



The effect of foliar application of microelements on phenological and physiological characteristics of Mung bean under drought stress

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Abstract

In order to investigate on the effect of foliar application of microelements on phenological and physiological characteristics of Mung bean under drought stress, factorial experiment based on completely randomized design with three replications was conducted in the Agriculture Faculty greenhouse of Islamic Azad University of Varamin-Pishva branch in 2013-2014. The first factor was drought stress at four levels include a control (without stress), applying of drought stress at flowering stage, pod and dough stages and the second factor was foliar application of microelements at three levels include a control (spraying of distilled water), 3 and 6 pm. The results showed that the drought stress decreases plant height, leaf area index, thousand grain weight, chlorophyll a, chlorophyll b and total chlorophyll and increases grain protein percentage and pro line accumulation. The effect of drought stress at flowering stage was higher than other stages. Also, the foliar application of microelements was increased the plant height, leaf area index, thousand grain weight, chlorophyll a, chlorophyll b and total chlorophyll, grain protein percentage and pro line accumulation in with and without stress conditions. Therefore, the most effective concentration of microelements was 6 pm.

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Introduction Mung bean (*Vigna Radiata* L. Wilczek) is a granulegume with short growing season and cultivated in dry land fields of central and south-eastern of Asia (De costa *et al.*, 1999). Mung bean is one of the valuable legumes, because it has high quality protein. Mung bean has an important role in the nutrition of low-income people in developing countries. This plant is using for enriching and fertilizing of soil through biological fixation of nitrogen, for prevent of soil erosion as a covering plant and for green fodder. Cultivating of this plant requires little inputs and has a short maturation period, therefore this plant is recommended in multi-product systems (Dodwad *et al.*, 1998). Plants are encountered with different stresses such as drought stress, excessive rain fall, temperature changes and lack of nutrient elements. The lack of water is the most important stress in the world which is limited plants production (Umar, 2006; Reddy *et al.*, 2004). Drought stress occurs when the moisture around the roots is reducing and plant cannot absorb the water from soil. On the other hands, the drought stress occurs when transpiration is more than water absorption (Benjamin, 2007). The drought stress cause to reduce dry matter and yield of plants, because decreases plant height, leaf area (Sreevalliet *al.*, 2001). Water stress had negative effect on many plant processes such as photo synthesis, evaporation, precursor accumulation (Ohashiet *al.*, 2006) and cause to substantial reduction in plant production (Reddy *et al.*, 2004). Therefore, one strategy for increasing water use efficiency and yield is irrigation at specific developmental stage and/or at all stress condition period. Studying on the effect of drought stress on biomass and harvest index of three Mung bean cultivars have been showed that the Mung bean plant height decreases under drought stress conditions (Sadeghipour, 2009). The growth stages of soybean affected by applying the water stress and increasing its height, but the effect of drought stress at the early flowering stage is more than seed formation, grain filling and the beginning of grain maturation stages (Kumudini *et al.*, 2002). The maintaining of leaf chlorophyll and durability of photosynthesis in drought stress is one of the

physiological tolerances to the stress (Pessaraki, 1993). Also, the positive effects of nutrient increasing of plants resistance to water deficit stress have been reported by researchers (Ruan *et al.*, 1997). One of the effects of drought stress is disturbance of nutritional in plant, the completion of the micro elements uses by foliar application of them can be improve the plants growth under stress conditions (Lewis and Farlane, 1986). Microelements are essential and important elements for plant growth and development that uses at lower values than macro nutrients such as nitrogen, phosphorus and potassium. These elements include iron, zinc, copper, molybdenum, boron manganese and chlorine (Hargert, 1999). The experiments showed that the planting of seeds were obtained from foliar application of microelement have more and stronger growth. Researchers have suggested that the effect of microelement fertilizer treatments affected seed number and number of pods in per plant and increases soybean seed yield (Gasemiyan, 2001). It was also reported that the application of microelements increases the yield and yield components of corn (Khalili Mahaleh *et al.*, 2004).

Iran is located in arid and semi-arid regions of the world and the water deficiency in these areas and possibility of drought stress in all stages of plants growth is more than other regions. Cultivation of plants with short growth period such as Mung bean can be useful in water management in these areas. According to the importance of microelements on increasing of qualitative and quantitative yield of plants and evaluate of the effect of it on water deficiency, this research is designed to investigation the effect of the foliar application of microelements and drought stress on Mung bean growth.

