RESEARCH ARTICLE

Effect of the type of potassium supply on the physiological response of cowpea (*Vigna unguiculata* L. Walp. var. KN-1) under hydraulic constraints

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The present study had the general objective of comparing KN-1 variety of cowpea cultivated according to the supply of different Potassium (K^+) solution in a situation of water constraint. The test was carried out in the experimental garden of the Unit of Training and Research in Health, Life and Technology (UFR/SVT) in the open air during the rainy period (July to September) in pots. Three types of solution namely water, Potassium Dihydrogen Phosphate (KH_2PO_4) and Potassium Nitrate (KNO_3) were used to supply the plants. Then, after 6 days, the plants were subjected to a water deficit by stopping watering in the vegetative phase. At the end of the water restriction, the morphophysiological and biochemical parameters were assessed on all the plants. Note that all the plants underwent water restriction.

Our work shows that the different potassium solutions had a positive effect on the production Root Biomass (RB) (3.093 ± 0.180 g for KNO_3 and 2.826 ± 0.146 g for KH_2PO_4) and therefore on cowpea adaptation to drought, compared to control plants (2.671 ± 0.099 g). Plants supplied with potassium solutions have a lower water potential (-1.528 ± 0.054 MPa for KNO_3 and -1.683 ± 0.029 MPa for KH_2PO_4) than that of control plants (-1.283 ± 0.058 MPa). Similarly, RB ($p<0,027$), chlorophyll A content ($p \leq 0,000$) and nodule mass ($p<0,009$) of plants were affected by the solution supply level in the KN-1 variety. Responses to water stress varied depending on the potassium solution used and the treatment supply level. On the basis of these results, potassium solutions and the KN-1 variety allow to improve cowpea production in Burkina Faso.

Key Words: *Cowpea; Potassium; Water deficit; Biomass; Water potential*

INTRODUCTION

Cowpea constitute an important source of minerals and proteins (20% to 25% of dry weight) and the high level of which is intended to plays a vital role in the nutritional balance of humans and animals, in sub-Saharan Africa [1]. As a result, it is considered "poor man's meat" by savannah population [2]. In various preparations, cowpea conceals pharmaceutical virtues [3]. Cowpea is an important source of foreign exchange [2,3]. Cowpea, through the presence nodules containing atmospheric nitrogen-fixing bacteria, allow the fertilization of the soil with nitrogen usable by plants [4,5]. In a word, it is a culture that can play a considerable role in the perspective of food self-sufficiency, diversifying crops and reducing Burkina Faso's trade balance. Known to be drought-tolerant, cowpea is sensitive to climatic hazards. Indeed, the growth and productivity of cowpea are considerably impacted by repeated water constraints [6,7]. In Burkina Faso, agriculture depends mainly on rain. In addition, rains are random and droughts are unpredictable severe. In addition to poor soil conditions, this accentuates the high fluctuation in cowpea production. The key to drought tolerance depends on the phenological and physiological adaptation of plant to its environment for rational management of water resources [8]. Thus, the efficient use of water resources and the search for conditions allowing plants to tolerate a water deficit constitute an essential issue for cowpea production in Burkina Faso.

In order to improve cowpea's adaptation to water deficits and boost agricultural production, mineral nutrition of plants, which integrate all the mechanisms involved in collection by the roots, transport, storage and use of mineral ions necessary for metabolism and plant growth can be considered. Thus, potassium one of the mineral elements with an electrochemical and catalytic function, is essential for good growth and development of plant playing a vital role in water management, but also for resistance disease [9,10].

In the search for solutions to the lack of water and study of the mechanisms for the proper utilization of soil water, the objective of our study is to compare the adaptive responses of cultivated cowpea as a function of the supply of different potassium solutions (K^+). Specifically, the aim is to study (i) The effect of the type of K^+ supply on the morphophysiological responses of cowpea; (ii) The variation in the morphophysiological responses of cultivated cowpea under water deficit conditions and; (iii) The difference in growth and transpiration of cowpea under the influence of K^+ solutions provided at different levels.

MATERIALS AND METHODS

Material used

The experiment was carried out in the experimental garden of the UFR/SVT located within the ground of Joseph Ki-Zerbo University, in natural temperature lighting and conditions hygrometry. The climate of the study area is dry tropical, characterized by a long dry season (October to May) and a short and irregular rainy season (June to September). Annual rainfall in the area varies between 800 mm and 1000 mm. Temperature and relative humidity varied between 21°C and 40°C and 38% and 91% respectively (Figure 1). The study site is located at 319 m altitude, with coordinates of 12°22'45.6" North latitude and 001°29'52.3" West longitude [11]. A translucent shed is used to protect plants in case of rain.

