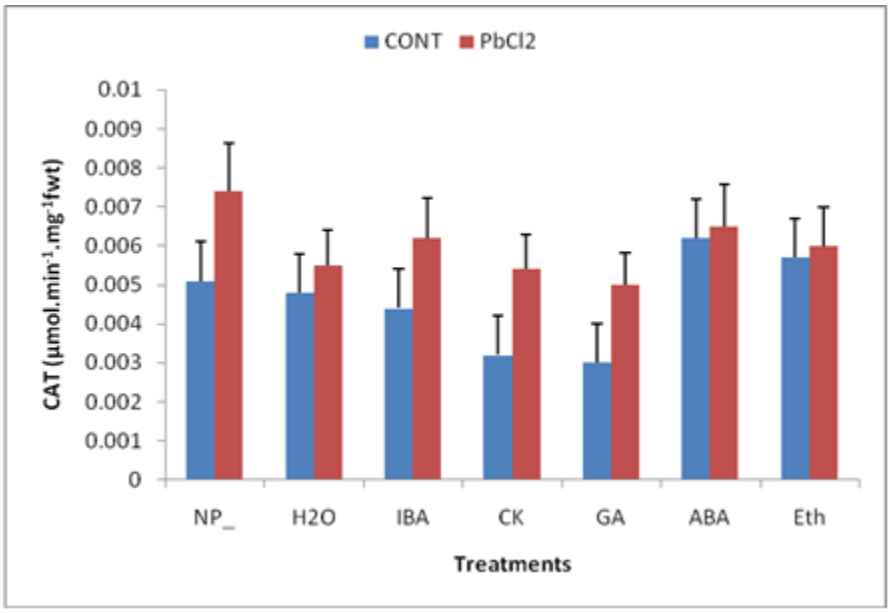

Figure 6: Catalase (CAT) activity of soybean seeds pretreated with auxin (IBA), cytokinin (CK), gibberellins (GA_3), abscisic acid (ABA) and ethylene (Eth) incubated in control conditions (CONT) or under lead stress ($PbCl_2$). (vertical lines indicate SD values).

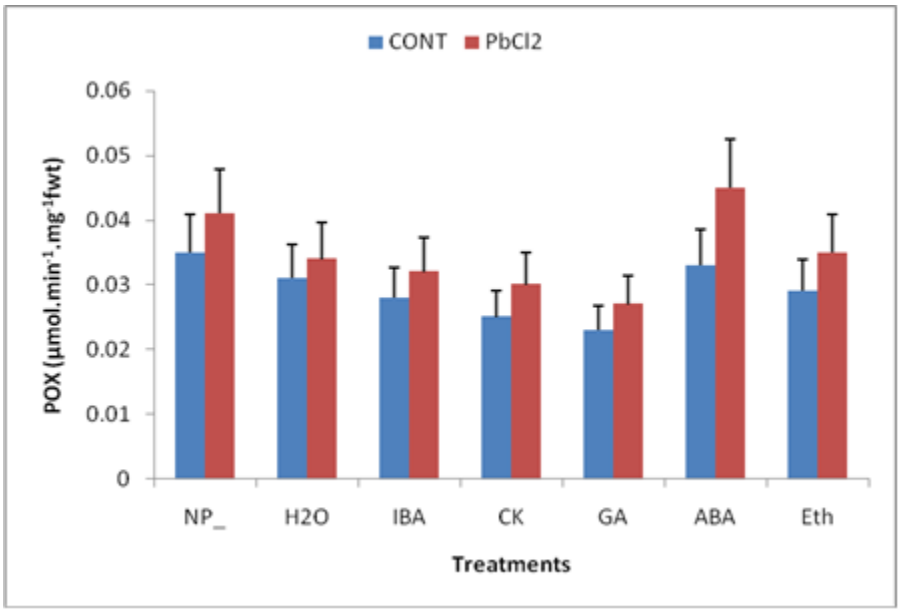

Figure 7: Peroxidase (POX) activity of soybean seeds pretreated with auxin (IBA), cytokinin (CK), gibberellins (GA_3), abscisic acid (ABA) and ethylene (Eth) incubated in control conditions (CONT) or under lead stress ($PbCl_2$). (vertical lines indicate SD values).

