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EFFECTS OF COPPER ON DROUGHT TOLERANCE OF THREE COMMON BEAN (*PHASEOLUS VULGARIS* L.) CULTIVARS AT SEEDLING STAGE

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Abstract

Common bean (*Phaseolus vulgaris* L.) is a legume, which is widely cultivated and consumed in the world. Copper (Cu) is one of the essential micronutrients required by crop plants. This article presents results obtained in a study on how micronutrient copper affects the drought tolerance of three common bean lines namely NHP04, NHP08 and GV11 at seedling stage. The study findings show positive effects of Cu on the abilities to cope with drought stress of these three common bean cultivars, in which the most drought tolerant was NHP08 with its relative drought tolerance index of 3215.20 in Formula I (FI) and 3964.26 as in Formula II (FII), followed by GV11 with 3068.54 in FI and 3320.82 in FII; and then NHP04 with only 2255.65 in FI and 2803.00 in FII. The study also supports the direct connection between Cu and the drought tolerance of common bean via some indicators such as leaf water content, leaf water retention capacity, leaf transpiration rate and root weight. The results are served as scientific basis for developing the drought tolerance of common bean varieties.

Key words : Common bean, copper, drought tolerance, indices, seedling stage.

Introduction

Common bean (*Phaseolus vulgaris* L.) is alegume native to Americas, is now widely cultivated in many countries, including Vietnam (Gepts and Debouck, 1991; Broughton *et al.*, 2003). Common bean is not only a rich source of nutrients for human beings (Sgarbieri, 1989; Hayat *et al.*, 2014), but also highly nutritious for animals and can be effectively used for soil conditioning (Blair *et al.*, 1990). While increase in common bean yield offers enormous benefits, weather and climate conditions, especially drought, are major limitations tobean yield (Gallegos and Shibata, 1989; Boutraa and Sanders, 2001).

Drought is a severe abiotic stress affecting the agricultural systems and global food production. Prolonged shortage of water supply (drought) can make changes in relatedmetabolic reactions, water retention capacity of the soil, agricultural product quality or evenmake the plants stunted (Farooq *et al.*, 2009). Therefore, studying cultivars with the capacityof handling water deficit stress has become adominant research topic among scientists (Subbarao *et al.*, 1995).

Trace elements (Zinc, Boron, Copper, etc.) are essential factors contributing to the development of cultivars (Alloway, 2008). There has been a vast number of studies worldwide which focus on the roles of trace elements in reducing the adverse consequences of drought for maize (Sajedi *et al.*, 2011; Ahmad *et al.*, 2015), chickpea (Jan mohammadi*et al.*, 2012) and wheat grain (Moeinian *et al.*, 2011). Hasanuzzaman *et al.* (2017) concluded that micronutrients could not only improve the physiological processes and development of cultivars but also contribute to enhancing their drought resistance. In Vietnam, several studies have been conducted to assess the contribution of micronutrients to enhanced water deficiency tolerance of peanut (Thi *et al.*, 2008; Le and Ngan, 2010) and sesame (Le, 2010). Le and Ngan (2010) confirmed that under the weather and climate conditions of the summer yield in Danang, the resistance of peanut to the heat and water shortage could be enhanced by

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adding $CaCl_2$ and the combination of Mo, B, Mn, Cu elements. Thi *et al*. (2008)'s study on how B, Mo, Zn influence physiological parameters and productivity of peanut in Thua Thien Hue concluded that B, Zn, Mo microelements foster the development of photosynthetic apparatus as well as the content of chlorophyll a and b. The results of Minh (2012)'s study support the good influences that Mo, Cu, Zn have on plant physiological indices right from the germination stage. These trace elements were also reported to promote the photosynthesis pigments and contribute to organic matter accumulation as well. Waraich *et al*. (2011) stated that micronutrient Cu could minimize the adverse effect that drought exerted on cultivars. According to studies, proper supplement of micronutrient can relieve the water deficiency stress for crops (Waraich *et al*., 2011; Ahanger *et al*., 2016).

