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Dry matter, growth, and mineral status of cowpea grown under drought and amino acid application

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A POT experiment was carried out in the greenhouse of the National Research Center, Dokki, Cairo, Egypt during summer season of 2018 to study the role of proline and glutamic acid on dry matter, photosynthetic pigments and mineral status of cowpea plants. Missing of irrigation negatively affected stem and top dry weight of cowpea plants. The depressive effect was more under missing of irrigation at flowering than at filling stage. This was true for the above mentioned criteria. Generally, application of amino acid (glutamic and proline) improved growth traits stem and top dry weight resulted from glutamic acid treatment exceeded those obtained from proline spraying. Missing of irrigation at any stage of growth under study did not affect the concentration of Chlorophyll (Chl. a and Chl. a + Chl. b), while, carotenoids and total Chlorophyll concentrations were slightly affected. However, (Chl. b) concentration increased under drought treatment during fruiting period but the difference not great enough to reach the level of significance. Chlorophyll a: Chl b ratio increased with the drought in the same period only. Application of glutamic acid showed the highest levels of Chl a, Chl. b total chlorophyll concentrations but carotenoids were slightly affected. Furthermore, Chl. a: Chl. b and Chl. a + Chl. b carotenoids ratios showed the similar responses. Nitrogen and phosphorus concentration in cowpea seeds decreased with drought and depression increased by delaying subjection plants to drought. For potassium, its concentration increased by drought but the increment was more when drought was done at flowering stages. The concentration of nitrogen increased by both treatment of amino acids but the increase by proline exceeded those by glutamic acid. Phosphorus increased only by proline treatment while potassium concentration rose approximately with similar trends either by proline or glutamic acid). A positive relationship was found between the concentration of N and spraying with amino acids. Under regular irrigation or drought at fruiting proline induced the high effect than glutamic acid or control treatment while under drought at flowering proline or glutamic acid induced approximately the same increase. Concerning K concentration in seeds of cowpea, it showed approximately the same response of P concentration with both amino acids exogenous spraying under moisture stress at flowering or fruiting stages as well as with the regular irrigation all over the growth stages of cowpea plants.

Keywords: Cowpea ((*Vigna Unguiculata L.*Walp*)*-Drought-Amino acids- Dry matter-Photosynthetic pigments-NPK concentration.

Introduction

Global climate changes are expect to increase the duration and occurrence of water stress in arid and semi-arid regions, it leading farmers and governments in different regions to face the increasing risk of food security. As possible technological alternatives, use of more drought-tolerant species and varieties associated with high nutrient-use efficiency may help cope with or at least ameliorate these abiotic stresses problems. Thus, efforts of ameliorate and increasing plant tolerance to these effects or diminishing reasons including drought should be made and to find alternatives in order to decrease the induced effects of water stress on crops (**Da Silva, *et al.*, 2011 and Hussein, *et al.*, 2019a**).

Plant growth is often suppressed as a result of root zone stress such as water logging, drought and salinity not only shows several physiological and biochemical disturbances associated with general reduction in size but also exhibits characteristic modifications in structure particularly of leaves **(Mamdouh, *et al*., 2002 Hussein, *et al*., 2019b and Gaber, 2003).** Water is vital for plant growth and development. Water-deficit stress, permanent or

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temporary, limits the growth and the distribution of natural vegetation and the performance of cultivated plants more than any other environmental factors **(Shao, *et al.*, 2008).**

Cowpea (*Vigna unguieulata L.* Walp) is consider one from the main leguminous crops grown in summer season and its water requirements less than many of the other leguminous crops and its seeds can use for human and the vegetative parts for animal feeding **(Emngour, 2007).** Cowpea is a major source of dietary protein and essential component of the cropping systems in Sub-Saharan Africa (Simi-arid regions) as feed or food (**Kyei-Boahen, *et al.*, 2017**). Cowpea seed contains about 25% protein and 64% carbohydrates (**Singh, *et al.*, 2012**). **Dakora (2012)** shows that trace elements and macronutrients tend to be high on cowpea leaves than grains. Total nitrogen and protein of pod and seed raised rapidly during early stages of growth and the concentration decreases as time of maturity reach. For instance, where cowpea was compar to other legume crops on their nutritional quality, it could be shown that cowpea had the highest content of potassium, magnesium and phosphorus. Many investigations were conducted and reported the adverse effect of water stress specially at flowering stage **(Pallardy, 2008). El-Bastawisy (1999)** mentioned that the differences in organic acid in response to water stress conditions may be a reflection on relative turgidity, water relation, dehydration or coagulation of protoplasm that would lead to disturbances in metabolic activities by ribosomes and enzymes. In addition, different water treatments so as wet and or water logging reduced significantly the carbohydrate fraction such as soluble sugars, sucrose, polysaccharides and total carbohydrates. Carbone dioxide (Co2) photo-assimilation indicated that soluble, insoluble and consequently total photosynthesis were reduced under the effect of water stress. A significant decreases in total-N and protein-N were observed in relation to the control treatment. It is clear from the results that the reductive effect with water stress on mineral ions content (K, Ca, Mg, Mn and Fe) of investigated plant was alleviated by foliar application of phytochemicals.