The seeds used came from the KN-1 cowpea variety (*Vigna unguiculata* (L.) Walp.) popularized in Burkina Faso by the cowpea improvement team of the Burkina Faso Institute for the Environment and Agricultural Research (INERA). This choice is justified by the fact that KN-1 is widely cultivated in areas often confronted with water deficit. KN-1 is a non-photoperiodic variety. This variety is resistant to diseases that attack other cowpea varieties. The taste of its seeds is pleasant and the seed very nutritious, especially for children. Its large leaf biomass is useful for human and animal food.

6 L pots were used, each pot contained 6 kg of soil beforehand sieved with a 2 mm mesh sieve. The bottom of each pot is meticulously perforated to allow water drainage after watering at the Crop Actual Consumption (CAC). The ground used is the land has not undergone any amendment. Before sowing, the soil was watered at 90% of the CAC. Four seeds were sown per pot and the seedlings were removed 10 Days after Sowing (DAS) to obtain only one plant per pot. The soil was irrigated with water every day until 28 days.

Experimentation conditions

Substrate characteristics: The granulometric composition of the substrate reveals a sandy-loam texture (68.8% sand; 20.3% silt; 10.9% clay). The

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quantity of nutrients (organic matter, nitrogen, phosphorus and potassium) from the substrate is sufficient to allow good of plant growth if water is available (Table 1). The good water retention capacity and ease of water absorption of the root system justified the choice of this substrate [12].

Climatic data were measured using a thermohygrometer and the physico-chemical characteristics of the soil were determined in the Bureau for the Use of Natural Resources soil analysis laboratory, BUNASOLS.

Our experiment took place from August to September (during the rainy season, with an average maximum temperature of 35°C and an average relative humidity of 86%) on a ferric Lixisol soil previously sieved to 2 mm. The soil is then put in plastic pots with a capacity of 6 l, whose bottom is carefully perforated to allow the water to drain off after watering. The trial was conducted in the open air, allowed the plants to be in natural conditions of temperature, lightening and humidity.

Experimental design

The trial was carried out using a randomized complete block design with 3 replications and two factors: The nutrient solutions used (distilled water (T0), Potassium Dihydrogen Phosphate (KH_3PO_4) and Potassium Nitrate (KNO_3) and the level of supply. The solution was supplied at three levels: The Soil (S), the fifth leaf from the crown (leaf A) and the fifth leaf from the apex (leaf B). Each block, made up of plants having the same level of supply of nutrient solution is made up of 36 pots. The experimental unit consisted of three (03) pots per nutrient solution.

Growing method and water deficit test

The seeds were sown in pots containing 6 kg of soil. This land was watered monitor at field capacity (CAC). Each pot contained four seeds. Young plants were on 10th Days after Sowing (DAS), leaving just one plant per pot, which was regularly watered. From 30 DAS, the plants were watered with three types of nutrient solution (T0, KH_3PO_4 and KNO_3). The nutrient solution was also administered at leaf level, where the mineral elements are administrated to the plant through the central petiole by through the buckets having cut its limb for the first category and at ground level for the second category. At leaf level, the supply of the solution was made at two levels: The fifth leaf from the collar (leaf A) and the fifth leaf from the apex (leaf B). After 10 days of treatment, the plants were subjected to 6 days of water stress from 40th to 45th DAS. On the last day of stress, leaves from the 3rd row was taken from the apex for biochemical and physiological analyses, one leaf per pot.

Measurement method

Measuring plant water status-leaf hydric potential: At the end of the experiment, the water status of the plants was determined by measuring the potential leaf water (Ψf). The baseline water potential (between 4 and 5 h) and water potential minimum (between 1 and 2 h) of the leaves were measured once at the end of the water stress, on one of the leaves of the 3rd stage, using the scholander chamber.

Determination of chlorophyll A content: To determine chlorophyll content, leaves from the 3rd tier was taken at 1.30 pm, when the plant transpires the most.

Chlorophyll A concentration was measured on the third leaf from the apex of plants in the different treatments using the McKinney method. 100 mg of fresh material was ground in 10 ml of 80% acetone. The solution obtained was filtered and its Optical Density (OD) measured using a spectrophotometer at 663 and 645 nm. Chlorophyll A (Chl.A) concentrations were deduced using the following formula:

$$[Chl.A] = 12 \times (OD_{663}) - 2.67 \times (OD_{645})$$

Measurement of above-ground dry biomass, roots and nodules: To quantify the biomass produced, we unloaded the plants. This unloading consisted of immersing the root surface of a plant in a container contening clear water for a few moments to separate the roots and nodules from the soil. When the roots were then carefully washed with water before cutting each plant at the collar. Once cut, the nodules are isolated from the roots and counted. Then the aerial part of the plant, the root and the nodules are dried in an oven set at 60°C for 72 h. Subsequently the completely dry samples were then weighed using a DENVER AC-1200D electronic balance.