Materials and methods

Plant material, planting conditions and treatments

This experiment was performed as a factorial experiment based on completely randomized design with three replications in Agriculture Faculty greenhouse of Islamic Azad University of Varamin–Pishva branch in 2013-2014. The experiment site was

located in 7 km distance from the Varamin city at 35° and 19' of the north longitudinal and 51° and 39' of the east latitude with 1000 m height above the sea. The treatments were included drought stress at four levels include control (without drought stress), the applying of drought stress at flowering stage, pod and dough stages and the foliar application of microelements at three concentrations in clued control (foliar application of distilled water), 3 and 6 ppm. The Part of cultivar of Mung bean have been used with 96% of viability, 99% of Purity and 40-45 gr of thousand grain weight and obtained Seed Providing and Plant Improvement Institute, Karaj, Iran.

For planting of Mung bean in green house, 36 plastic pots with 30 cm height and 25 cm diameter were prepared and after washing, disinfected with 2% of sodium hypochlorite. Then 7 kg of soil with a ratio of $\frac{2}{3}$ field soil and $\frac{1}{3}$ blown sand sterilized for 25 minutes at 121° C and soil characteristics were measured and poured into each pot. The soil characteristics shown in Table 1.

In each pot 10 Mung bean seeds were planted in 4 cm depth at 1. After seed germination and seedling establishment (after two weeks), the seedling were thinned and reduced to 5 seedlings in each pot. The drought stress was applied according to the growth stage at flowering stage on 21th July for 10 days, pod

filling stage on 28th July for 10 days and dough stage on 7th August for 10 days. The foliar application of micronutrients at 0, 3 and 6 ppm were used at flowering stage by nutrient solutions MICROMAX was produced with OMEX England.

Chlorophyll, protein and proline analysis

After maturation, the plants were separated from pots and plant height measured by ruler. The leaf area index was measured by Eijelkamp-ci202 at 2 plants in each pot. The chlorophyll, protein and pro line content of leaf were measured by Arnon (1949), Bradford (1976) and Bates *et al.* (1973) methods respectively.

Statistical analysis

The data analysis were performed using SAS Statistics software and mean comparison were carried out by using Duncan's multiple range test at probability levels of 0.05.

Results

Plant height

According to the variance analysis results (Table 2), drought stress and foliar application of microelements had significant effect ($P < 0.01$) on plant height, but the interaction of drought stress with foliar application of microelements was not significant.

Table 1. Physical and chemical characteristics of the used soil in the pots for Mung bean. Cultivation.

Tissue	pH	EC (ds/m)	OC (%)	Clay (%)	Silt (%)	Sand (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)	B (mg/kg)	Zn (mg/kg)	Se (mg/kg)
Silt-Loam	7	0.35	0.5	20	20	60	3.3	6.4	155	0.83	0.58	12.2

The results showed that the drought stressed creases plant height (Fig. 1). The highest plant height was of 31.82 cm at control. The results indicated that the application of drought stress in different stages of plant growth was not significant. The lowest plant height was 29.29 cm obtained by applying drought stress. The drought stress has significant effect on turgor pressure and cells growth and increases plant height and growth. On the other hand, drought stress at flowering stage decreases the plant height 8.63%

than the control. Also, the results showed that foliar application of microelements increases the plant height. So that, the plant height increases 6.9% than the control by 6 ppm foliar application of microelements (Fig. 2).

Leaf area index

The drought stress and foliar application of microelements had significant effect on leaf area index at $P < 0.01$ and the interaction of them has

significant effect at $P < 0.05$ (Table 2). The results showed that drought stressed creases leaf area index but the foliar application of microelements increases the leaf area index in presence and absence of drought stress. So that, the highest amount of leaf area index obtained in control condition and foliar application of 6 pm microelements and the lowest amount of that was obtained in drought stress at flowering stage and foliar application of distilled water (Fig. 3). Also, the results indicated that the foliar

application of microelements in drought stress conditions increases leaf area index 17.64% than the control at flowering stage.

Thousand seed weight

The variance analysis results showed that drought stress and foliar application of microelements significantly affected thousand seed weight at $P < 0.01$ and the interaction of them significantly affected at $P < 0.05$ (Table 2).

Table 2. The variance analysis of microelements on phenological and physiological characteristics of Mung bean plant under drought stress.

