Plant Archives Volume 20 No. 1, 2020 pp. 253-259 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ROLE OF SOAKING SEEDS WITH COBALT AND ASCORBIC ACID IN ALLEVIATION OF MUNG BEAN UNDER WATER STRESS EFFECT.

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Abstract

A Field experiment was conducted in the testing ground of department of Field Crop Science \ Collage of Agriculture Engineering Science (ALJADERIA), Baghdad University during 2017's autumn aiming for studying the effect of gradient concentrations on soaking seeds with Cobalt and Ascorbic acid to decrease the effect of water stress in some phenotypic growth traits and root's dried weight of local species Mung Bean (KHADRAWI). Using Split Plot Design in The Randomized Complete Block Design (RCBD) with three replications, the irrigation treatments represent the Main Plots which it is the irrigation after depletion 50%, 60% and 70% of available water that referred I1, I2 and I3 respectively. Furthermore, the concentrations of soaking seeds with Cobalt are (4, 8, 12) $mg.L^{-1}$ and with Ascorbic acid (40, 50, 60) $mg.L^{-1}$ which referred CO_1, CO_2, CO_3 and ASA1, ASA2, ASA3 respectively. As well as the two comparison treatments, Dry seed and Distilled water sequentially for the sub-plot, in which the seeds was soaked for 24 hrs. in soaking treatments, then dried to its original humidity. The results shows insignificant differences between irrigation treatment I1 (after depletion 50% of available water) and irrigation treatment I2 (after depletion 60% of available water) for 2017's autumn season, with regard to the days from planting to 50% of flowering (51.83 and 51.75) day, plant height (69.17 and 66.92) cm, the number of leaves (75.38 and 72.54) leaf $plant^{-1}$, the leaf area (543.7 and 523.3) cm2 and the dried weight of total root (7.17 and 6.72) gr $plant^{-1}$ respectively. While the irrigation treatment I3 (after depletion 70% of available water) yield less average of studied traits. While the treatments of seeds soaking with cobalt (CO) and ascorbic acid (ASA) significantly affect in most of studied traits except the days from planting until 50% of flowering, since the treatment CO_2 significantly exceeded at concentration (8 $mg.L^{-1}$) with highest average of plant height it reach 77.89cm, leaves number 80.44 leaf $plant^{-1}$, leaf area 580.2 cm^2 and dry weight of total root 7.91 gr $plant^{-1}$ and by insignificant difference with treatment I3 at concentration (12 $mg.L^{-1}$). In the other hand treatment of soaking with ascorbic acid ASA3 exceeded at concentration (60 $mg.L^{-1}$) by increasing the number of branches and dry weight of total root compared with measurement treatments (Seed dry) and (Distilled water) for autumn season respectively. The overlap of irrigation treatments and soaking treatments had a significant effect on all studied growth traits. So we recommend in case of limited water the possibility of irrigate the Mung Bean plants after depletion 60% of available water without needed to irrigation after depletion 50% of available water without significant effect on growth traits, as well as the possibility of treat the Mung Bean plants with cobalt at concentration CO_2 (8$mg.L^{-1}$) and CO_3 (12$mg.L^{-1}$) and with ascorbic acid at concentration ASA3 (60$mg.L^{-1}$) when soaking seed and this indicator of its role in reduced the effects of water stress in some vegetative traits of Mung Bean, this may reflect positively on the productivity of this crop.

Key words : Mung Bean, Water stress, Soaking seeds, Cobalt, Ascorbic acid.

Introduction

The aggravating water crisis form in present and future great challenges in agricultural sector to achieve sustainable development in arid and semi-arid areas, especially in Middle East including Iraq, the agricultural sector constitute the main consumer of water it is 65% of consumed water in world. While the agriculture consume 92% of available water in Iraq and this adversely affect in food production when the population is increasing, the expectations suggest increase in importation of food in these areas in circumstance of population growth and lack of production under imperfect growth circumstances. The long term water deficit lead to changes in natural environment of plants in general and then changes in physiological doing and reduction in output more than other stresses combined. And this demand take care of

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available water resources, not to waste it and water rationing to get highest productivity with lowest water amount. Therefore, researchers and specialist in agricultural field persevere to using some agricultural applications that aims to exclude or overcoming the physiological symptoms of grown plants in harsh environment like dryness or water deficit to facilitate resistance of stress and giving good crop under water insufficiency.