This treatment also enhanced the rate and efficiency of germination under Pb stress condition; therefore it can be used as a good alternative efficient to improve seed soybean germination in Pb contaminated areas.

Activity of antioxidant enzymes

Catalase activity

Data recorded in Fig (6) indicated clearly that catalase activity increased under $PbCl_2$ stress condition as compared to Pb non-stressed condition (control). The highest values of CAT activity were obtained at NP treatment and ABA priming treatment. However, NP was more pronounced in increasing CAT activity than ABA priming. The CAT activity increased with NP by about 45% as compared with CONT treatment. It was obvious also that ABA and Eth enhanced theactivity of CAT as compared with other hormones, either at $PbCl_2$ treatments or CONT treatment, while CK and GA_3 showed least values of enzyme activities, particularly at CONT treatment. Generally, the effect of seed priming was more pronounced at $PbCl_2$ treatments than at CONT treatments.

Peroxidase activity

Peroxidase activity was found to increase significantly under $PbCl_2$ stress condition (Fig. 7). The NP treatment tended to stimulate the activity op POX more than H_2O treatment. The highest values of POX activities were obtained at NP and ABA priming under $PbCl_2$ stress. But ABA in this case was more pronounced than NP, since the former caused an increase in POX activity of about 29%, while NP caused an increase of about 15%, as compared with CONT treatments. Data indicated also that, among hormones the ABA and Eth were the most pronounced priming treatments causing significant increases in POX activity, while CK and GA_3 priming showed lower activities.

DISCUSSION

Results of this study showed clearly that plant hormones are effective in stimulating soybean seed germination grown in Pb polluted soils. And using the phytohormonal primed seeds, one can overcome the deleterious effect of heavy metal stress on seed germination. The present study showed that IBA, CK, GA_3, ABA and Eth were effective to enhance soybean seed germination in the presence of Pb heavy metal.

It is well known that IBA is a plant hormone plays a regulatory role in many vital processes within plants such as growth, development and vascular and pollen formation. Its function on growth, including embryo development is controlled by its transport which is regulated by transcriptional factors (Hayashi 2012). Another important function of the IBA is its role in cell elongation in the growing embryo (Hauvermale et al. 2012). Auxin by itself is not a necessary hormone for seed germination; however, according to the analyses regarding the expression of auxin related genes, auxin is present in the seed radicle tip during and after seed germination. Although, Hentrich et al. (2013) stated that auxin might not be necessary for seed germination, in our opinion it is necessary for the growth of young embryo and seedlings. And, the accumulated auxin in the seed cotyledon is the major source of that for the seedling growth. In this regard, Miransari and Smith (2014) found that amide products were the major source of auxin in mature legume seeds.

Cytokinins are plant hormones having the ability to regulate a range of cell activities including cell division and seed germination. They were found to be active in all stages of germination and they can also enhance the activity of meristemic cells in epicotyls and hypocotyls (Heyl et al. 2012). It was found also that Cks are able to stimulate seed germination by alleviating the stresses found in germination media such as drought, salinity and heavy metals as well as oxidative stress (Peleg and Blumwald 2011; Miransari and Smith 2014).

Gibberellins are diterpenoid, regulating seed germination and plant growth through its antagonistic effects with ABA. In the present study GA_3 priming was found to enhance seed germination, may be through its effect on stored food within seeds and makes it available for embryos during germination processes. The endosperm within seeds becomes available to the embryo via the activities of some hydrolase enzymes. It is well known that GA_3 stimulates the synthesis and production of the hydrolases, especially α-amylase, resulting in the germination of seeds. This conclusion is almost in line with that reported by Voegele et al. (2011). In this regard, Yamaguchi (2008) found that gibberellins were able to induce a range of enzymes necessary for seed germination including amylase, protease and glucanase. Moreover, seed germination is often controlled through suppression effects of excess ABA on the expansion of embryo organs caused by inhibition

of GA effects on the growth of radical and hypocotyle (Voegele et al. 2011).