S.O.V	df	Plant height	Leaf area index	Thousand seed weight	Seed protein	Chlorophyll a	Chlorophyll b	Total chlorophyll	Proline
Application (a)	2	13.66**	3.08**	69.75**	7.83**	0.02**	0.18**	0.35**	427.86**
Drought stress (b)	3	12.72**	0.14**	54.91**	15.92**	0.008**	0.05**	0.1**	5.78**
a*b	6	1.2 ^{ns}	0.041 [†]	5.13 [†]	0.94 [†]	0.001**	0.005 [†]	0.01**	0.48 [†]
Error	24	2.47	0.015	2.08	0.35	0.0003	0.001	0.003	0.17
CV%		5.23	4.41	12.89	3.33	2.41	2.43	2.2	4.07

** , * and ns are significantly at 1%, 5% and not significant, respectively.

The results showed that the drought stress decreases thousand seed weight and foliar application of microelements increases it. Also, foliar application of microelements increases thousand seed weight under drought stress. According to the results, the highest amount of thousand seed weight was 54.98 gr obtained at control conditions and foliar application of 6 pm microelements and the lowest amount of that was 43.29 gr obtained at drought stress conditions at flowering stage and foliar application of distilled water (Fig. 4).

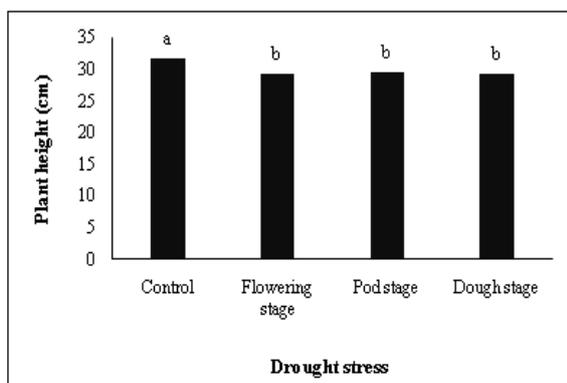


Fig. 1. The effect of drought stress on plant height of Mung bean.

Percentage of seed protein

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The variance analysis results indicated that drought stress and foliar application of microelements had significant effect on seed percentage of protein at $P < 0.01$ and interaction of them had significant effect at $P < 0.05$ (Table 2). According to the results the drought stress conditions and foliar application of microelements in drought stress conditions increases percentage of seed protein. The results showed that the highest percentage of seed protein was 19.4% at drought stress treatment applying in flowering stage and foliar application of 6 pm microelements and the lowest percentage of seed protein was 14.31% obtained at control conditions and foliar application of distilled water (Fig. 5).

Chlorophyll a, b and total Chlorophyll

According to the variance analysis results, drought stress and foliar application of microelements had significant effect ($P < 0.01$) on chlorophyll a, b and total Chlorophyll and also the interaction of them had significant effect ($P < 0.05$) on chlorophyll a and total Chlorophyll (Table 2). The results showed that the drought stress decreases chlorophyll a, b and total Chlorophyll and foliar application of microelements

increases them. The highest negative effect of drought stress was observed at flowering stage and the highest positive effect of foliar application of microelements was observed at 6 ppm (Fig. 6, 7and8).

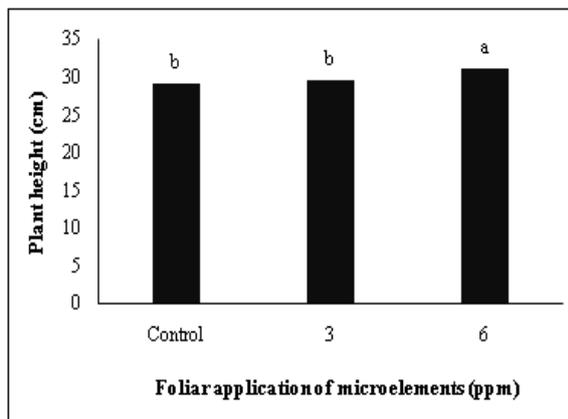


Fig. 2. The effect of foliar application of microelements on plant height of Mung bean.

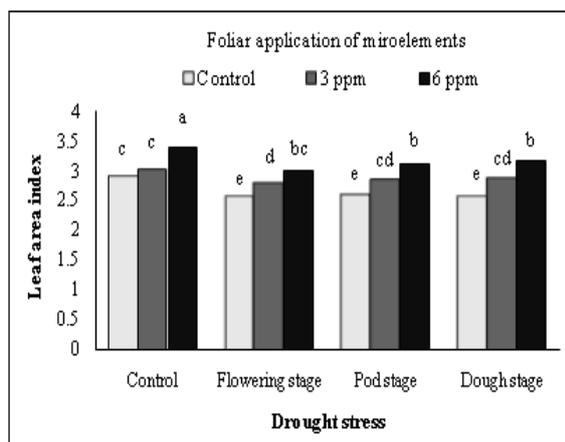


Fig. 3. The effect of foliar application of microelements and drought stress on leaf area index of Mung bean.