Thus, it will use seed soaking technology which mean soaking seed by the water or nutrient solutions and vitamins for certain period before the agriculture and it is one of the most important ways that helped in seed growth control by response to previous adaptations when various stresses occur including water deficit and then damage reduction that caused by this stress in morphological, physiological, anatomical and biochemical aspects than result in decreasing plant size, leaf area, stems, fruits and roots development and result reduction (Cazares and others, 2010; Mafakheri and others, 2010; Alemu and Tekle, 2016), in addition of this technology give strong and homogenous gestures and good established field and almost give high result or without occurrence of huge decrease in seeds results compared with plants result from dried seeds and applicable, low cost and important application in arid and semi-arid regions.

Cobalt is one of the minor beneficial nutrients which has an effective role in facing the stress because it improve the water balance of cultivated plant cell under water and thermal stress and salinity that may be due to its importance in increase plant efficiency by strengthen plant growth processes including delay plant aging and improve nutrients movement from root to vegetative parts and vice versa and increase efficiency of growth regulators and increase production, as well as its specially importance of legume crops because it is the main element in synthesis of vitamin B12 which is important in forming root nodules and then the efficiency of nitrogen fixation increase which positively reflect in plant growth and result (Gad and El-Metwally, 2015).

The vitamins are considered one of the tools that can be used in facing the harmful effect of environmental stresses, especially water stress so functioning as anti-oxidant like vitamin C (ascorbic acid) which is one of non-enzymatical anti-oxidants that the plant use it under stress circumstances to deal with adverse effects that imposed by reactive oxygen types, as well as it role as co-factor to many enzymes and regulator to plant hormonal signal during transitional phase from vegetative stage to reproductive stage. Ascorbic acid has an important role in increasing cells volume and division and activate photosynthesis (Magahase and Church, 2006).

Materials and Methods

A field experiment was conducted in the testing ground of department of Field Crop Science \ Collage of Agriculture Engineering Science (ALJADERIA) \ Baghdad University during 2017's autumn, in a soil that's physical and chemical properties are illustrated in (chart 1) aiming to study the effects of graded soaking concentrations to soak the seeds with cobalt and ascorbic acid linked with the water stress in some of the appearance characteristics of growth and the dried weight of the roots of the mung bean crop of the local species (KHADRAWI). Randomized complete blocks design (RCBD) was used by split plots design, represent three level of water stress for the main plot and depletion 50%, 60% & 70% of the available water referred to as I1, I2 and I3 respectively while the soaking concentrations of seeds with cobalt (4, 8 and 12) $mg.L^{-1}$ and ascorbic acid of (40, 50 and 60) $mg.L^{-1}$ and referred to as CO_1, CO_2, CO_3, ASA1, ASA2, ASA3 respectively, as well as comparison treatments of dry seeds and soaking with distilled water for the sub plot, where seeds were soaked for 24 hours in the soaking treatments and then dried to its original degree of moisture and depletion treatments were applied at stage 6. Soil and crop process were conducted such as plowing and smoothing and settlement then the experiment ground was divided into experimental units each unit with an area of (2*3) m^2 including 6 lines the length of which is 3 meters and are 0.30 meters apart and there's a 0.25 meters between each cavern to attain a plant density of 133333 plants H^{-1}, a distance was left between experimental unit plots 1m and 2m between the main treatments, same with repeaters to block water leakage and fertilizers crossing. Mung bean seeds (local species) were planted then a super triphosphate fertilizer (P_2O_5 26%) was added with an average of 75kg P_2O_5 H^{-1} in one batch mixed with the soil before planting. While the nitrogen fertilizer was added in two batches in the form of urea (N 46%) with an average of 40kg N H^{-1} the first batch is added when planting and the second is added at the stage of flowering (Ali, 2012).

