Phytotoxicity of Copper on the Physiological Parameters of two Varieties of broad bean (*Vicia faba*)

S. Benouis, H- AH. Regueig Yssaad

***Abstract*—Copper is one trace element potentially toxic in too strong concentration, the accumulation of this heavy metal in the biosphere disturbs the development of the plants and causes a fall of production. The Cu phytotoxicity appears mainly through the rhizotoxicity, and by an inhibition of the growth on the level of the air part. The principal symptoms observed at the plants following a metal exposure are related to the metal ions and is characterized by the molecular and metabolic reactions at various levels of organization of the whole plant. The study was undertaken on two varieties of broad bean (*Vicia faba. L)* Sidi Aïch and Super Aguadulce. The device was installed on altered substrate contaminated artificially by the addition of three amounts of copper 0,200 and 400 ppm in the form of copper sulfate [Cu SO4, 5:2 O]. The major objective of our work is to estimate the effects provided by the copper on the physiological behavior of the broad bean (vicia faba. L). The results obtained show a decrease of the productivity parameters (photosynthesis and protein) and an accumulation osmo (proline and soluble sugars). These results suggest that the presence of copper in high doses modifies the operation and the normal development of the broad bean by disturbances on the Physiologically.**

***Keywords*—phytotoxicity, copper, stresses, variety, broad bean, parameters physiological.**

I. Introduction

The human activities considerably increased the release of various molecules in the environment of which some appear toxic for all the living organisms. Among the introduced molecules, heavy metals where copper occupies a considerable place.

The plants need for their growth and their development certain metal ions such as for example the copper, which uses the composition of certain enzymes where to be used to them as Co-factors [43]. This element, necessary in small quantity, proves however toxic even lethal when it is present in strong concentration [37]. At the plant, the phytotoxicity can be expressed on the level racinaire (rhizotoxicity) and/or on the level of the air parts and is characterized by molecular, and metabolic reaction and/or at various levels of organization of the whole plant [27].

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Copper would be responsible for an inhibition of photosynthesis [11], synthesis of chlorophyll, and while interfering on the level of the photosystèmes [5]. Copper blocks the respiratory processes, slows down the biosynthesis of proteins and the transfers of elements by decreasing the membrane activity [18]. It is also able to catalyze the production of free radicals which are powerful oxidants [3]. The various tests carried out for the realization of work presented consist of the estimate of the effects gotten by copper on the behavior of broad bean. For a better valorization of these effects, the introduction of a genotypic variability is essential, indeed two varieties of origin and of behavior controversy are led.

II. Materials and Method

A. Vegetable material:

The study carried out relates to two varieties of broad bean (*Vicia faba.* L) of origin and behavior, different. A local variety (V1), sidi Aïch, provides by institutes technical field crops (ITGC) of Sidi Bel Abbès, Algéria. And another variety of Spanish origin (V2), super Aguadulce.

B. Method of test:

The experimentation takes place in a semi-automatic greenhouse to the level of the Faculty of Science of nature and life of university IBN KHALDOUN of Tiaret, Algéria.

The seeds of *Vicia faba*, of the two varieties are sterilized in a hypochlorite solution of sodium with 12%, rinsed with distilled water and crumbs in germination. After germination, seeds its mended in PVC cylinders (90cm length, 11cm of diameter) and filled of a reconstituted substrate of sand, ground and compost with proportions (8-1-3) a low holding capacity, 24,54%. A sample is analyzed to determine the properties of the ground (Table I).

TABLE I
PHYSICOCHEMICAL PROPERTY OF THE SOIL USED.

Granulometry % (Pipette Of Robinson)	
- < 2µm	05.26
- 2 µm to 20 µm	07.89
- 20 µm to 50 µm	44.18
- 50 µm to 200 µm	17.52
- 200 µm to 2 mm	25.15
pH (AFNOR X31-103 Soil/Water:2/5)	07.67
Electric conductivity (Ms/Cm) (Iso:11265 Soil/Water:1/10)	0.416
Organic matter (%) (Walkley Method)	0.13
Nitrogen Total (%) (Kjeldahl ISO:11261)	0.18
Assimilable phosphorus (ppm) Joret Hebert AFNOR:X31-161	N.D
Total limestone (%) Calcimeter Bernard ISO:10693	04.41
Active limestone (%)	0.75
cation exchange capacity (CEC) In Meq/100g of Sol	10
U.S.D.A Texture:	Silt Loam

N.D: not determined

C. the experimental device

The cylinders are divided into three levels. The first level is used as witness (D.0) and the others two undergo a metal stress, with two intensities, 200 ppm (D.1) and 400 ppm (D.2). Each treatment is composed of 20 cylinders, representing ten repetitions for each of the two varieties. The whole of the cylinders are maintained with field capacity by daily contribution of a quantity of water estimated by weighing of the cylinders, in order to bring back them to maximum capacity holding. The water of irrigation is substituted each three days by a commercial nutritive solution of type ACTIVEG.