Moreover, **Ali, *et al.*, (2007)** found that seeds sowing or spraying different plants with amino acids enhanced its vegetative characters or dry weight and yield. Although research and practices aimed at improving water-stress resistance and Water-Use Efficiency (WUE) have been carried out for many years, the mechanism involved is still not clear. Therefore, several researches have been done in different fields, one of them the use of phytochemical as exogenous application for elevating plant tolerant to moisture stress **(Emngour, 2007).**

Therefore, the current study designed to investigate the effect of exogenous application of two amino acids (proline and glutamic acid) on growth and mineral status of cowpea plants grown under water stress condition.

Materials and Methods

A pot experiment was carried out in the greenhouse of the National Research Center, Dokki, Cairo, Egypt during summer season of 2018 to study the role of proline and glutamic acid on dry matter, photosynthetic pigments and mineral status of cowpea plants.

The experiment included 9 treatments which were the combination of 3 water stress treatments (without, flowering and Fruiting) and two amino acids treatments (200 ppm of proline and glutamic acid) and distilled water sprayed as a control) with 6 replicates. Metallic ten pots 40 cm. in diameter and 60 cm. in depth were use. Every pot contained 30 Kg. of air dried clay loam soil. The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and metal. The physical and chemical properties of clay soil and sea water were illustrated in Table 1and 2 using the methods described by **Cottoniee, *et al.*, (1982).**

Seeds of (*Vigna unguiculata L.*Walp) were sown in the 1st day of March. Plants were thinned twice, the 1st after 8 days from sowing and the 2nd 12 days latter to leave five uniform plants /pot. Calcium super phosphate (15.5% P205) and potassium sulfate (48.5 % k20) in the rate of 3.0 and 1.50 g/pot were added twice, before sowing and two weeks later. Omitting of irrigation at flowering and fruiting stages more than regular irrigation as a control. Proline and glutamic acid treatments were sprayed twice 20 and 35 days after sowing. Chlorophyll A, B and total carotenoids concentrations were by the methods described by **Wellburn (1994).**

Two plants from every treatment were collected, cleaned, and dried in electric oven at 70 C and ground in stainless steel mill. Seeds powder was digested and nutrients were determined using the methods of **Cottoniee, *et al.*, (1982).**

All collected Data was subjected to the proper statistical analysis using the methods described by **Snedecor and Cochran (1982).**

Table 1. Some physical and chemical characteristics of the soil used in this study.

A- Soil mechanical analysis.

Course >200 µ %	Fine 200-20 µ %	Silt 200-2 µ %	Clay <2µ %	Soil texture
9.8	22.2	21.5	46.5	Clay loam

B- Soil chemical analysis

pH 1:2.5	EC dSm^{-1}	$CaCO_3$ %	OM %	Soluble cations Meq/100g soil				Soluble anions Meq/100g soil			
				Na^+	K^+	Ca^{++}	Mg^{++}	CO^{--}_3	HCO^-_3	Cl^-	SO^-_4
7.87	2.21	2.43	0.97	2.43	0.44	2.58	1.24	0.0	0.94	1.96	3.79

Available macro-nutrients%			Available micro-nutrients ppm			
N	P	K	Fe	Zn	Mn	Cu
0.76	0.19	0.98	4.9	3.8	8.6	3.98

Table 2. Chemical analysis of sea water used in irrigation.

Source	pH	EC dSm-1	Soluble cations (mM)				Soluble anions(mM)				Total soluble salts mg/L
			Na^+	K^+	Mg^{++}	Ca^{++}	CO^{--}	HCO^-_3	Cl^-	SO_4^{--}	
Sea water	7.94	50	475	9.70	56..0	10.0	2.50	2.30	536	28.0	32.0

Results and Discussion

Dry Matter

• ***Drought***

Data presented in Table (3) showed that missing of irrigation negatively affected plant height, number of green leaves and stem, leaves and top dry weight of cowpea plants. The depressive effect was more under missing of irrigation at flowering than at filling stage. This was true for the above mentioned criteria.

Kandil, *et al*., (2010) reported that plant height, number of leaves and dry matter of leaves and bulbs of onion plants increased by the increasing number of irrigation. Plant response to water stress depends mainly on the severity and duration of the stress and the growth stage of the plant. Different physiological and biochemical processes are altered by water stress, such as water relation (**Camilia, *et al*., 2013**), gas exchange, photosynthesis (**Pagter, *et al*., 2005 and Hussrin, et al**) and the metabolism of carbohydrates, protein, amino acids and other organic compounds (**Hussein, *et al*., (2009) and Hussein, *et al*., (2011)** noticed that the depression on growth parameters may be due to the nutrients absorption translocation and distribution or nutrients in plant tissues and this intern on dry matter and yield.

Table 3. Response of dry matter of cowpea plants to drought by missing of irrigation at some growth stages.