Soil capacity of water retention was estimated by the relationship between the structural tension for a sample of soil and the moisture content when tightness of 0, 33, 100, 500, 1000, 1500 kilo pascal by which the water content for the soil was calculated from the difference of the moisture content at the field capacity and the wilting point.

The volumetric method was used to measure the moisture content of the soil and to observe the moisture changes and to determine the time of irrigation depending on the depletion level of irrigation treatments. By taking soil samples in an auger on day before irrigation and two days after from the depth of 0-20 cm and 0-30 cm until the end of the experiment then placed in an aluminum can and weighed while moist in a micro water oven for 12 minutes after calibration of the drying duration with the electrical oven according to the suggested method by (Zein, 2002) for samples drying then weighed after drying and the moisture content was estimated according to the following equation:

$$Qv = Qw \times \partial b \qquad ...1$$

Whereas

Qv is the moisture content based on volume

Qw is the moisture content based on weight

∂b is the apparent density of soil (mega-gram^{-3})

Irrigation was performed by plastic tubes attached to an electrical pump, equal amount of water was added to every plot when depletion reaches 50% of the available water at the depth of 0-20 cm when planting until the plant reaches stage 6 with constant monitoring of the moisture content of soil. Irrigation process was continued until the plant reaches stage 6 at which plants need to be exposed to the water tension when depletion 50%, 60%, 70% of the available water at depths of 20 cm and 30 cm according to the growth stages mentioned previously, the amount of irrigation water for the depth of 20 cm for the treatments I1, I2, I3 was (97.2, 116.4, 136.8) liter /6 m^2. While the amount of water for 30 cm depth was (146.8, 174.6, 205.2) liter/6 m^2 until the last irrigation time when the plant reaches physiological maturity.

The water depth that should added to compensate depleted moisture was calculated according to (Allen and others, 1988) equation:

$$d = (\theta_{fc} - \theta_I) \times D \qquad ...2$$

Whereas

d is the depth of added water (mm)

θ_{fc} is the volumetric humidity at field capacity (cm^3 cm^{-3})

θ_I is the volumetric humidity before irrigation (cm^3 cm^{-3})

D is the depth of soil that equal the effective total root depth (mm)

Measured Traits

1. Number of days from implantation until 50% flowering
2. Plant length (cm)
3. Number of branches (branch.plant^{-1})
4. Number of leaves (leaf.plant^{-1})
5. Leaf area (cm2.plant^{-1})
6. Dried weight of root (gr.plant^{-1})

Statistical Analysis

The experiment data was analyzed statistically according to variance analysis method in split plot design of R.C.B.D and low significant difference L.S.D was chosen in the scale 0.05 to compare between arithmetic averages of treatments (Steel and Torrie, 1980) using Genstat software.

Results

The results of table 2, 3, 4, 5, 6, 7 shows presence of significant effect of available water depletion treatments in all studied traits which are number of days from implantation until 50% flowering, plant height, number of branches, number of leaves, leaf area and dried weight of total root of 2017's autumn, so treatment I3 (depletion 70% of available water) recorded lowest average of days to reach 50% flowering reached 48.54 day, plant height 47.33 cm, number of branches 6.23 branch.plant^{-1}, number of leaves 53.33 leaf.plant^{-1}, leaf area 315.5 cm^2 and dried weight of total root 5.21 gr.plant^{-1} respectively and significantly differed from treatments I1,I2 (depletion 50%, 60% of available water) which recorded highest average for all these traits reached 51.83 and 51.75 day, 60.17 and 66.92 cm, 8.45 and 7.55 branch.plant^{-1}, 75.38 and 72.54 leaf.plant^{-1}, 533.6 and 504.0 cm^2, 7.17 and 6.72 gm.plant^{-1} respectively and not significantly differed from each other except trait of number of branches.

As the results of tables 2, 3, 4, 5, 6, 7 illustrate the presence of significant effect of seeds soaking treatments in all studied traits except trait of number of days from

Table 2: The effect of available water depletion and soaking treatments in average of number of days from implantation until 50% flowering for 2017's autumn.