Shortage of water supply at seedling stage poses a threat to water imbalance in crops, thus adversely influencing their physiological functions, stunting plant growth and causing reduction in productivity (Khanh and Bang, 2008; Anjum *et al*., 2011). Therefore, studying the effect of drought on seedlings occupies a crucial role in promoting yield and enhancing stress tolerance in cultivars.

Study on the influence of Cu on the ability to handle water deficit stress of some common bean cultivars at seedling stage contributes to working out solutions to improve the drought resistance of these varieties, thus boosting the productivity.

Materials and methods

Research materials

The selected common bean lines were NHP04, NHP08, GV11 by Legumes Research and Development Center, Vietnam National University of Agriculture.

$CuSO_2.5H_2O$0.03%.

Pot-in-pot planting method

Pots sized 20 × 40cm with 6-7 side and bottom holes (0.5 cm in diameter) were used. Each pot contained the mixture of soil and sand for the purpose of providing nutrients and controlling the water supply to the plants during the drought treatment. The pots were placed on the groundwith rain shelter and classified into two lots corresponding to two experimental formulas:

+ Formula I (FI): drought treatment without supplement of $CuSO_2.5H_2O$ 0.03% (control group).

+ Formula II (FII): drought treatment with supplement of $CuSO_2.5H_2O$ 0.03%.

Experimental pots were provided with care corresponding to experimental formulas. Everyday FI samples was watered and FII ones was sprayed with $CuSO_2.5H_2O$ liquid 0.03%.

Artificial drought method

When the cultivars reached the stage of two true leaves, artificial drought treatment was provided by stopping watering or spraying with $CuSO_2.5H_2O$ liquid 0.03% and applying rain shelter. The duration of the drought was determined when the first wilted leaf appeared and lasted for 1 day, 3 days, and 5 days. After the drought treatment, cultivars were re-watered and supplied with $CuSO_2.5H_2O$ 0.03% in each formula after 1 day, 3 days and 5 days of recovery.

Sampling

Samples were collected in the morning using the five point sampling method, and leaf samples were selected from nodes of the same level.

Evaluating drought tolerance at seedling stage

The percentage of non-wilted and recovered plants after 1 day, 3 days, 5 days under drought condition were calculated.

Relative drought tolerance of cultivars was demonstrated in a radar chart including a, b, c, d, e, g axes which represented a_n, b_n, c_n, d_n, e_n, g_n variables, respectively. The relative drought tolerance was calculated by formula:

$$S = 1/2\sin\alpha(a_nb_n + b_nc_n + c_nd_n + d_ne_n + e_ng_n + g_na_n)$$

In which: α: the equal inner angle of the radar chart $\alpha = 360^o/6$

S : Relative drought tolerance index

a : % of non-wilted plants after 1 day under drought condition

b: % of recovered plants after 1 day under drought condition

c: % of non-wilted plants after 3 days under drought condition

d: % of recovered plants after 3 days under drought condition

e: % of non-wilted plants after 5 days under drought condition

g: % of recovered plants after 5 days under drought condition

n: codes of the selected varieties in the study

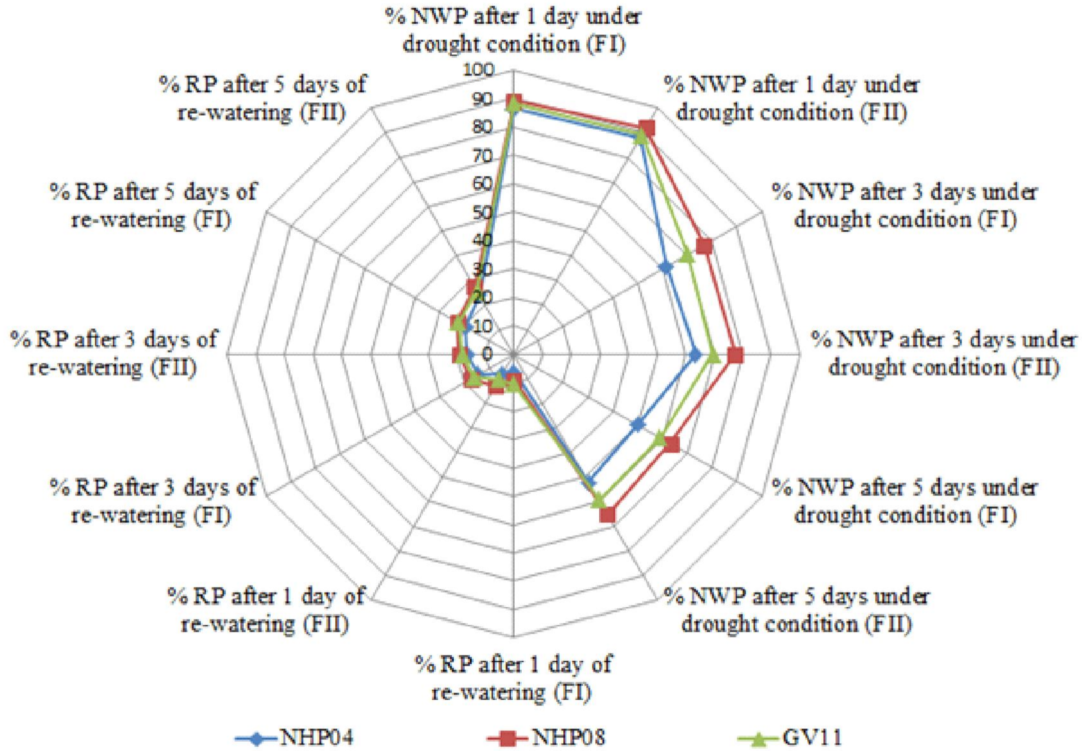

Fig. 1 : Radar chart depicting different tolerance levels in response to drought of three common bean cultivars in FI and FII under the impact of Cu.

Analysis and determination of physiological indicators related to drought tolerance of common bean in the seedling stage

*Determination of leaf water retention capacity

Leaf samples of each cultivar were collected from nodes of the same level, which was conducted 3 times. After being detached from plants, leaf samples were immediately transferred to plastic bags in order to prevent moisture from escaping. Samples were then brought to laboratory and initial fresh weight (B) was recorded. Leaves were let dry at room temperature for 3 hours before weighed again to get the fresh weight after wilting (b). Next, the weighed samples were put into a drying oven at the temperature of 105°C and weighed until its unchangeable weight (V) (Ma *et al.*, 2013). The leaf water retention capacity (a) was calculated by the following formula:

$$a\% = \frac{B-b}{B-V} \times 100$$

In which, a represents the leaf water retention capacity (the percentage of water loss weight/ the total water weight) (%)

B : initial leaf fresh weight (g)

b : leaf fresh weight after 3 hours of wilting (g)

V : dry leaf weight after drying (g)

*Determination of water content in plants

Plants of each variety were placed in plastic bags to avoid dehydration and brought to laboratory where they were weighed to get the initial fresh weight (B). Weighed plants were then transferred to a drying oven at the temperature of 105°C until they reached the unchangeable weight which was the plant dry weight (b) (Ma *et al.*, 2013). The water content in plants was calculated by the following formula :

$$A\% = \frac{B-b}{B} \times 100$$

In which, A: water content in plant (%)

B: plant initial fresh weight (g)

b: plant dry weight after drying(g)

* Determination of the transpiration rate

The transpiration rate was measured by the CI-340 system from CID Bio-Science, USA.

*Determination of the root weight

Plants of each line were transferred to plastic bags and brought to the laboratory where the roots were detached. After soil and dirt were removed from the roots, weighing were processed to get the root fresh weight.

Statistical analysis

Data collected were analyzed by the software IRRISTAT (version 5.0) for windows computer (IRRI, 2005).