Omitting of irrigation	Dry weight (g):		
	Stem	Leaves	Total
Without	5.31	6.35	11.66
flowering	3.45	4.60	8.05
Fruiting	3.63	5.14	8.77
LSD at 5%	1.41	N.S.	2.11

Drought affected the most physiological processes inter the plant. Furthermore, the negative effect of drought may be attributed to some other effects such as its effect on water adjustment (**Vendruscolo, *et al*., 2007)** photosynthesis (**Bloch, *et al*., 2006**) hormonal balance (**Hussein, *et al*., 2009**), protein building (**Silvaria, *et al*., 2001**), enzymes activity (**Liu, *et al*., 2009**) or oxidative defense (**Celik, *et al*., 2007**).

• ***Glutamic and proline acids***

Table (4) showed the effect of proline and glutamic acid spraying and its effect on dry matter of cowpea plants. Generally, application of amino acid improved growth traits. Stem and top dry weight resulted from glutamic acid treatment exceeded those obtained from proline spraying. **Kim, *et al*., (2010)** noticed that while glutamine did not affect root growth, over 0.1 mM glutamate inhibited severe root growth. However, when the amino acid solution was adjusted to pH 5.7 and added into medium, *Arabidopsis* seedlings show normal growth pattern on medium containing glutamate or aspartate. These results demonstrated that inhibition of the root growth by high concentration of exogenous glutamate was a result of the low pH toxicity caused by acidic amino acid, although low concentration (0.05 mM) of glutamate has an inhibitory effect on the primary root growth.

Table 4. Response of dry matter of cowpea plants to spraying with glutamic acid and proline.

Amino acids (200 ppm)	Dry weight (g):		
	Stem	Leaves	Total
Without	3.36	4.56	7.92
Proline	4.06	5.98	10.04
Glutamic acid	4.97	5.55	10.52
LSD at 5%	0.55	1.43	2.06

Cao, *et al.*, (2010) revealed that glutamic acid solution at the concentration of 0.5-10 mmol/L decreased nitrate contents in Chinese chive leaves. Especially, the treatment of Glu. at 5 mmol/L produced an obvious increase of 15.1%. However, the total-N content increased in Chinese chive leaves as increasing of glutamic acid concentration at the range of 0.5-10 mmol/L Meanwhile, activity of nitrate reductase (NR) and glutamine synthetize (GS) raised by a wide margin, and chlorophyll content increased from 13.7 to 29.5%. In addition, the contents of free amino acid, soluble protein, and soluble sugar also increased.

Foliar application of proline increases plant yield, plant height, leaf number and stimulates the growth of root system (**Teixeira da Silva, 2013**), as well as activates nitrogen uptake and the synthesis of amino acids and chlorophyll (**Takeuchi *et al.*, 2008**). The optimal proline level was 50mM. Exogenous application of proline significantly increased plant growth and nitrogen assimilation; however, the effect depends both on the plant species and the cultivar of treated crop.

• ***Drought x Glutamic and proline***

Data showed in Table (5) and Fig. (1) Presented the interaction effect of drought and spraying of glutamic and proline acids on dry matter of cowpea plants. Generally, application of both amino acids increased the growth characters of cowpea plants under different irrigation treatments i.e. regular irrigation or omitting of irrigation at flowering or fruiting periods. The improvement caused by glutamic acid spraying in dry weight of stem exceeded those obtained by proline spraying under drought or regular irrigation while on leaves under regular irrigation only. Moreover, glutamic acid treatment induced the same response of stem on stem+leaves dry weight.

Table 5. Response of dry matter of cowpea plants to drought by missing of irrigation at some growth stages and spraying with glutamic acid and proline.

Omitting of irrigation	Amino acids (200 ppm)	Dry weight (g):		
		Stem	Leaves	Total
Without	Without	4.62	5.43	10.05
	Proline	5.36	6.65	12.01
	Glutamic acid	5.95	6.96	12.91
Flowering	Without	2.31	3.82	6.13
	Proline	3.17	5.44	8.61
	Glutamic acid	4.87	4.55	9.42
Fruiting	Without	3.16	4.43	7.59
	Proline	3.65	5.85	9.50
	Glutamic acid	4.09	5.13	9.22
LSD at 5%		0.96	N.S	N.S