The low values of seed germination rates that were recorded at high ABA concentration (100 µM) treatment, even in the absence of Pb stress, were probably due to the known inhibitory effect of ABA on the activity and /or the synthesis of some enzymes involved in the degradation of endosperm cells such as α-amylase within seeds, which considered an important process for seed germination. This interpretation is in line with those reported by Staroske et al. (2016) and Sneideris et al. (2015) who found that ABA inhibited the activity of many enzymes involved in seed germination of grains and pigeon pea, respectively.

While ABA positively affects seed dormancy and plant activities under biotic and abiotic stresses (Popko et al. 2010), it negatively affects seed germination process. High ABA concentrations can inhibit seed germination in many species (Chiu et al. 2016). While, the negative interaction of other hormones such as GAs, Eth and CKs with ABA, can positively regulate seed germination process (Kucera et al. 2005; Hermann et al. 2007). It was found that the inhibition of seed germination at high levels of ABA was through inhibition of the radicle expansion and suppression of some transcriptional factors, which can negatively affect the process of seed germination (Graeber et al. 2010).

It seems that, seed responses to Eth priming are complex phenomena. In this regard, it was documented, in previous studies, that seed priming with ethylene precursor ACC (1-aminocyclopropane-1-carboxylic acid) increased the rate of germination in lettuce seeds (Nascimento et al. 2004), but it didn't affect significantly the rate of germination in ryegrass (Tiryaki et al. 2004). Concerning roles of Eth as seed priming, it has been found that ethylene and gibberellins affect embryo radicle growth, with gibberellins being the most important phytohormone in germination processes. Although gibberellins are essential in seed germination for the production of mannanase, that is necessary for seed germination, Eth is not (Wang et al. 2005). However, under abiotic stress conditions and in GA_3 deficient mutants, ethylene can play same role and act similar to GAs, therefore Eth priming makes seeds able to germinate completely at such conditions (Matilla and Matilla-Vazquez 2008). In spite of its effect on many plant activities including seed germination, tissue growth and development, how ethylene influences seed germination is not yet understood. According to earlier studies, ethylene was reported to be necessary for the process of seed germination (Baskin et al. 2003). And it can regulate plant responses under a different stress conditions (Keunen et al. 2016). It was found that ethylene concentration increases during seed germination process of many species including wheat, corn, soybean and rice; the rate of seed germination was also affected (Zapata et al. 2004). The amicocyclopropane-1-carboxillic-acid (ACC), the precursor of Eth, can enhance seed radicle emergence through the production of ethylene which produced in the radicle (De Poel and der Straeten 2014). It has been found that during seed germination ethylene is produced in different plant species and it can contribute to the germination of seeds after dormancy. Ethylene level was found to increase under heavy metal stress conditions and it can control many processes in plants, including seed germination (Keunen et al. 2016).

Generally seed priming could stimulate soybean seed germination as shown in this study. During dehydration, lipid peroxidation may be inhibited mainly via production of antioxidants and repair enzymes. In this connection, McDonald (2000) stated that membrane repair by phytohormones is often occurring. This study showed that phytohormonal priming could stimulate soybean seed germination. An explanation for decreased germination time (AGT) by hormonal priming (Fig. 4) is that during priming treatments seed dormancy may be broken and the bio-chemical processes within seeds may begin, which might lead to earlier germination and faster emergence (Farooq et al. 2006b). Seed priming could ensure proper seed hydration resulted in enhanced activity of some enzymes such as α-amylase and protease that hydrolyzed starch and protein molecules into simple forms available for the embryo to germinate. This availability to the germinating seed gave a energetic start that is indicated by lower T_{50} and AGT in treated seeds (Farooq et al. 2006c). Early emergence, as indicated by lower T_{50} and AGT, in some primed seeds may be due to the faster synthesis and production of DNA, RNA, proteins and other metabolites (Graeber et al. 2010).