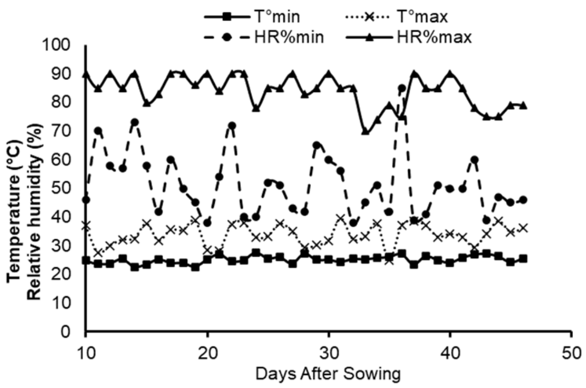

Figure 1) *Evolution of temperatures (°C) and relative humidity (%) minimum (min) and maximum (max) during the experiment conducted in the experimental garden of the UFR/SVT on the number of Days After Sowing (DAS)*

TABLE 1

Physico-chemical characteristics of the culture substrate

Parameters	Quantity
Physical properties	
Clay (%)	68,8
Silt (%)	20,3
Sand (%)	10,9
Chemical properties	
Total organic matter (%)	2,286
Total carbon (%)	1,326
Total nitrogen (%)	0,176
Total phosphorus (ppm)	1042,85
Total potassium (ppm)	2698,41
Cation exchange capacity (méq/100 g)	8,48
pH (H_2O)	6,43

RESULTS AND DISCUSSION

Air temperature and relative humidity

To quantify the influence of environmental constraints on plants, temperature and relative humidity of the air were recorded daily using a HANNA HI 9564 thermo-hygrometer during the test. Measurements were made daily every 3 h between 6 am and 6 pm, but only the minimum and maximum daily values were recorded. Figure 1, shows the daily variation in minimum and maximum air temperature and relative humidity during of the experimentation.

Effect of nutrient solutions on morphophysiological parameters

The effect induced by the use of nutrient solution was generally an increase in vegetative growth in a natural environment in the open air (Tables 1 and 2). The statistical analysis has revealed a significant effect of the nutrient solution for Above-Ground Biomass (ABS) ($p<0.001$) and the nodule number ($p<0.008$). The level of nutrient solution application has significantly influenced root Biomass Ratio (BR) ($p<0.027$), BR/ABS ratio ($p<0.016$), chlorophyll A content ($p<0.0001$) and nodule mass ($p<0.009$). The arial biomass values of plants treated with the KNO_3 solution are more followed by those treated with T0 and lower in plants treated with KH^2PO. However, it should be noted that the value above-ground biomass at the level of plant having received the KNO_3 solution at the level of the fifth leaf from the apex (leaf B). For root biomass and chlorophyll A content, although the differences are not significant, the values are higher in plants treated with potassium solutions than in those treated with T0 (Table 1), except for plants having received T0 on the ground (Table 2). The data in Table 1, show that plants treated with the KNO_3 solution produced significantly fewer nodules ($p<0.008$) but the dry mass of these nodules was relatively higher, particularly in feet having received KNO_3 at leaf level (Table 2). Likewise, the plants that received nutrient solutions at leaf level B had a relatively lower number of nodules, on the other hand the dry mass of the nodules is significantly higher in these same plants ($p<0.009$) at 45 days after harvest (Table 1).

Effect of nutrient solutions on leaf water potential

The water status of the plants in the different treatments was determined at 45^+ Potassium Nitrate (KNO_3), regardless of the treatment level, produced DAS by measuring the basic (Ψb) and minimum (Ψm) leaf water potential. The basic water potential (-0.569 ± 0.055 MPa) of plants watered with all nutrient solutions is higher than their minimum water potential (-1.498 ± 0.047 MPa) (Figure 2). Figure 2, also shows that the minimum leaf water potential at 45th DAS of plants that received potassium solutions is significantly lower ($p<0.0001$), regardless of the level of treatment. The highest minimum leaf water potential was observed at the level of having plants received water at ground level.