Results and Discussion

Evaluating of drought tolerance at seedling stage of three common bean cultivars by artificial drought method

The ability to handle water deficiency of common bean seedlings were analyzed based on the percentage of wilted plants after 1 day, 3 days, 5 days of the drought treatment and the percentage of recovered plants after 1 day, 3 days, 5 days of re-watering. Accordingly, the relative drought tolerance indices were calculated as shown in table 1.

NHP08 ranked first in the list as the most tolerant cultivar with the indicators of 3215.20 in FI and 3964.26 in FII, followed by GV11 with 3068.54 in FI and 3320.82 in FII, and the last line was NHP04 with a considerably low drought tolerance indices of only 2255.65 in FI and 2803.00 in FII. The higher drought tolerance indicator a cultivar gets, the more tolerant it is to the shortage of water supply and vice versa. In other words, among the selected common bean lines in the study, NHP04 had the most limited drought resistance capacity, followed by GV11. In contrast, the most tolerant one was NHP08. The relative drought tolerance indices calculated in FI were higher than those in FII, representing the statistical difference which proved the positive impact of Cu on the ability to handle and recover from water deficit stress of the study common bean varieties.

The relative drought tolerance indices were calculated in another way by using radar chart area. The radar chart depicted how commonbean seedlings tolerated water shortage after 1 day, 3 days, 5 days of drought.

The results of artificial drought method at seedling stage suggest that each common bean line gives different response to water deficit stress. NHP08 is the highest droughttolerant cultivar with the highest relative droughttoleranceindex, followed byGV11, and NHP04 is the lowest relative droughttoleranceindex cultivar.

Effects of Cu on some physiological indicators of common bean in seedling stage

*** Effects of Cu on total leaf water content**

Studying leaf water content at seedling stage serves as the basis for assessing the drought tolerance of plants. The higher the leaf water content is, the higher the drought tolerance of the plants will be. This is because the plants are provided with sufficient amount of water to tolerate under drought condition (Gardner *et al*., 2017). The results are shown in tables 2 and 3.

Table 1 : Influence of Cu on percentage of non-wilted plants, recovered plants and relative drought tolerance indices of three common bean cultivars at seedling stage.

Cultivar	**NHP04**		**NHP08**		**GV11**	
Formula	**FI**	**FII**	**FI**	**FII**	**FI**	**FII**
% NWP after 1 day under drought condition	86.22	88.25	89.26	92.16	88.36	89.25
% NWP after 3 days under drought condition	61.51	63.45	76.54	77.28	70.21	69.43
% NWP after 5 days under drought condition	50.04	52.19	63.03	65.27	58.78	59.32
% RP after 1 day ofre-watering	6.45	8.12	9.34	12.86	10.74	10.27
% RP after 3 days ofre-watering	14.52	16.34	17.38	19.26	16.27	17.89
% RP after 5 days ofre-watering	19.35	24.19	22.66	27.28	22.31	25.56
Drought tolerance index	2255.65	2803.00	3215.20	3964.26	3068.54	3320.82

Note: NWP: Non-wilted plants; RP: Recovered plants.

Table 2 : Effects of Cu on leaf water content of common bean under drought condition (%).

Cultivars	**After 1 day under drought condition**		**After 3 days under drought condition**		**After 5 days under drought condition**	
	FI	**FII**	**FI**	**FII**	**FI**	**FII**
NHP04	75.33^{b}	78.18^{c}	74.58^{bc}	75.32^{b}	72.49^{b}	72.68^{b}
NHP08	78.97^{a}	80.82^{a}	77.82^{a}	79.53^{a}	75.21^{a}	76.64^{a}
GV11	77.34^{ab}	79.02^{b}	75.71^{b}	76.21^{b}	74.17^{a}	75.42^{a}

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent differences in significance (p=0.05) by Tukey test.

Table 3 : Effects of Cu on leaf water content of common bean after re-watering (%).