D. Application of the metal stress

The plants were stressed after 25 days of sowing by solutions of copper sulfate Cu SO4, 5H2O with the following amounts; 0 ppm (D.0) 200 ppm (D.1) and 400 ppm (D.2). The whole of measurements are carried out during one period equivalent to 80 days after sowing.

E. measured parameters

1. Proportioning of the chlorophyllian pigments

The concentrations of chlorophylls and the carotenoids are determined by spectrometry according to the procedure quoted by Lichtenthaler [16]

The following equations are used to calculate the concentrations of chlorophyls and carotenoids in the sheets (in mg/g) [19]:

- Chlr.a = 12,25 × A663 - 2,79 × A645
- Chlr.b = 21,50 × A645 - 5,10 × A663
- Chlr.a+b = 7,50 × A663 + 18,71 × A645
- CRTN = (1000 × A470 - 1,82 × Chlr.A - 85,02 × Chlr.B)/198.

2. Total protein rate of seeds:

The rough protein determination is carried out after determination of total nitrogen by the method Kjeldhal [17] according to the following formula:

Rough proteins (%) = NR % X 6,25.

3. Proportioning of the proline

The proline is determined by the method of Troll and Lindsley [42], simplified and developed by Dreir and Goring [41], the contents are expressed of (mg of proline/g MF), after the reading of the optical density with 528 nm by means of a VISIBLE spectrophotometer UV.

4. Proportioning of soluble sugars

Simple sugars (glucose, fructose, and saccharose) are proportioned by the method of Shields and Burnett [36]. The absorptance is read with spectrophotometer UV VISIBLE with a wavelength of 585 nm and the concentration is expressed in $mg.g^{-1}$ of statistical MF.

F. Treatment statistical:

The whole of given obtained are studied by stat box version 6.40, an analysis variance and a comparison of average is made. It is considered that the results are significant when P≤ 0,05.

III. RESULTS

A. Content of Chlorophyll Pigments

TABLE II
VARIANCE OF THE PHYSIOLOGICAL PARAMETERS

Variables	Cu dose (F1)	Variety (F2)	Interaction (F1xF2)
Chl a	0.0043 ***	0.169 NS	0.762 NS
Chl b	0.0442*	0.739 NS	0.508 NS
Carotenoids	0.0114*	0.932 NS	0.461 NS
Proteins	0 ***	0 ***	0.08
Proline	0.0266*	0.176 NS	0.360 NS
Sugars Soluble	0.0046 ***	0.001 ***	0.981 NS

ns: insignificant
*: significant
*** highly significant

1. Chlorophyll a:

The content Chl a (Table III) is affected by the copper amounts at the two varieties of broad bean, it decreases régressivement by 17,17 mg/g for the D.0 with 13,12 mg/g for the D.1 treatment and 8,79 mg/g for the D.2 treatment at the V1 variety. For the other V2 variety, the averages of Chl a recorded is of a lower order, in comparison with those recorded for the first variety. Thus, in D.0, one records 14,73 mg/g. In the treatments D.1 and D.2, this parameter regresses respectively with 9,86 and 8,14mg/g. Variance analysis (Table II), watch that the factor variety and the interaction of the two factors influence in a way very highly significant the variation of this element ($p<0,005$).

2. Chlorophyll b:

The exposure of the plants to copper also induces a lowering of the content Chl b at the two varieties tested (Table III). For D.0, content Chl b varied between 5,59mg/g (V1) and 4,79 mg/g at V2. For D.1 and D.2, the recorded median values express regressive evolutions compared to the control.V1 variety records the strongest reduction in the content Chl b in the level D.2, 50,08% are reached. The study of the released results (Table II) shows that the lowering of the content Chl b is narrowly dependant on the intensity of copper ($p<0.05$).

3. Carotenoids:

The average results obtained, show that, the content carotenoids decreases gradually with the intensity of copper (Table III). On the level of the D.0 amount, one records the highest values with 5,41 mg/g for V1 and 4,84 mg/g for V2. On the other hand, the application of the metal treatment D.2, the contents carotenoids prove to be the most affected by copper, by registering the most important reductions of this element, with rates respective of 43,62% (V1) and 23,55% (V2). The statistical study of the results (Table II), watch that the metal treatment induced by copper causes a significant effect on the variation at the level of the carotenoids ($p<0,05$).