Proline content

Variance analysis results showed that the foliar application of microelements and drought stress had significant effect ($P < 0.01$) on Mung bean pro line content and interaction of drought stress with foliar application of microelement had significant effect at $P < 0.05$ (Table 2). The results showed that drought stress and foliar application of microelements increases pro line accumulation. The highest of pro line content was 37 mg.gFW^{-1} obtained at drought stress treatment at flowering stage and foliar application of microelements at 6 ppm concentration and the lowest pro line content was 5.92 mg.gFW^{-1}

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obtained at control conditions and foliar application of distilled water (Fig. 9).

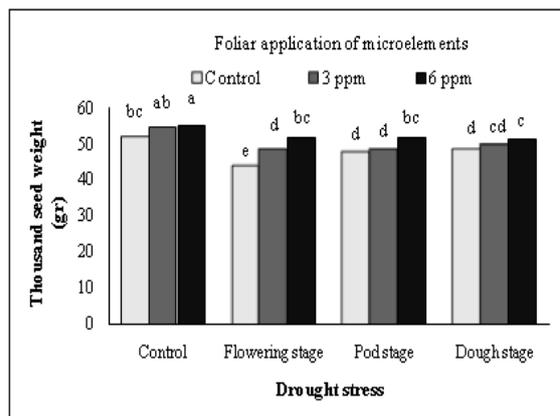


Fig. 4. The effect of foliar application of microelements and drought stress on the thousand seed weight of Mung bean.

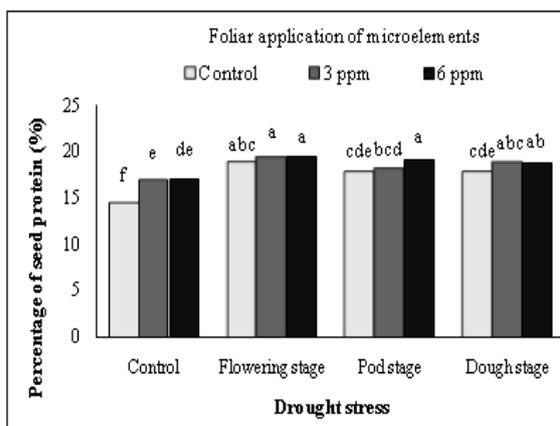


Fig. 5. The effect of foliar application of microelements and drought stress on the percentage of seed protein of Mung bean.

The foliar application of microelements prepared precursor for pro line synthesis. Therefore, in foliar application of microelements treatments the pro line content was higher than foliar application of distilled water. In drought stress conditions pro line produce for maintains of osmotic potential for absorb required water. Pro line is an amino acid that naturally is found in many higher plants and usually its concentration is increases in response to environmental stress.

Discussion

During the water stress, photosynthesis is limited by stomata closure. Studies showed that water deficiency

cause to disruption in communication between proteins and membrane lipid and lead to decrease in enzyme activity and lipid bilayer membrane transmission capacity that ultimately lead to decreasing of plant growth. The similar results reported by Costa and Morel (1994). The microelement mobility in the soil is decreases by drought stress and according to the root growth limitation, the plant could not absorption them from soil. Therefore, the plant has microelements deficiency. By foliar application of microelements the deficiency of them compensated. Zinc and iron plays an important role in protecting of plant cells. Cell size decreasing in drought stress leads to decreases the tissue and organs size such as stem.

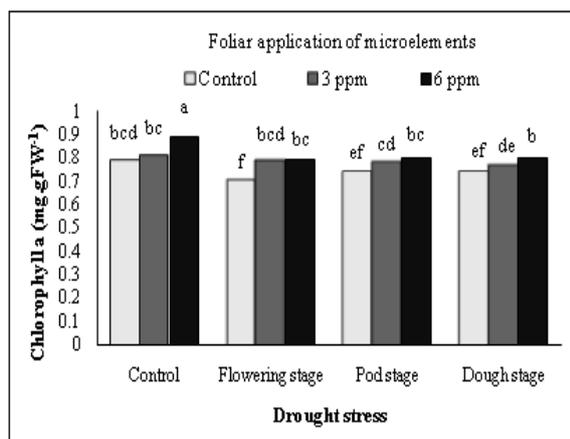


Fig. 6. The effect of foliar application of microelements and drought stress on the chlorophyll a of Mung bean.