The supply of water and mineral salts and the influence of high temperatures are constituted factors limiting agricultural production, particularly in sub-Saharan Africa. In addition to light, the mineral elements, water and temperature constitue the most important factors in the physical environment of plants. They exert a strong influence on growth and development of plant. Thus, the variation in temperature and relative humidity measured during the experiment showed that the application of water stress to the different plants coincided with a relatively hot period, with average temperatures exceeding 35°C. Furthermore, the rainfall recorded during this period

TABLE 2

Average Above-Ground Biomass (ABS) and Root Biomass (RB), Total Chlorophyll A (T. chlo. A), number of nodules, mass of nodules at 45 days after sowing under natural conditions for nutrient solutions and plant supply level

	ABS (g)	RB (g)	RB/ABS	T. chlo. A	Number of nodules	Mass of nodules (g)
			Type of solution			
T0	8,607 ± 0,105	2,671 ± 0,099	0,310 ± 0,010	19,667 ± 1,024	207,333 ± 10,145	0,666 ± 0,003
KNO_3	8,785 ± 0,115	3,093 ± 0,180	0,351 ± 0,017	20,390 ± 0,084	169,556 ± 5,172	0,682 ± 0,043
KH_2PO_4	8,124 ± 0,107	2,826 ± 0,146	0,348 ± 0,017	21,198 ± 0,521	201 ± 8,639	0,656 ± 0,005
p	0.001	0.139	0.124	0.284	0.008	0.756
			Level of application			
Soil	8,542 ± 0,093	2,797 ± 0,129	0,328 ± 0,016	21,534 ± 0,262	198,667 ± 13,714	0,613 ± 0,024
La	8,563 ± 0,209	3,17 ± 0,110	0,370 ± 0,00B	18,420 ± 0,737	193,333 ± 9,176	0,677 ± 0,009
Lb	8,411 ± 0,104	2,623 ± 0,164	0,311 ± 0,016	21,301 ± 0,328	185,889 ± 4,906	0,713 ± 0,024
p	0.727	0.027	0.016	0.000	0.664	0.009

Note: T0: Plants watered with distilled water; H_3PO_4: plants watered with Potassium Dihydrogen Phosphate solution; KNO_3: plants watered with Potassium Nitrate solution; Soil: Plants with the nutrient solution at ground level; L_a: Plants with the nutrient solution at the level of the fifth leaf from the collar; L_b: Plants with the nutrient solution at the level of the fifth leaf from the apex.

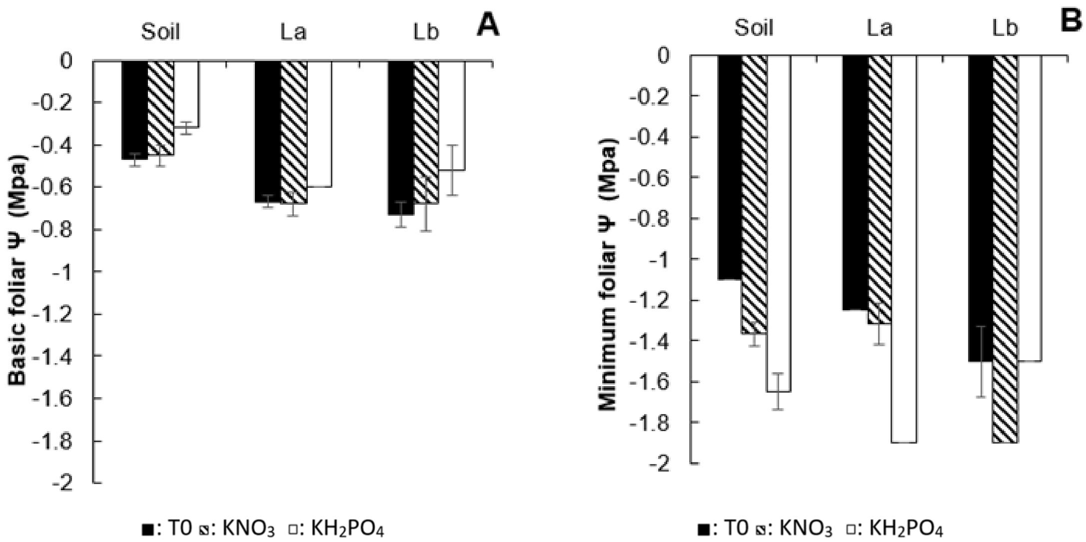

Figure 2) *(A): Variation of basic water potential of plants watered with all nutrient solutions and (B): Variation of minimum leaf water potential depending of the type of nutrient solution at the 45th Days after Sowing (DAS)*

explains the higher relative humidity.