TABLE III
CHLOROPHYLL RATE DEPENDING ON THE DOSE OF CU FOR V1 AND V2

Variety	Cu Dose (ppm)	Chl a (mg/g)	Chl b (mg/g)	CRTN (mg/g)
V1	0	17,17 ± 4,12	5,59 ± 1,84	5,41 ± 0,77
	200	13,12 ± 3,25	4,23 ± 0,69	4,16 ± 0,84
	400	8,79 ± 1,34	2,80 ± 0,86	3,05 ± 0,46
V2	0	14,73 ± 4,24	4,79 ± 1,68	4,84 ± 1,20
	200	9,86 ± 2,77	3,66 ± 1,27	3,98 ± 1,12
	400	8,14 ± 1,51	3,15 ± 0,17	3,70 ± 0,11

B. *Rate of total proteins of seeds*

The observation of the average results (Fig. 1) reveals a reduction in the rate of proteins according to the intensity of the Cu amounts applied. At the V1, the D.0 registers an evaluated average with 30.95%. In the D.1 and D.2, the averages of these rates are in a respective order 23,91% and 20,95%. At the V2, recorded average rates of proteins are more important, in comparison with the variety V2. Thus, for D.0, the recorded average is of 38.67%. For the treatments D.1 and D.2, the median values of the rate of proteins register in a respective order 31,89% and 27,14%. The analysis of the variance (Table II) indicates that the variation of the amounts of Cu affect the content of total proteins in a highly significant way ($p<0,005$).

C. *Content of proline:*

The average results obtained (Fig. 2), indicate that the application of copper increases in the contents of proline at the two varieties. The quantities highest are noted with the D.2 for V1 with 157,65 µg/g and with the level D.1 for V2 with 122,35 µg/g. Variance analysis (Table II), watch that the variation of the contents of proline is significantly influenced by the application of copper ($p<0,05$).

D. *Sugar content soluble:*

Besides proline , application of copper to deferential dose causes an increase in levels of soluble sugars , this increase is more marked at the V2 variety than at V1 (Fig. 3). On the D.0 this element records 125,33 µg/g for V1 and 75,71µg/g (V2). Increases of 19,76% and 52,96% one noted respectively with the levels D.1 and D.2 for V1 and 26,43% and 80,26% for V2. The average results obtained (Table II) show that the sugar content soluble is significantly influenced by the intensity of copper and the nature of led variability.