Cultivars	After 1 day of re-watering		After 3 days of re-watering		After 5 days of re-watering	
	FI	FII	FI	FII	FI	FII
NHP04	73.33[c]	74.25[c]	74.53[b]	75.43[b]	75.28[b]	77.80[b]
NHP08	75.65[a]	76.57[a]	76.79[a]	77.71[a]	77.54[a]	79.67[a]
GV11	74.24[b]	75.14[b]	76.92[a]	77.14[a]	77.05[a]	79.51[a]

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent differences in significance (p=0.05) by Tukey test

Table 4 : Effects of Cu on leaf water retention capacity of leaves of common bean under drought condition (%).

Cultivars	After 1 day under drought condition		After 3 days under drought condition		After 5 days under drought condition	
	FI	FII	FI	FII	FI	FII
NHP04	26.31[a]	25.67[a]	25.07[a]	24.06[a]	29.39[a]	26.68[a]
NHP08	22.49[c]	18.56[c]	19.16[c]	16.86[c]	23.86[b]	21.56[b]
GV11	24.05[b]	22.48[b]	23.22[b]	21.34[b]	24.19[b]	22.75[b]

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent differences in significance (p=0.05) by Tukey test

Table 5 : Effects of Cu on leaf water retention capacity of leaves of common bean after re-watering (%).

Cultivars	After 1 day of re-watering		After 3 days of re-watering		After 5 days of re-watering	
	FI	FII	FI	FII	FI	FII
NHP04	30.16[a]	28.09[a]	27.43[a]	24.86[a]	26.54[a]	25.49[a]
NHP08	25.63[b]	23.93[b]	24.69[b]	22.21[c]	23.45[b]	22.75[b]
GV11	26.56[b]	25.32[b]	24.33[b]	23.87[b]	24.02[b]	23.46[b]

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent differences in significance (p=0.05) by Tukey test

Monitoring the leaf water content of common bean cultivars after 1 day, 3 days and 5 days under drought condition, it can be seen that the leaf water content of the cultivars in FII was always higher than that in FI. After 1 day under drought condition, NHP08 had the highest water content at 78.97% in FI and 80.82% in FII, followed by GV11 and NHP04 (the water content of NHP04 was the lowest).

The leaf water content of the cultivars decreased after 3 and 5 days under drought condition. After 3 days, NHP08 had the highest leaf water content at 79.53% in FII, followed by that of GV11 at 76.21% in FII and at 75.71% in FI. Both of these cultivars have the higher water content than the one of NHP04. After 5 days under drought condition, the highest leaf water content of NHP08 was 76.64% in FII and 75.21% in FI, the leaf water content of GV11 and NHP04 took the second and the third place, respectively.

The results of table 3 show that after re-watering, the leaf water content of the cultivars increased after 1 day, 3 days and 5 days. In FII, water content of the cultivars was higher when compared to the controls, which showed statistically significant differences.

In short, Cu had effects on leaf water content of the common bean cultivars. That the total water content in FII was higher than that in FI is because Cu is associated with the increase in leaf water content under drought condition. Under the influence of Culeading to an increase in osmotic bound water content and a reduction of osmotic pressure of the fluid increased the viscosity of protoplasm, thereby increasing the drought tolerance (Khanh and Bang, 2008). This research result was similar to the one fromthe study of Le (2010) in which Cu increased drought tolerance and heat tolerance of sesame.

***Effects of Cu on leaf water retention capacity**

Leaf water retention capacity is one of the criteria to ensure water balance in plants,which represents the water retention capacity of protoplasm against dehydration (Khanh and Bang, 2008; Gardner *et al.*, 2017). The findings are presented in tables 4 and 5.

The smaller percentage of water loss/totalamount of

Table 6. Effects of Cu on the leaf transpiration rate of common bean under drought condition ($mmol/m^2/s$).

Cultivars	After 1 day under drought condition		After 3 days under drought condition		After 5 days under drought condition	
	FI	FII	FI	FII	FI	FII
NHP04	6.42[b]	5.37[b]	5.22[b]	4.68[b]	5.04	4.08[a]
NHP08	6.94[a]	5.83[a]	5.56[a]	5.27[a]	4.92	4.65[a]
GV11	6.21[b]	4.92[b]	5.36[b]	4.34[b]	4.52	3.76[b]

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent differences in significance (p=0.05) by Tukey test

Table 7 : Effects of Cu on the leaf transpiration rate of common bean after re-watering ($mmol/m^2/s$).