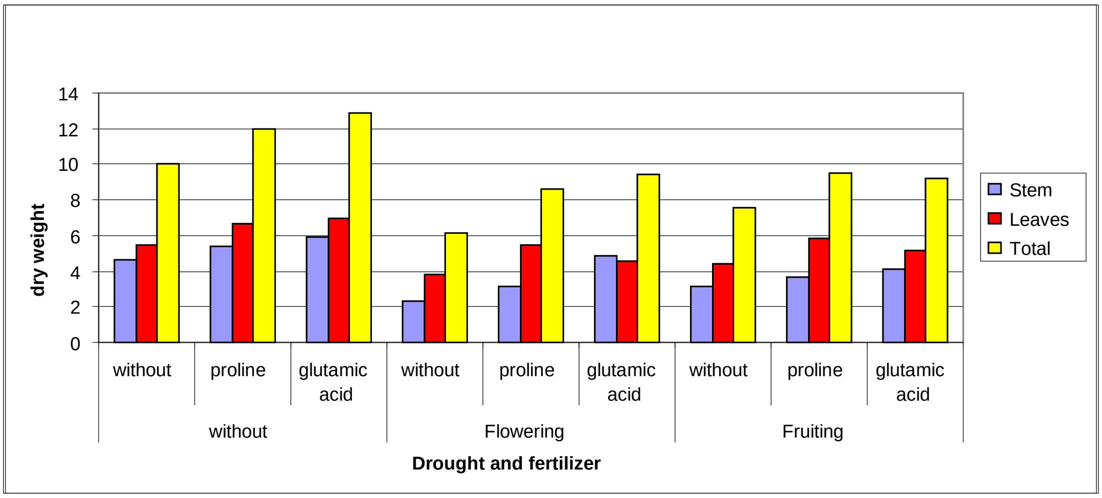

Fig. 1. Effect of drought and fertilizer on dry matter of cowpea.

Except under drought through fruiting period which treatment of proline showed the highest percentage of improvement. Various growth regulators are used for plant stress reduction. They help to maintain the osmotic pressure required in cells and protect against increased saline concentration in the nutrition medium (**Tripolskaja and Razuka, 2019**).

Ali, *et al.*, (2008) concluded that it is evident from different reports that exogenous application of proline induces abiotic stress tolerance in plants.

In unstressed seedlings, proline uptake was detected only at higher (1 mM) concentration of applied L-proline. However, proline uptake was promoted at all (1 µM to 1000 µM) concentrations of applied L-proline under osmotic stress conditions. Amongst other exogenous amino acids, L-leucine, L-glutamic acid, L-alanine, and L-histidine enhanced endogenous levels of proline, while exogenous hydroxyl proline and γ-amino butyric acid reduced it. **Kamaran, *et al.*, (2009)** pointed out that exogenous application of proline as a pre-sowing seed treatment improved shoot and root fresh and dry weights, shoot length and grain yield under both non-stress and stress conditions and total leaf area per plant only under stress conditions. Overall, drought stress adversely affected the plant biomass, gas exchange characteristics and grain yield of all wheat cultivars under investigation. Exogenous application of proline as a pre-sowing seed treatment mitigated the adverse effects of drought on growth and yield. Increase in growth with proline was not found to be associated with net CO2 assimilation rate. The various proline levels used for pre-sowing seed treatment, 40 m*M* was resulted in more effective in enhancing growth of wheat cultivars.

Proline, which is an indicator of stress, is often considered as a good parameter for the testing of plants with good drought tolerance capacity. Thus, exogenous application of proline is a possible technique to avoid the deleterious effects of the drought on plant growth (**Bekka, *et al.*, 2018**).

Chlorophyll and carotenoids

• ***Drought***

Missing of irrigation at any stage of growth under study did not affect the concentration of Ch. a, Ch. a + Ch. b while, carotenoids and total chlorophyll concentrations were slightly affected. However, Ch. b concentration increased under drought treatment during fruiting period but the difference not great enough to reach the level of significance. Chlorophyl a: Ch. b ratio increased with the drought in the same period only (Table 6).

Table 6. Response of photosynthetic pigments of cowpea plants to drought by missing of irrigation at some growth stages.

Drought	Chl. a	Chl.. b	Carot.	Chl. a + Chl. b	Chl.a: Chl. b	Chl. a + Chl. b: carot,
Reg. Irr	6.79	2.80	456	9.59	2.39	2.10
At flowering	6.87	2.59	4.63	9.46	2.65	2.04
At fruiting	6.39	3.92	5.13	10.31	1.63	2.01
LSD at 5%	N.S	N.S	N.S	N.S	…..	……

The abiotic stress affected chlorophyll content which abiotic stresses can affect director and indirectly physiological processes of plants (**Chutia and Borach, 2012).** Drought affect the photosynthetic process through its effect on stomatal closures which control the transpiration and this intern affect the CO2 basses and limiting photosynthesis activity and on chlorophyll content (**Li, *et al.*, 2007). Macar and Ekmekc (2008**) reported that the plants stressed for 3 days exhibited a rapid drop in their relative and absolute water contents. The quantum efficiency of PSII open centers in the dark-adapted and light-saturated state, excitation energy trapping of PSII and electron transport rate decreased significantly from the 5th may to the end of the drought treatments. Plants drought-stressed for 7 days brought about a marked increase in non-photochemical energy dissipation and a marked decline in photochemical quenching. After rewarding all chlorophyll a fluorescence characteristics except for FM completely recovered and reached the control values. Under 5 and 7 days of drought, the anthocyanin content increased gradually while the total chlorophyll content of leaves declined compared to the controls. The total carotenoid content remained unchanged during the experiments**.** The total chlorophyll content expressed per unit dry weight increased insignificantly during the first two periods of drought but decreased by 13–15% later on. This decrease was not accompanied by changes in chlorophyll a/b ratio **(NikolaevaS, *et al.*, 2010).** However, **Liu, *et al.*, *(*2018)** found that chlorophyll content, fluorescence, AhPORA protein level and genes related to chlorophyll biosynthesis and photosynthesis declined markedly under drought conditions, but all increased during recovery, but other reported the increase in chlorophyll as a responses to water stress, while others noted the depressive effect on chlorophyll concentration (**Fayes and Bazaid, 2014 and Hussein, *et al.*, 2014)**. This may be depending upon the varieties and species.