Under stress conditions, it was found that ROS that produced during germination often interact with the phytohormone priming. This

interaction between ROS and phytohormones could be antagonistic or synergistic (Golldack et al. 2013). An early investigation showed that germination of pigeon pea seeds was substantially improved when primed with phytohormoes under both control and cadmium stress conditions (Sneideris et al. 2015). Therefore, the role of phytohormonal priming during seed germination is still a subject of further research.

It is well known that heavy metals stimulate the production of ROS leading to an oxidative stress causing a physiological damage to plant tissues. In addition, heavy metal induced imbalance between the production of ROS and their scavenging process through the antioxidative defense mechanism. In this regard Heidari and Sarani (2011) showed that this antioxidative defense mechanism offers an effective system to detoxify and scavenging the toxic ROS through the antioxidative enzymes such as peroxidase, catalase and others. Such enzymes often convert the H_2O_2 produced under Pb heavy metal stress to water and oxygen useful to the embryo, theyb also prevent lipid peroxidation under such conditions (Keser and Saygideger 2010). Recorded data showed that activities of the catalase and peroxidase antioxidant enzymes were increased under $PbCl_2$ stress condition. These findings were similar to those indicated by Malar at al. (2014) who found that heavy metal stress including Pb effect caused an increase in the activity of some antioxidant enzymes in *Eichhornia,* in which peroxidase activity increased by about 63% under Pb as compared with control plants.

The present study showed that the activities of catalase and peroxidase increased significantly with the phytohormonal seed priming, particularly under Pb stress conditions, as compared with those recorded under control conditions. In this regard, early studies indicated that seed priming resulted in higher activity of antioxidant enzymes in okra (Sharma et al. 2014) and rice (Jisha and .Puthur 2016). Moreover, seed priming was found to alleviate the deleterious effects of seed aging and abiotic stress (Wojtyla et al. 2016). In this regard, Chmielowska et al. (2015) found that germination percentage of cotton seeds improved when primed with water and ascorbic acid and this was associated with lowering lipid peroxidation and activating antioxidant enzyme activities, especially catalase, peroxidase and glutathione reductase. Similarly, Kibinza et al. (2011) showed that priming of sunflower seeds improves germination percentages due to the significant drop in H_2O_2 accumulation and to the restoration of catalase activity within seeds. The authors concluded that catalase enzyme protects against damages caused by ROS activities in stressed seeds exposed to priming treatments.

Besides the effects of plant hormones on seed germination, studies have indicated that under both stress and non-stress conditions, phytohormonal priming can enhance seed germination through enhancing some enzymes such as amylase activities (Miransari and Smith 2014). Hormonal priming was found also to reduce the oxygen reactive species (ROS) produced under $PbCl_2$ stress thus, it can alleviate the stress by controlling the oxidative damage, similar to the effects of antioxidant enzymes such as catalse (CAT), peroxidase (POX) and superoxide dismutase (SOD), on seed germination and embryo growth under stress conditions (Sajedi et al. 2011).

CONCLUSIONS

Seed germination is important process affecting soybean production. This process is affected by heavy metals stress such as Pb stress, and is also influenced by phytohormones. The phytohormones, including auxin, cytokinins, gibberellins ABA and ethylene, can positively or adversely affect soybean seed germination, while interacting with Pb. It was clear from the results that priming improves seed germination and seedling growth of soybean. Even hydro-priming is an easy and useful technique for enhancing germination rate and percentage of soybean. The effects of hydro and hormonal priming can improve seedling establishment and field performance of soybean as an important food legume. Hormonal priming using AUX, CK and GA_3 were the most appropriate priming treatments for soybean (*Glycine max*) seeds grown under Pb stress conditions.

REFERENCES

Abdel Latef AA. and Tran LP, 2016, Impacts of priming with silicon on the growth and tolerance of maize plants to alkaline stress. *Front Plant Sci.,* *7*, 243. doi: 10.3389/fpls.2016.00243.