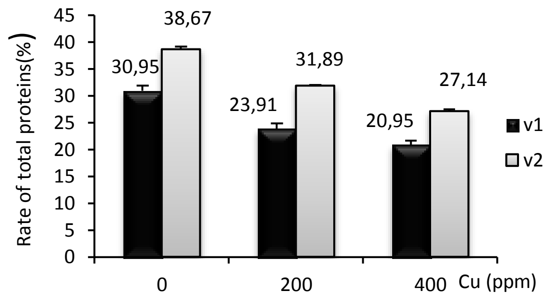

Fig.1. Evolution of Total Proteins Rate According copper Dose for V1 and V2

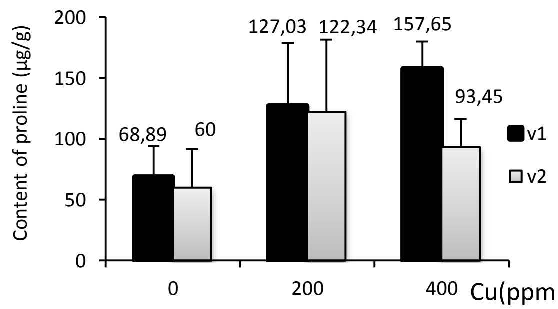

Fig.2. The Proline Evolution According copper Dose for V1 and V2

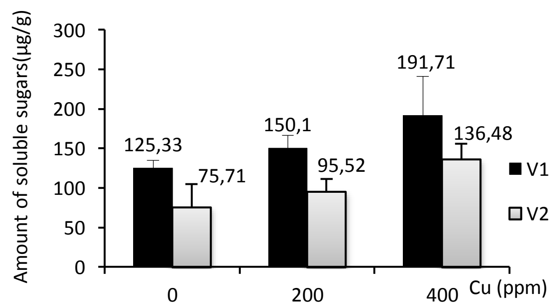

Fig.3. Evolution of Soluble Sugars Amount According copper Dose for V1 and V2

IV. DISCUSSION

The results obtained show that the copper excess caused a reduction in Chl a and Chl b of the sheet, but this reduction was more in Chl b that Chl a. Many studies found the reduction of chlorophyll by the treatment of copper [12]–[38]–[13].The reduction of chlorophyll in broad bean under treatment of copper can be due to the inhibition of the enzymes acting on the synthesis of the chlorophyll or the degradation of chlorophyll [30].Copper decreases the foliar chlorophyll rate and modifies chlorophyllian fluorescence and the activity of the photosystème II, it is responsible for an inhibition of photosynthesis by the peroxidation of the lipids of the membranes of the thylakoïdes, the blocking of the activity of the ribulose, 1-5, biphosphate carboxylase-oxygénase [5]–[11]–[8]. The reduction in the contents in chlorophyll in the sheets is accompanied by a reduction by the contents out of carotenoids [23][6][45] which at summer is in harmony with our results.

Copper seems to affect the biosynthesis of chlorophylls more than the cadmium at *Lemna trisulca* [29] and *Chlamydomonas reinhardtii* [28]. However, the effects observed seem to depend on the age of the sheets, the sheets masts being more sensitive than the newly formed sheets [46].

Copper is one of the protein components implied in photosynthesis like plastocyanines of the chloroplasts. Its role of cofactor in the transport of electrons of the photosystème II is also paramount [14]. He was recognized like an essential cofactor of the synthesis of a great number of proteins implied in the reactions of oxydoreduction, binder or activator of molecular oxygen. He is the coenzyme many metal-worker-proteins like the ascorbate oxydase and tyrosinease [10]. The Cu excess in the cells is at the origin of a stress oxidizing [9]–[31]. Cu will induce the production of reactive species of oxygen (ROS) and of free radicals. The ROS are dangerous for the cells because if they are not reduced they deteriorate proteins and amino-acids, the DNA, the ARN [31]. The results of this study revealed a reduction of the protein rates of seeds under the conditions of stress, this reduction proves to be proportional with the copper intensity. Studies showed that in answer to the oxydative damage, the activities of the endoprotéases are stimulated at plants [39]–[25] just as in answer to the presence of heavy metals such as Cd, Hg or Pb [20]–[22].

The immobilization of copper on the level of the cellular walls and the vacuoles blocks the diffusion of the complexes Cu-proteins [2] implied in many enzymatic processes. The toxicity of copper results thus in the fixing of metal on the grouping thiol of proteins what causes the inhibition of their activity, modifies their structure, or causes a deficiency in another element with which copper in excess enters in competition [15]. It results a reduction from it from the protein concentration, out of starch [40].

The accumulation of proline is usually observed at the time of environmental stresses like the presence of heavy metals, the oxydative stress, dryness [32]. The results raised in this study, show a relative increasing in the contents of the proline according to the increase in the copper amounts applied in the substrate. Aleksandra and al [35] detected an accumulation of proline in the sheets of broad bean in the majority of the cases of treatment with heavy metals (Cu, Pb, Zn, Cd, Ni, C).

Indeed, the proline is an amino-acid often considered as a biomarquor of stress [33]–[21], and considered as chelating of metals thanks to the grouping thiol (- HS) and a guard of the structure subcellulaire [26], that is related to its protective role of compatible aqueous solution implied in the stabilization of the membranes and of the proteins [7]–[24]. But it was as shown the proline could be implied in the reduction in the quantity of free radicals [47], in the trapping of the reactive species of oxygen [44], or a function in the osmotic adjustment at certain species [21]–[34].

In addition to the changes of acid content amino, an accumulation of soluble glucids was reported in this present study. Likewise, Arafat and Mona (2013) [1], recorded a notable accumulation of sugar soluble in the sheets and the roots of broad bean following an exposure to the stress induced by copper. In the same way, Alaoui-Sossé and al (2004) [4] brought back an accumulation of soluble sugars to the level of the sheets of cucumber after the treatment with copper.

V. Conclusion

These results suggest that the presence of copper in strong amounts modifies the operation and the normal development of broad bean by disturbances on the physiologically. This study showed that the metal stress induced by copper causes, on one hand, a reduction in the photosynthetic activity (chlorophyll content a, b and the carotenoids) and a reduction by the rates by proteins on the levels by seeds, on the other hand. In addition, the results recorded in this study revealed an increase in the osmoregulators (prolines and soluble sugar) which are accumulated following the presence of copper in the medium.

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