• ***Glutamic acid and Proline***

Application of glutamic acid showed the highest levels of Ch. A, Ch. B, total chlorophyll concentrations but carotenoids were slightly affected. Furthermore, Ch. A: Ch. B, and Ch. A+ Ch. B: carotenoids ratios showed the similar responses (Table 7).

Table 7. Response of photosynthetic pigments of cowpea plants to spraying with glutamic and proline.

Amino acids	Chl. a	Chl. b	Carotenoids	Chl.a+Chl.b	Chl.a : Chl.b	(Chl.a+Chl.b) : carot.
D.W.	4.18	2.24	4.45	6.42	1.87	1.44
Proline	6.66	3.44	4.94	10.10	1.94	2.05
Glutamic.A.	8.37	3.96	4.92	12.33	2.11	2.51
LSD at 5%	2.46	N.S	N.S	2.83	….	….

Awad *et al.*, (2007) concluded that the increased in chlorophyll pigments may be attributed to the high level of amino acids in the plant tissues. Exogenous application of amino acids or yeast increased chlorophyll content of date palm plantlets **(Darwish, 2013). Sadak, *et al.*, (2015)** found that increased the concentration of sprayed amino acid increased the concentration of Ch. A, Ch. B, total chlorophyll and carotenoids. **Ahmed, *et al.*,(2011**) revealed that supplementary application of proline led to mitigate the negative effect on photosynthetic activity and chlorophyll on young olive plants. Meanwhile, **Haroun, *et al.*, (2010)** in cultured tissues of French bean during entire period of experiment showed a significant increase in response to 1 mM glutamic acid but a decrease in photosynthetic pigments with 2-5 mM of this amino acids.

Proline plays a crucial role in osmoregulation and osmo tolerance (**Szabados and Savouré, 2009**). Exogenous proline increased the chlorophyll content, especially in bread wheat seedlings (**Bekka, *et al.*, 2018).** These results are in accord with **Shahid *et al.*, (2014**) showed that exogenous proline improved the growth by increasing photosynthesis. The effect of exogenous proline on chlorophyll contents may also have been due to stabilizing photosynthetic reactions **(Abdelhamid *et al.*, 2013).**

• *Drought x Glutamic acid and Proline*

Data showed that in (Table 8) and Fig. 2. No significant differences were detected on Ch. B total chlorophyll and carotenoids concentrations as result of the interaction effect of omitting of irrigation and spraying proline and glutamic acids, but Ch. A was significantly affected. The concentration of this pigment the higher with spraying glutamic acid with the regular irrigation and when missing of irrigation done in the fruiting period while when omitting of irrigation done at flowering period spraying proline gave the highest Ch.A concentration. Furthermore the concentration of Ch. A as a result of proline treatment, the was lesser than that of the control treatment.

Table 8. Response of photosynthetic pigments of cowpea plants to drought by missing of irrigation at some growth stages and spraying with glutamic acid and proline.

Drought	Amino acids	Ch[. a,	Chl. b,	Carotenoids	Ch[.a+Chl.b	Chl.a: Chl.b	Chl.a+Chl.b : carot.
Reg. Irr.	D.W.	5.17	2.30	4.06	7.47	2.25	1.84
	Proline	4.11	3.07	5.43	7.18	1.34	1.32
	Glutamic.A.	11.09	3.03	4.20	14.12	3.66	3.36
At flowering	D.W.	3.41	2.24	4.21	5.65	1.52	1.34
	Proline	9.58	2.70	4.52	12.20	3.55	2.70
	Glutamic.A.	7.62	3.84	5.16	11.46	1.98	2.22
At fruiting	D.W.	3.95	2.19	5.09	6.14	1.80	1.21
	Proline	6.29	4.55	4.88	10.84	1.38	2.22
	Glutamic.A.	8.93	5.02	5.41	13.95	1.78	2.58