Cultivars	After 1 day of re-watering		After 3 days of re-watering		After 5 days of re-watering	
	FI	FII	FI	FII	FI	FII
NHP04	4.48[b]	3.69[b]	5.24[a]	4.12[b]	6.12[b]	5.84[a]
NHP08	4.72[a]	4.09[a]	5.06[a]	4.74[a]	6.78[a]	5.44[b]
GV11	3.81[c]	3.65[b]	4.48[b]	4.04[b]	6.56[a]	5.12[c]

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent differences in significance (p=0.05) by Tukey test.

wateris the higher the water retention capacity. After 1 day under drought condition, the percentage of water loss/ total water content of NHP08 was the lowest at 18.56% in FII and 22.49% in FI, followed by that of GV11 at 22.48% and 24.05% in FII and FI, respectively. NHP04 had the lowest values of 25.67% in FII and 26.31% in FI.

The water retention capacity of cultivars increased after the third day under drought condition and sharply declined after the fifth day. At this time, NHP08 still had the greatest capacity to hold water, followed by GV11 and NHP04. The reason for this was that water loss increases the content of osmotically active substances such as reducing sugar, proline amino acid, etc. which were capable of creating high pressure, increasing cells' water intake and retention capacity as well as the expansion of colloidal system (Khanh and Bang, 2008). This process, however, did not last long because of the extension of drought condition. After 5 days under drought condition, the water retention mechanism began to work improperly, which led to an increase in the amount of water loss and a decrease in water retention of tissues and cells. The results were completely consistent with the conclusion of the Huyen and Khanh(2011)'s study on water retention capacity of 20 sesame varieties under drought condition and Giang *et al*. (2009)'s study on water retention capacity of vegetable soybean DT – 02 under drought condition.

Being re-watered after 5-day drought condition, the water retention capacity of the cultivars began to increase gradually and significant differences between FI and FII were recorded.

To sum up, under the influence of Cu, the common bean cultivars have better water retention capacity as well as faster recovery, which proves that Cu affects the drought tolerance of varieties of common bean. This is similar to conclusion of Le and Ngan (2010)'s study that Cu increases drought tolerance of peanuts.

*** Effects of Cu on leaf transpiration rate**

Leaf transpirationcreates upper impetus for uptake process and reduces leaf temperature, which stimulates the plant's physiological processes (Gates, 1968). This is the basis for an increase in plants' biomass, crop yield and tolerance. Whether the transpiration speed is high or low is shown by transpiration rate. The results are presented in tables 6 and 7.

It can be seen that there was differences in transpiration rate of cultivars in FI and FII. After 1 day under drought condition, the transpiration rate of NHP08 was at the highest value of 6.94 $mmol/m^2/s$ in FI and 5.83 $mmol/m^2/s$ in FII, followed by that of GV11 and NHP04. The transpiration rate of the cultivars decreased gradually after 3 and 5 days under drought condition. Nevertheless, the figures in FII where Cu was added were lower than those in FI. This is due to a significant decrease in free water content. That Cu increased the bound water content of the leaves led to changes in colloidal system of Protoplasm; therefore, the leaf water retention capacity increased while and the transpiration

Table 8 : Effects of Cu on root weight of common bean under drought condition (g)

Cultivars	After 1 day under drought condition		After 3 days under drought condition		After 5 days under drought condition	
	FI	FII	FI	FII	FI	FII
NHP04	0.26^b	0.29^b	0.35^b	0.41^b	0.43^b	0.52^c
NHP08	0.38^a	0.42^a	0.46^a	0.51^a	0.59^a	0.66^a
GV11	0.28^b	0.32^b	0.31^b	0.42^b	0.47^b	0.59^b

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent differences in significance (p=0.05) by Tukey test

Table 9 : Effects of Cu on root weight of common bean after re-watering (g).