Abdul-Qados AS, 2015, Mechanism of nanosilicon-mediated alleviation of salinity stress in faba bean (*Vicia faba L.*) Plants. *American Journal of Experimental Agriculture*. *7*(2), 78-95.

Ansari O, Azadi MS, Sharif-Zadeh F and Younesi E, 2013, Effect of hormone priming on germination characteristics and enzyme activity of mountain rye (*Secale montanum*) seeds under drought stress conditions. *J Stress Physiol Biochem 9*, 61-71.

Arya SK and Roy BK, 2011, Manganese induced changes in growth, chlorophyll content and antioxidants activity in seedlings of broad bean (Vicia faba L.). *J Environ Biol 32*, 707–711.

Baskin CC, Baskin JM, Chester EW and Smith M, 2003, Ethylene as a possible cue for seed germination of *Schoenoplectus hallii* (Cyperaceae), a rare summer annual of occasionally flooded sites. *Am. J. Bot., 90*(4), 620-627

Chiu RS, Saleh Y and Gazzarrini S, 2016, Inhibition of FUSCA3 Degradation at High Temperature Is Dependent on ABA Signaling and Is Regulated by the ABA/GA Ratio*. Plant Signal Behav., 11* (11), e1247137.11.

Chmielowska BJ, Izbia´nska K and Deckert J, 2015, Products of lipid, protein and mRNA oxidation as signals and regulators of gene expression. *Front.PlantSci., 6*, 405. doi:10.3389/fpls.2015.00405

De Poel VB and der Straeten VD, 2014, 1-aminocyclopropane-1-carboxylic acid (ACC) in plants: more than just the precursor of ethylene. *Front Plant Sci., 5*, 640. doi: 10.3389/fpls.2014.00640.

Farooq M, Basra SMA and Tabassum R, 2006a, Enhancing the performance of direct seeded fine rice by seed priming. *Plant Prod. Sci*., *4*, 446-456.

Farooq M, Basra SMA, Ahmad N and Hafeez K, 2005b, Thermal hardening: A new seed vigor enhancement tool in rice. *J Integr. Plant Bio.I*, *47*, 187-193.

Farooq M, Basra SMA, Tabassum R and Ahmed N, 2006c, Evaluation of seed vigour enhancement techniques on physiological and biochemical basis in coarse rice (*Oryza sativa* L.). *Seed Sci. Technol*., *34*, 741-750.

Galmés J, Medrano H and Flexas J, 2006, Germination capacity and temperature dependence in Mediterranean species of the Balearic Islands. Invest Agr Sist Rec For 15: 88-95.

Golldack D, Li C, Mohan H, and Probst N, 2013, Gibberellins and abscisic acid signal cross talk: living and developing under unfavorable conditions. Plant Cell Rep 32: 1007–1016. Doi:10.1007/s00299-013-1409-2.

Graeber K, Linkies A, Muller K, Wunchova A, Rott A and Leubner-Metzger G, 2010, Cross-species approaches to seed dormancy and germination: conservation andbiodiversity of ABA-regulated mechanisms and the Brassicaceae DOG1 genes. Plant Mol Biol 73: 67–87.

Hadi F and Aziz T, 2015, A Mini Review on Lead (Pb) Toxicity in Plants. Journal of Biology and Life Science 6(2): 91-101.

Hauvermale AL, Ariizumi T and Steber CM, 2012, Gibberellin signaling: a theme and variations on della repression. Plant Physiol 160: 83–92.

Hayashi K, 2012, The Interaction and integration of auxin signaling components. Plant Cell Physiol 53: 965–975.

Heidari M and Sarani S, 2011, Effects of lead and cadmium on seed germination, seedling growth and antioxidant enzymes activities of mustard (*Sinapis arvensis* L.). ARPN Journal of Agricultural and Biological Science 6: 44-47.

Hentrich M, Boettcher C and Duchting P, 2013, The jasmonic acid signaling pathway is linked to auxin homeostasis through the modulation of YUCCA8 and YUCCA9 gene expression. Plant J 74: 626–637.