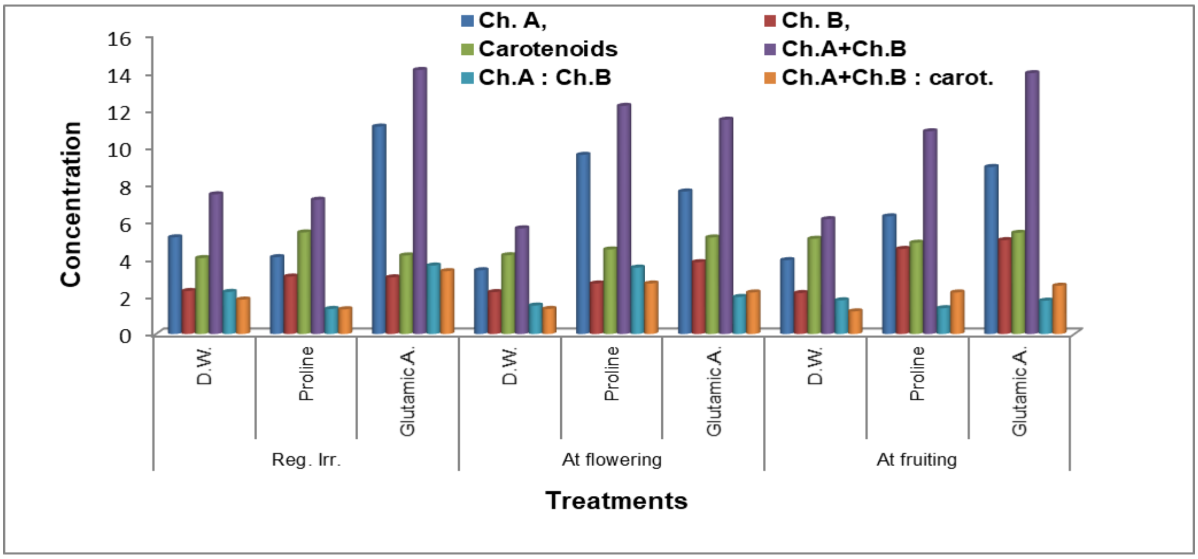

Fig. 2. Response of photosynthetic pigments of cowpea plants to drought by missing of irrigation at some growth stages and spraying with glutamic acid and proline.

It is considered as biomarker of stress. An increase in free proline content during drought stress conditions due to PEG has also been shown in different crops as maize **(Meeta *et al.*, 2013)** and bread wheat **(Ji *et al.*, 2014).** The results of **Bekka, *et al.*, (2018)** showed that water deficit affected both species leading to a reduction in growth, chlorophyll content and relative water content. **Rasheed *et al.*, (2014**) noted that the addition of 20 mM proline under oxidative stress causes an increase in the chllorophylls and carotenoids contents in leaves of bread wheat.

Ali, *et al.*, (2007) revealed that application of 30 mM proline proved to be more effective in inducing water stress tolerance as compared to the other level. Photosynthetic rate of water stressed plants of both maize cultivars was also enhanced due to foliar applied proline which was positively associated with sub-stomatal CO2 (Cv) and stomatal conductance (gs) as well as photosynthetic pigments. **Sadok, *et al.*, (2015**) concluded that the ameliorated effect of amino acid to salt stress (which salt stress included osmotic adjustment effect) may be related to its effect on chlorophyll content which affected photosynthesis process and intern reflected on biomass accumulation. **Hussein, *et al.*, (2017).**

Abiotic stresses reduce plant development by affecting different biochemical and physiological mechanisms like photosynthesis, antioxidant systems, and hormonal signaling (**Sharma, *et al.*, 2016**). Similarly, proline accumulation is also one of the mechanisms or responses in many plants under various stresses (**Anjum, *et al.*, 2017**). It is thus important to understand the mechanisms that control die rent processes and mechanism underlying abiotic stress tolerance in plants.

Proline accumulates in cytosol and protects enzymes and membranes from stress. It also stabilizes the membranes and acts as free radical scavenger under abiotic stress conditions. Recently, reports have shown higher accumulation of proline in *Arabidopsis altissima* seedlings in the presence of salt and less water conditions **Filippou, *et al.*, (2014**).]. Under stress conditions, accumulation of proline acts as a sink for excess reductants, which provides NAD+ and NADP+ required for regulating respiration and photosynthesis (**Sharma, *et al.*, 2019**).

The total chlorophyll and carotenoids contents were significantly decreased under drought stress in both species. Under stress conditions, the addition of proline resulted in a significant increase. leaf chlorophyll contents of the wheat seedlings (+43.87 %) and carotenoids (+54.42 %) in comparison to the stressed without proline. In contrast, for the lentil seedlings, no positive effect has been observed. Under normal conditions and with exogenous proline, Clh. a: Ch. b ratio did not vary significantly for both species. However, under water stress conditions, proline application on bread wheat increased this ratio but it had no effect on lentil (Bekka, *et al.*, 2018).Recently, **Abid, et al (2020)** found that β-aminobutyric acid (BABA) is a non-protein amino acid that may be involved in the regulation of plant adaptation to drought stress The application of BABA increased the leaf relative water content, leaf photosynthesis rate (A), transpiration rate (E), and stomatal conductance .

NPK concentration in seeds

• ***Drought***

Under conditions of water stress, roots are unable to take up many nutrients from the soil due to a lack of root activity as well as slow ion diffusion and water movement rates (**Dubey and Pessarakli, 2001**). Water in liquid form allows the diffusion and mass flow of solutes and is therefore essential to the translocation and distribution of nutrients and metabolites throughout the entire plant (**Mengel and Kirkby, 2001).**

Table 9. Effect of drought on N, P and K concentration (%) in cowpea seeds.