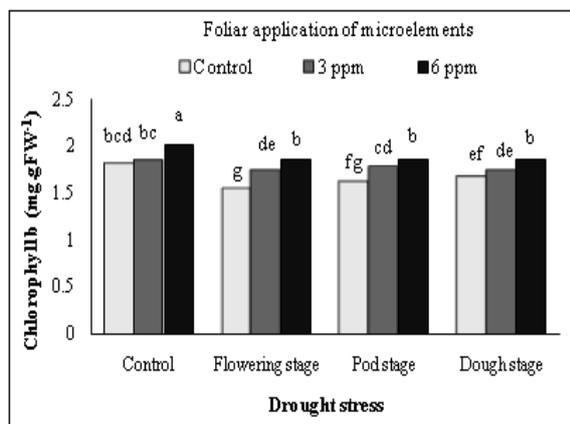


Fig. 7. The effect of foliar application of microelements and drought stress on the chlorophyll b of Mung bean plant.

Drought stress decreases leaf area index by decrease

of leaf production and growth. This results similar with Cakir (2004) results. A decrease of leaf area index is the first mechanism of plants for resistance against drought stress. So that, the increasing of leaf area index by application of micronutrients due to providing the some elements such as zinc and iron, which leads to the synthesis auxin hormone in leaves. Since the auxin is an inducer of cell division can be effective in increasing of leaf area index. The similar results were obtained by Mortvedth (2003).

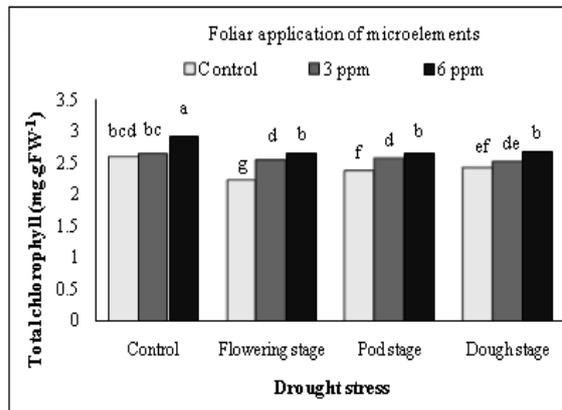


Fig. 8. The effect of foliar application of microelements and drought stress on the total chlorophyll of Mung bean.

Drought stress is affected degree of stomata opening, decreases the Calvin cycle enzymes activity and decreases the photo-assimilates production (Pessaraki, 2001) and directly decreases the weight of seeds (Emam and Niknejad, 2004; Seilsepoor *et al.*, 2006). The use of microelements increases the plant leaf area durability and prevents the decreases of thousand seed weight. Similar results reported by Ghorashi Nasb *et al.* (2009).

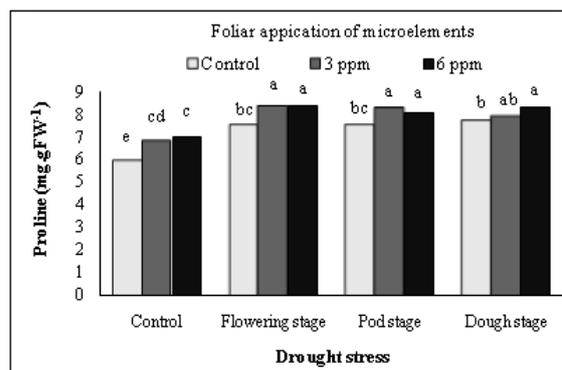


Fig. 9. The effect of foliar application of microelements and drought stress on the proline content of Mung bean.

The results indicated that the percentage of seed protein increases by drought stress. The studies showed that the drought stressed creases carbohydrate accumulation and increases percentage of protein (Bahrani *et al.*, 2009). The increasing of percentage of protein by microelements application is reported by other researchers (Babhulkar *et al.*, 2000; Sawan *et al.*, 2001).

Drought stress increases the oxygen free radicals in the chloroplast and causes to destruction of the chlorophyll molecules and chloroplasts membranes that lead to decreases the photo synthesis and growth which reported by Takamiya *et al.* (2000). On the other hand, chlorophyll destruction increases by active oxygen species. Also, the chlorophyll destruction was performed with separating of phytolchainofporphyrinring by oxygen free radicals and/or chlorophyll as enzyme. In the sensitive plants expression of chlorophylls enzymes genes are increases (Benedetti and Arruda, 2002). Leaf chlorophyll preservation and photosynthesis durability in drought stress are physiological tolerance indices (Pessaraki, 1993). Therefore, the using of nutrient containing with iron and magnesium, which are the main component of Chlorophyll under drought stress conditions can be prevented this organelle decreases.

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