Seeds soaking treatments	Available water depletion			Average
	I1	I2	I3	
Dry Seed	51.00	51.33	49.33	50.22
Distilled water	52.00	51.33	48.67	50.67
CO_1	53.00	52.33	48.00	51.11
CO_2	53.00	51.33	48.67	51.00
CO_3	52.33	51.00	49.33	50.89
ASA1	51.33	52.00	48.33	50.55
ASA2	50.00	52.00	49.00	50.33
ASA3	52.00	52.67	48.00	50.89
L.S.D 0.05	1.78			N.S
Average	51.83	51.75	48.54	
L.S.D 0.05	1.36			

Table 3: The effect and overlap of available water depletion and soaking treatments in average of plant height (cm) for 2017's autumn.

Seeds soaking treatments	Available water depletion			Average
	I1	I2	I3	
Dry Seed	48.00	55.67	35.67	46.44
Distilled water	50.33	55.67	35.67	47.22
CO_1	79.33	67.33	41.67	62.78
CO_2	81.33	86.33	66.00	77.89
CO_3	88.67	76.33	58.33	74.44
ASA1	70.33	71.33	49.00	63.56
ASA2	75.00	62.00	49.00	62.00
ASA3	60.33	60.67	43.33	54.78
L.S.D 0.05	7.40			4.26
Average	69.17	66.92	47.33	
L.S.D 0.05	3.74			

Table 4: The effect and overlap of available water depletion and soaking treatments in average of number of branches ($branch.plant^{-1}$) for 2017's autumn.

Seeds soaking treatments	Available water depletion			Average
	I1	I2	I3	
Dry Seed	6.33	5.50	5.31	5.71
Distilled water	7.25	6.50	5.83	6.52
CO_1	9.50	8.00	5.85	7.78
CO_2	9.00	8.08	7.50	8.19
CO_3	9.83	8.98	7.36	8.72
ASA1	8.16	7.33	7.25	7.58
ASA2	8.50	7.83	5.13	7.15
ASA3	9.08	8.18	5.60	7.62
L.S.D 0.05	1.26			0.66
Average	8.45	7.55	6.23	
L.S.D 0.05	0.88			

implantation until 50% flowering, so the treatments of soaking with cobalt at concentration CO_2, CO_3 - insignificant difference between each other and with ascorbic acid at concentration ASA1 significantly exceeded in giving highest average of plant height reached 77.89 and 74.44 and 63.56 cm with increasing percentage reached (67.72 and 64.95%) and (60.29 and 57.64%) and (36.86 and 34.60%) by measuring with the two comparison treatments Dry seed and Distilled water respectively (Table 3). Also, the seeds soaking process before implantation led to increase in number of branches of mung bean crop (Table 4), the treatment of cobalt at concentration CO_3 recorded highest average of number of branches reached 8.72 $branch.plant^{-1}$ which doesn't significantly differ from treatment of cobalt at concentration CO_2, but significantly differed from the treatment at concentration CO_1. While the treatment of ascorbic acid at concentration ASA3 recorded average reached 7.62 $branch.plant^{-1}$ which doesn't significantly differ from treatments ASA1, ASA2, CO_1 by measuring with the two comparison treatments Dry seed and Distilled water that recorded lowest average of number of branches reached 5.71 and 6.52 $branch.plant^{-1}$ respectively.

While the results of table 5, indicated the significant overcoming of cobalt treatment CO_1, CO_2, CO_3 without significant difference between them and ascorbic acid treatment ASA1 by giving highest average of number of leaves it reached 73.44, 80.44, 80.00, 70.67 $leaf.plant^{-1}$ measured by the two comparison treatments respectively. However the results in (table 6) shows that the soaking treatment with cobalt and ascorbic acid led to significant increase in average of leaf area measured by the two comparison treatment Seed Dry and Distilled Water which recorded lowest average for this trait reached 363.3, 319.7 $cm^2.plant^{-1}$ respectively. Whilst the average of leaf area reached its highest limit at soaking treatment with cobalt CO_2 it is 580.2 $cm^2.plant^{-1}$ that doesn't significantly differ from CO_3, also the results in (Table 7) shows that the average of dried weight of total root affected by soaking treatment with cobalt and ascorbic acid before planting, the treatments CO_2, CO_3, ASA3 recorded highest average of dried weight of total root reached 7.91, 7.80, 7.57 $gr.plant^{-1}$ and doesn't differ from each other's measured by the two comparison treatment Dry Seed and Distilled Water that recorded lowest average of leaf area reached 5.36, 6.01 $gr.plant^{-1}$ respectively. The results of previously mentioned tables indicate the significant effect of overlap between the two studying factors in all indications of studied vegetative growth for 2017's autumn.