Drought	N	P	K
Reg. Irr	3.43	0.057	2.20
At flowering	3.06	0.053	3.13
At fruiting	3.18	0.048	2.78

Data in Table (9) showed that nitrogen and phosphorus concentration in cowpea seeds decreased with drought and depression increased by delaying subjection plants to drought. For potassium, its concentration increased by drought but the increment was more when drought was done at flowering stages. **Prasertsak and Fukai (1997)** conducted an experiment in order to investigate the interaction of N application and water stress on growth and grain yield of two rice cultivars and found that nitrogen application resulted in the rapid development of water stress, decreasing the leaf water potential and causing high leaf death during the stress period. However, some studies have found that N supply can minimize the effects of drought on plants. **DaMatta *et al.*, (2002**) found that Coffea canephor plants (cloneINCAPER-99) cultivated with high nitrogen nutrition increased their cell wall rigidity under drought conditions, thereby exhibiting some ability for osmotic adjustment. Water-use efficiency in plants is greatly influenced by the nitrogen supply. Nitrogen deficiency reduces the ability of a crop to convert available water into yield. According to the Potash and Phosphate Institute, the early uptake of nitrogen under conditions of low moisture enhances both shoot and root development, which is critical to the final yield (**Jonhston, 2002**), increased K and Ca concentrations in leaf tissue. **Pimratch *et al.*, (2008)** cite a number of studies in which drought reduced nitrogen fixation in leguminous species. However, the same authors also suggest that the maintenance of high N2 fixation under drought stress could be a means for a legume genotype to achieve high yield under conditions of limited water. Some researchers have pointed out that a good N supply can alleviates the effects of water deficit on the plants.

A good supply of water is required for phosphate availability and absorption by plants. Phosphate ions move through soils primarily through diffusion and if the water content in the soil decreases, the radii of water-filled pores decrease, tortuosity increases and P mobility decreases (**Faye *et al.*, 2006**). Drought causes a reduction in P absorption and transport in plants. A decrease in available P forms and increase in occluded P in the soil reduces P uptake and consequently induces lower foliar P content

(**Sardans and Peñuela 2004).** They added that a 22% reduction in soil moisture produced 40% decrease in the accumulated aboveground P content in plants. Water conditions in plants influence the K^+ accumulation in leaves and interact with K^+ nutritional status in some plant species (**Restrepo-Diaz, *et al.*, 2008**). The stomatal opening mechanism is governed by the K^+ concentration (**Mengel, 2007**). It has been demonstrated that water is the main factor determining the availability of mineral nutrients such as K^+ in the soil as well as absorption by plants and translocation from the roots to the shoot. However, some studies have shown that higher levels of K fertilization may allow plants such as maize (**Premachandra, *et al.*, 2008**) and potato (**Khosravifar, *et al*., 2008**) to tolerate water stress.

- ***Amino acids***

The examination of Data in Table (10) cleared that the concentration of nitrogen increased by both treatment of amino acids but the increase by proline exceeded those by glutamic acid. Phosphorus increased only by proline treatment while potassium concentration rose approximately with similar trends either by proline or glutamic acid. Little attention has been given to its role in affecting the uptake and accumulation of inorganic nutrients in plants (**Okuma, *et al.*, 2000 and Khedr, *et al.*, 2003).**

Table 10. Effect of drought on N, P and K concentration (%) in cowpea seeds.

Amino acids	N	P	K
Distilled water	3.13	0.052	2.75
Proline	3.31	0.057	3.20
Glutamic acid	3.24	0.050	3.15

Thus, it was hypothesized that the exogenous application of proline might regulate uptake of mineral nutrients in plants subjected to water deficit conditions. **Ramadan, *et al.*, (2019)** in comparative study found that arginine or SNP at all tested concentrations decreased significantly plant content of Na, while K and P highly significantly increased and Ca, Mg, and N non-significantly increased. L-arginine (arg.) is one of the most functionally diverse amino acids and a precursor for the biosynthesis of polyamines (PAs) and the cell signaling molecule nitric oxide (NO). It seems that some positive effects of arg. are related to the production of nitric oxide, polyamines, or proline (**Liu, *et al.*, 2006**). The PAs modulate several biological activities in plants, throughout participation in cellular defense against oxidative damage via the inhibition of lipid peroxidation and scavenge of free radicals (**Velikova, *et al.*, 2000**). **Demir and Kocacaliskan (2002)** noted that the addition of 10 mM exogenous proline under salt stress causes an increase in the protein content in the bean leaf. **Khedr *et al.*, (2003)** showed that 10 mM exogenous proline improves the salt-tolerance of *Pancratium maritimum L.* by protecting the protein turnover machinery against stress-damage and up-regulating stress protective proteins. Proline has been shown to act as a chemical protein chaperone and to prevent protein aggregation and the rmode naturation. (**Ignatova and Gierasch, 2006).**

- ***Interaction between treatments***

Data showed that in (Table 11 and Fig. 3) a positive relationship was found between the concentration of N and spraying with amino acids. Under regular irrigation or drought at fruiting proline induced the high effect than glutamic acid or control treatment while under drought at flowering proline or glutamic acid induced approximately the same increase. Concerning K concentration in seeds of cowpea, it showed approximately the same response of P concentration with both amino acids exogenous spraying under moisture stress at flowering or fruiting stages as well as with the regular irrigation all over the growth stages of cowpea plants .