Hermann K, Meinhard J, Dobrev P, Linkies A, Pesek B, Heß B, Machackova I, Fischer U and Leubner-Metzger G, 2007, 1-minocyclopropane-1-carboxylic acid and abscisic acid during the germination of sugar beet (*Beta vulgaris* L.) – A comparative study of fruits and seeds. J Exp Bot 58: 3047–3060.

Heyl A, Riefler M, Romanov G and Schmulling T, 2012, Properties, functions and evolution of cytokinin receptors. Europ J Cell Biol 91: 246–256.

Hu YF, Zhou G, Na XF, Yang L, Nan WB, Zhang YO, Li JL and Bi YR, 2013, Cadmium interferes with maintenance of auxin homeostasis in Arabidopsis seedlings. J Plant Physiol 170: 965-975.

Hussain S, Khan F, Cao W, Wu L and Geng M, 2016a, Seed Priming Alters the Production and Detoxification of Reactive Oxygen Intermediates in Rice Seedlings Grown under Sub-optimal Temperature and Nutrient Supply. Frontiers in Plant Science 7: 439. doi:10.3389/ fpls.2016.00439.

Hussain S, Khan F, Hussain HA and Nie L, 2016b, Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. Front Plant Sci 7: 116. doi: 10.3389/fpls.2016.00116.

Jisha KC and Puthur JT, 2016**,** Seed priming with beta-amino butyric acid improves abiotic stress tolerance in rice seedlings. Rice Science 23(5): 242–254**.**

Jisha KC, Vijayakumari K and Puthur JT, 2013, Seed priming for abiotic stress tolerance: an overview. Acta Physiol Plant I: 1381-1396.

Keser G and Saygideger S, 2010, Effects of lead on the activities of antioxidant enzymes in watercress, Nasturtium officinale R. Br. Biol Trace Elem Res 137: 235–243.

Keunen E, Schellingen K, Vangronsveld J and Cuypers A, 2016, Ethylene and Metal Stress: Small Molecule, Big Impact. Front Plant Sci., 7: 23. doi:10.3389/fpls. 2016.00023.

Khan HA, Ayub CM, Pervez MA, Bilal RM, Shahid MA and Ziaf K, 2009, Effect of seed priming with NaCl on salinity tolerance of hot pepper (*Capsicum annuum* L.) at seedling stage. Soil Environ 28: 81-87.

Kibinza S, Bazin J, Bailly C, Farrant JM, Corbineau F, and El-Maarouf- Bouteau H, 2011, Catalase is a key enzyme in seed recovery from ageing during priming. Plant Sci., 181: 309–315.doi:10.1016/j.plantsci.2011. 06.003

Kucera B, Cohn MA and Leubner-Metzger G, 2005, Plant hormone interactions duringseed dormancy release and germination. Seed Sci Res 15: 281–307.

Malar S, Vikram SS, Favas PJC and Perumal V, 2014, Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths (*Eichhornia crassipes* (Mart.). Botanical Studies 55: 54-68.

Matilla AJ and Matilla-Vazquez MA, 2008, Involvement of ethylene in seed physiology. Plant Sci 175: 87–97.

McDonald MB, 2000, Seed priming. In: Seed Technology and Its Biological Basis (Eds. M. Black and J.D. Bewley), Sheffield Acad. Press, Sheffield, England, pp: 287-326.

Miransari M and Smith DL, 2014, Plant hormones and seed germination. Environmental and Experimental Botany 99: 110–121.

Nascimento WM, 2004, Condicionamento osmótico de sementes de hortaliças: potencialidades e implicações. Hortic Bras 16: 106-109.

Nawaz J, Hussain M, Jabbar A, Nadeem A, Sajid M, Subtain M and Shabbir I, 2013, Seed priming a technique. Intl J Agri Crop Sci 6(20): 1373-1381.

Noctor G and Foyer CH, 1998, Ascorbate and glutathione: keeping active oxygen under control. Annual Review in Plant Physiology and Molecular Biology 49: 249–279.