Discussion

It becomes obvious from the tables (2, 3, 4, 5, 6, 7) that the higher soil moisture treatment (low stress) designated by I1 (depletion of 50% of the available water) and the treatment I2 (depletion of 60% of the available water) didn't differ fundamentally in all the studied indicators except that the number of plant branches, as it has enhanced the vegetative growth of the mung bean. as the availability of appropriate moisture in the soil has an important role in the growth of the growth of the roots, its deepening, the absorption of water and nutrients and its distribution inside the plant and its reflection in the growth of the plant cells, its division, its enzyme activities and the constancy of the photosynthesis and then increasing the accumulation of the dry matter of the plant. as all parameters of vegetative growth has declined in the over mentioned tables with a decrease in the amount of the added water at I3 (depletion of 70% of the available water), results from table 2 have shown a reduction of the number of days from the time of implantation to 50% flowering under the effect of moisture depletion indices,

Table 5: The effect and overlap of available water depletion and soaking treatments in average of number of leaves (leaf.plant^{-1}) for 2017's autumn.

Seeds soaking treatments	Available water depletion			Average
	I1	I2	I3	
Dry Seed	58.00	67.67	49.67	58.44
Distilled water	49.67	55.00	42.33	49.00
CO_1	98.67	66.67	55.00	73.44
CO_2	86.33	96.33	58.67	80.44
CO_3	90.33	89.00	60.67	80.00
ASA1	78.00	76.00	58.00	70.67
ASA2	75.67	62.67	51.00	63.11
ASA3	66.33	67.00	51.33	61.56
L.S.D 0.05	9.92			5.75
Average	75.38	72.54	53.33	
L.S.D 0.05	4.78			

Table 6: The effect and overlap of available water depletion and soaking treatments in average of leaf area (cm^2) for 2017's autumn.

Seeds soaking treatments	Available water depletion			Average
	I1	I2	I3	
Dry Seed	353.3	397.0	305.8	353.8
Distilled water	418.8	488.2	358.6	421.8
CO_1	711.4	481.0	397.1	529.8
CO_2	622.6	694.6	423.4	580.2
CO_3	651.4	641.8	437.8	577.0
ASA1	562.6	658.2	418.6	509.8
ASA2	545.8	452.2	368.2	455.4
ASA3	478.6	483.4	370.7	444.2
L.S.D 0.05	71.4			41.4
Average	543.7	523.3	385.0	
L.S.D 0.05	34.4			