Amino acids are considered to be osmolytes, nitrogen sources, and hormone precursor (**Maeda, et al 2012 and Ssandriana, et al 2020).** Studies have also suggested that exogenously applied proline induces the endogenous levels of various important osmolyte that promote growth and decreases uptake of Na+ and Cl- ions (**Nounjan, *et al.*, 2012 and Shahbaz, *et al* ., 2013).** They found also that the lowest concentration of N was found in the seeds of the control treatment plants while the seed yield from these treatments was the highest as compared with the N concentration and yield of seeds from plants subjected to water stress during flowering and pod filling stages.

Table 11. Effect of drought and amino acid spraying on N, P and K concentration in cowpea seeds.

Drought	Amino acids	N	P	K
Regular irrigation	Distilled water	3.31	0.060	2.48
	Proline	3.55	0.060	3.62
	Glutamic acid.	3.43	0.050	3.50
At flowering	Distilled water	2.98	0.045	2.84
	Proline	3.09	0.060	3.27
	Glutamic acid	3.11	0.055	3.28
At fruiting	Distilled water	3.09	0.050	2.93
	Proline	3.29	0.050	2.72
	Glutamic acid	3.17	0.045	2.68

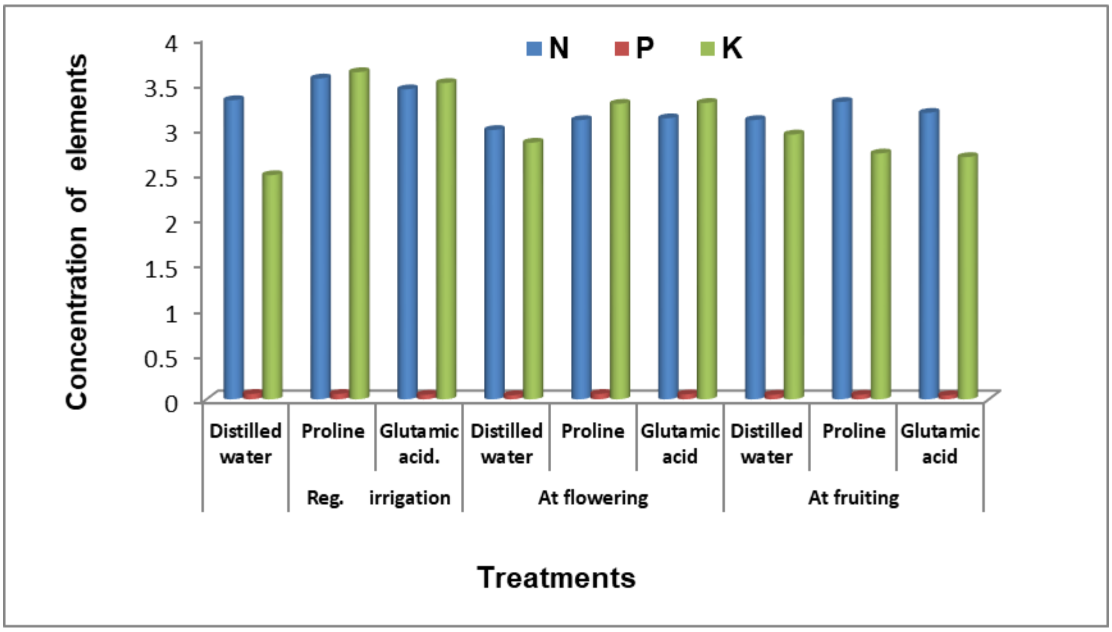

Fig. 3. Effect of drought and amino acid spraying on N,P and K concentration in cowpea seeds.

The concentration of N in the seeds was inversely related to the seed dry weight yield. In this regard, **Hussein, et al (2009**) revealed that both drought treatments increased the concentration of N, P, Na, K, Fe anb Zn but the increment when omitting of irrigation was done at milk ripe stage more than that when done at elongation stage. However, Mn concentration decreased by drought treatment but the response to the two irrigation treatments was approximately similar. The uptake of macro and micro nutrients except Mn showed the same trends of that in the concentration of these elements. Under conditions of water stress, roots are unable to take up many nutrients from the soil due to a lack of root activity as well as slow ion diffusion and water movement rates as detected by **Dubey and Pessarakli (2001**).

Conclusion

The experiment conducted to evaluate the effect of amino acids application (proline and glutamic acids) on cowpea plants dry matter grown under drought condition, stress affected dry matter, chlorophyll and total carotenoids and intern photosynthesis in plants and NPK concentration in seeds. From the obtained results it could be concluded that spraying amino acid used led to increase the water stress amelioration of cowpea plants trough its effects on improving dry mass, activating photosynthetic pigments more than its induced in seeds NPK nutrients.

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