Oves M, Saghir Khan M, Huda Qari A, Nadeen Felemban M and Almeelbi T, 2016, Heavy Metals: Biological Importance and Detoxification Strategies*.* J Bioremed Biodeg 7: 334. doi: 10.4172/2155-6199.1000334.

Peleg Z and Blumwald E, 2011, Hormone balance and abiotic stress tolerance in crop plants. Curr Opin Plant Biol 14: 290–295.

Popko J, Hänsch R, Mendel R, Polle A and Teichmann T, 2010, The role of abscisic acid and auxin in the response of poplar to abiotic stress. Plant Biol 12: 242–258.

Rahimi R, Mohammakhani A, Roohi V and Armand N, 2012, Effects of salt stress and silicon nutrition on cholorophyll content, yield and yield components in fennel (*Foeniculum vulgar* Mill.). Int J Agri Crop Sci 4 (21): 1591-1595.

Rakesh Sharma MS, Raju NS, 2013, Correlation of heavy metal contamination with soil properties of industrial areas of Mysore, Karnataka, India by Cluster analysis. Int Res J Environ Sci 2(10): 22–27.

Sajedi N, Ardakani M, Madani H, Naderi A and Miransari M, 2011, The effects of selenium and other micronutrients on the antioxidant activities and yield of corn (*Zea mays* L.) under drought stress. Physiol Mol Biol Plants 17: 215–222.

Sharma AD, Rathore SVS, Srinivasana K and Tyagia RK, 2014, Comparison of various seed priming methods for seed germination, seedling vigour and fruit yield in okra (*Abelmoschus esculentus* L. Moench). Scient

Hort 165: 75–81.
Silva S, Pinto G and Santos C, 2016, Low doses of Pb affected *Lactuca sativa* photosynthetic performance. Photosynthetica 2016, doi:10.1007/s.
Snedecor GW and Cochran GW, 1980, Statistical Methods, 7th edition. The Iowa State University Press, Ames, Iowa.
Sneideris LC, Gavassi MA, Campos ML, Damião VA and Carvalho RF, 2015, Effects of hormonal priming on seed germination of pigeon pea under cadmium stress. Anais da Academia Brasileira de Ciências, 87(3), http://dx.doi.org/ 10.1590/0001-3765201520140332.
Staroske N, Conrad U, Kumlehn J, Hensel G, Radchuk R, Erban A, Kopka J, Weschke W and Weber S, 2016, Increasing abscisic acid levels by immunomodulation in barley grains induces precocious maturation without changing grain composition. Journal of Experimental Botany 7 doi:10.1093/ jxb/erw102.
Tiryaki I, Korkmaz A, Ozbay N and Nas MN, 2004, Priming in the presence in the plant growth regulators hastens germination and seedling emergence of dormant annual ryegrass (*Lilium multiflorum* Lam.) seeds. Asian J Plant Sci 3: 655-659.
Urbanek H, Kuzniak-Gebarowska E and Herka K, 1991, Elicitation of defense responses in bean leaves by Botrytis cinerea polygalacturonase. Acta Phys Plant 13: 43-50.
Voegele A, Linkies A, Muller K and Leubner-Metzger G, 2011, Members of the gibberellin receptor gene family GID1 (GIBBERELLIN INSENSITIVE DWARF1) play distinct roles during *Lepidium sativum* and *Arabidopsis thaliana* seed germination. J Exp Bot 155: 1851–1870.
Wang AX, Wang XF, Ren YF, Gong XM and Bewley JD, 2005, Endo-bmannanaseand b-mannosidase activities in rice grains during and following germina-tion, and the influence of gibberellin and abscisic acid. Seed Sci Res 15: 219–227.
Wojtyla Ł, Lechowska K, Kubala S and Garnczarska M, 2016, Different modes of hydrogen peroxide action during seed germination. Front Plant Sci 7: 66. doi: 10.3389/fpls.2016.00066.
Zapata PJ, Serrano M, Pretel MT, Amorós A and Botella MA, 2004, Polyamines and ethylene changes during germination of different plant species under salinity. Plant Sci 167: 781–788.