the reduction of water with the temperature and the increase in wind speed and the decrease in the relative moisture have increased the speed of the biological processes that undergoes inside the plant which has induced a faster flowering, which is one of the plant mechanisms to withstand the water stress and its known as the plant escape from stress, meaning the ability of the plant to complete its life cycle before the presence of a critical water stress, this goes with what was found by (De Craene, 2010; Al-Kaisy and Al-Mentafji, 2011), whose have assured that the time form implantation until 50% flowering for the mung bean has been reduced with the reduction in the irrigation water amount and it has been noted a reduction in the height and the number of branches and leaves and the leaf area and the dried weight of total root in (Tables 3, 4, 5, 6, 7) successively by the depletion treatments, this could be attributed to the short period from implantation until 50% flowering (Table 2) which undergoes within it the period of growth and widening of the leaves and elongation of the stem cells and the water stress has reduced the relative water content (unreleased data) which determines the division, widening and elongation of the stem cells, as well as a reduction in number and area of the leaves (Tables 5, 6) which has led to a reduction in the in the plant cover which allowed the light to reach down to the vegetation and this didn't give the chance for the growth hormone Auxin to act by elongating the internodes because it underwent photolysis deceasing the height of the plant and the number of branches, as well as the water stress can contribute in the generation of free radicals which leads to a disturbances in the enzymatic system which then decreases photosynthesis and stops division, elongation and growth development (Gupta, 2011), at the same time the reduction in the leaves area (Table 4) is attributed to the reduction in the absorbed water which acts to reduce the leaf water potential which reduce the energy production in the light reactions (Verma and Verma, 2010) and this in turn leads to a reduction in the dried weight of total root and this is attributed to the to negative effect of water stress form different aspects including weakening of roots supply from the materials produced by the shoot system and hormonal defect which inhibits the growth of roots due to a reduction in the levels of auxin coming from the shoot system, as well as a reduced plant height and the number of branches and leaves and the leaf area (Tables 3, 4, 5, 6) successively, which has been reduced by the increase in the water stress which is reflected on the growth of roots, as the root growth depends on the products of metabolism in the shoot system, this goes with the results of (Habibzadeh and others, 2013; Al-Hussaini and Alsaadawi, 2013; Anjum and others, 2015; Al-Karkhi, 2017) which have found a reduced vegetative growth indices for the mung bean under the effect of water stress, form the results previously mentioned we notice that the seed irrigation factors have contributed to a fundamental increase in all the studied characters under study except for the number of days from cultivation to 50% flowering which didn't show an obvious differences, the seeds irrigation by cobalt has led to an enhanced vegetative growth characters by increasing the levels of seed irrigation and this is attributed to the positive role of cobalt which had increased the plant height (Table 3) and number of branches (Table 4) and number of leaves (Table 5) and the leaf area (Table 6) and the dry weight for the root system (Table 7) and this is attributed to role of cobalt in enhancing plant efficiency by enhancing the plant growth process including a delayed aging for plants and an enhanced mobilization of food elements from the root to the vegetative parts and vice versa and enhancing form the growth regulators and enhancing production (Gad and

Table 7: The effect and overlap of available water depletion and soaking treatments in average of dried weight of total root (gr2) for 2017's autumn.

Seeds soaking treatments	Available water depletion			Average
	I1	I2	I3	
Dry Seed	4.73	6.17	5.18	5.36
Distilled water	6.13	7.22	4.67	6.01
CO_1	5.26	4.69	3.59	4.51
CO_2	10.00	6.67	7.07	7.91
CO_3	10.09	6.92	6.40	7.80
ASA1	6.03	6.00	5.08	5.70
ASA2	6.08	7.59	4.59	6.09
ASA3	9.05	8.54	5.11	7.57
L.S.D 0.05	1.41			0.78
Average	7.17	6.72	5.21	
L.S.D 0.05	0.86			

El-Metwally, 2015) this goes with the results of other studies which has showed that cobalt usage on the soybean plant (Kandil and others, 2013) and fenugreek (Gad and Abdel-Moez and others, 2015) and fava beans (Gad and others, 2017) and the increased concentration of ascorbic acid leading to an enhanced traits of plant height and number of branches and the leaf area and the dried weight of total root is attributed to the positive role of ascorbic acid in enhancing the vegetative growth qualities by increasing cell division and expansion and activating the metabolism process and delaying aging for the leaves because it effect is similar for the plant growth regulators and this has been reflected in net effect of increase in the dried weight of total root (Abrahamian and Kantharajah, 2011; Zhang, 2012). We conclude that the I2 treatment (depletion of 60% of the available water) has given the same effect in the studied traits and without a significant difference than I1 treatment (depletion of 50% of the available water) and in another meaning it's possible to get the same effect with a lesser amount of added irrigation water, to meet the requirements of growth for the mung bean plant and this clarify the functional role for technology of irrigation the seeds with a solutions rich in cobalt and ascorbic acid and that's because of their role in decreasing the deleterious effects of water stress *via* enhancing the water equilibrium inside the plant cells, enhancing the efficiency of metabolism and enhancing the important enzymes in the different biological processes which has led to an enhancement in the vegetative growth for the mung bean plant represented by plant height, number of branches,number of leaves and the leaf area which was reflected in summation to a generation of a deep root system to absorb the largest amount of water and nutrient elements and its transportation to the plant via the increase in its dried weight.

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