Cultivars	After 1 day of re-watering		After 3 days of re-watering		After 5 days of re-watering	
	FI	FII	FI	FII	FI	FII
NHP04	0.48^b	0.58^c	0.59^b	0.66^b	0.62^b	0.76^b
NHP08	0.62^a	0.75^a	0.73^a	0.80^a	0.79^a	0.85^a
GV11	0.57^a	0.68^b	0.61^b	0.75^a	0.68^b	0.78^b

Note: In the same data column, values with similar letters represent non-significant differences, values with different letters represent differences in significance (p=0.05) by Tukey test.

rate decreased. After 3 days under drought condition, NHP08 had the highest transpiration rate at 5.56 $mmol/m^2/s$ in FI and only 5.27 $mmol/m^2/s$ in FII. After 5 days under drought condition, the highest transpiration rate, which still belonged to NHP08 was at 4.92 $mmol/m^2/s$ in FI and 4.65 $mmol/m^2/s$ in FII while the lowest values was recorded in GV11 with 4.52 $mmol/m^2/s$ in FI and 3.76 $mmol/m^2/s$ in FII.

After re-watering, transpiration rate of common bean increased but there was also differences between FI and FII and among distinct cultivars. After 1 day of re-watering, NHP08 has the highest transpiration rate,followed by NHP04 and GV11.

*** Root weight**

Development of root systems in acquiring water has been recognized crucial for crop plants to cope with drought conditions (Serraj *et al*., 2004; Kashiwagi *et al*., 2006). Under the condition of water stress, common bean often grows biomass of the root, thereby increasing the root/stem ratio. Studying results of Cu's effects on root weight were presented in tables 8 and 9.

Tables 8 and 9 shows that the root weight of the cultivars increased after 1 day, 3 days and 5 days under drought condition. This result is consistent with the study of Jiang *et al*. (2001), at an appropriate concentration, Cu is capable of stimulating the development of Zea mays's roots. After 1 day under drought condition, the root weight of NHP08 was the highest at 0.38g in FI and 0.42g in FII, followed by that of GV11 at 0.28g in FI and 0.32g in FII; NHP04 had the lowest value of 0.26g in FI and 0.29g in FII. After 3 days under drought condition, root weight increased. To be more specific, the figures of NHP08 with the highest at 0.46g in FI and 0.51 in FII, the lowest was GV11 at 0.31g in FI and 0.42g in FII. After 5 days under drought condition, NHP08 had the highest root weight of 0.59g in FI and 0.66g in FII. These results show that in FII, the root weight of the cultivars was higher when compared to the control results, which showed statistically significant differences. Cu strongly stimulated root growth of common bean varieties and increased osmotic pressure at the same time so that roots could absorb water to create a favorable environment for biochemical reaction (Sancenón *et al*., 2004; Khanh and Bang, 2008).

After re-watering, the root weight of the cultivars continued to increase. After 1 day, 3 days, 5 days of re-watering, NHP08 has the highest root weight, followed by GV11 and NHP04.

The results of the study on Cu's effects on root weight show that Cu is directly related to the weight of common bean roots and increase the plants' tolerance under drought conditions.

Conclusion

Relative drought tolerance index at seedling stage of some common bean cultivars was determined when Cu was added. NHP08 had the highest relative drought tolerance index at 3215.20 in FI and 3964.26 in FII, followed by that of GV11 at 3068.54 in FI and 3320.82 in FII. NHP04 had the lowest values of 2255.65 in FI and 2803.00 in FII.

Under drought conditions during the seedling stage of some common bean cultivars, Cu had effects on physiological indicators such as leaf water retention capacity, leaf water content, transpiration rate and root weight. In particular, when watered, these cultivars were able to quickly recover in terms of research criteria, especially in the formula of Cu fertilization, which proves that Cu affected the drought tolerance mechanisms of common bean.

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