The above-ground biomass of the different plants was significantly influenced by the different treatments. Plants treated with Potassium Nitrate (KNO_3), regardless of the treatment level, produced more above-ground biomass. This high biomass is due to the fact that the different plants having received KNO_3 reacted differently under stress condition water. This shows that KNO_3 improves plant tolerance to drought, by better water efficiency in the plant. This tolerance in all the more important when KNO_3 is made available to plants at leaf level. The limitation of leaf dry matter production when irrigation water quantities are limited is a known phenomenon in several cultivated species. Of even, the conclusions establish by on cowpeas go in the same direction. KNO_3, containing nitrate nitrogen, which is the most soluble form and mainly absorbed by the plant, produced more above-ground biomass. This contribution allowed the plant to boost the carbohydrate and protein syntheses, leading to cell proliferation and a subsequent increase in above-ground biomass. According to Ketterings et al., fertilizing of the soil with mineral elements causes significant mineralogical changes [13]. This will affect the availability of certain soil elements. Increased nitrogen mineralization makes the nutrients necessary for plants and simultary promote an evolution the pH towards neutrality.

The root system of plant species is generally present a great sensitive to water factor. Plants satisfy their water needs within the useful water margin i.e., between the holding capacity and the permanent wilting point. The reduction of this water margin dries out the soil and establishes of a gradient water potential, resulting in a state of stress around the roots. This state of stress induces several of responses, including a reduction in height and total dry biomass and an allocation of biomass towards the roots, to the detriment of the above-ground parts [14]. These observations go to the same direction our results. Indeed, at the end of the stress in the vegetative stage, the measurement of the root biomass of the different plants shows that the production of this root biomass is relatively important in plants treated with potassium solutions than in those having received water. We note that when processing is applied to the leaves, the plants treated with KNO_3 have a higher root biomass with a value of 3.607 g/plant when the plant receives the solution at the level of the fifth leaf from the apex (leaf B) and 3.24 g/plant when the plant receives the solution at level of the fifth leaf from the collar (leaf A). Plants having received KNO_3 at ground level had the lowest value, which is 2.4 g/plant. This situation would be due, on the one hand, to the high solubility of KNO_3, thus leading to leaching by drainage water of the quantity of KNO_3 that was not absorbed by the plant and on the other hand, to the efficient use of water by plants that received the KNO_3 at ground level. Plants treated with KH_2PO_4 and plants treated with distilled water at the leaf level had approximately the same root biomass, regardless of the leaf used. However, when the solutions are brought to the soil, it was the plants treated with KH_2PO_4 that produced significantly more root biomass. Indeed, phosphorus plays role capital in activating root growth [15]. It therefore acts in synergy with the potassium in the development of the root system.

Cowpea roots have nodules containing nitrogen-fixing bacteria, which play an important role in fertilizing the soil where they grow. The number of nodules of plants treated with water on the ground in the vegetative stage was greater than in plants treated with potassium solutions. Likewise, the same observations are made when the treatments are carried out at level of the leaves A. However, when the solutions are brought the level of leaves B, the plants treated with KH_2PO_4 produced a larger number of nodules. According to Divito et al., nitrogen accumulation in nodules as well as and the number of nodules per plant varied as potassium content increased [16]. The studies of the effect of potassium on the fixation at atmospheric nitrogen by nodules other species show that potassium improves the supply of carbohydrate from nodules and the supply of Adenosine Triphosphate (ATP), as well as the reduction of electrons required by the nitrogenase [17-19]. Furthermore, the stimulation of nodulation by phosphorus has already been observed. Thus, Thuynsma et al., and Kouassi et al., shown a very significant increase in nodulation in the presence of phosphorus [20,21]. By stimulating plant growth, phosphorus stimulates nodulation and nitrogen fixation. The potassium ion introduced with phosphate fertilisation is an important element for plants. But once soil acidity increases, potassium becomes deficient, leading to a reduction in symbiotic nitrogen fixation. Potassium, acting in synergy with phosphorus, will then see its action inhibited by the acidity of the environment. This hypothesis corroborates our results when the KH_2PO_4 solution is brought to the ground.

In an agricultural system, the atmospheric nitrogen-fixing potential in a symbiosis is reduced when nitrogen is combined. Nitrate nitrogen was an inhibitory effect on the legume-*rhizobium* symbiosis, which would cause nodulation to be block during our experimentation. In our experiment, the KNO_3 used at ground level made nitrate available to the plant root system, which was directly assimilated. This would have prevented the proliferation of the rhizobial population and therefore consequently, the number of nodules. Hence the reduction in the number of nodules produced by plants treated with KNO_3 at soil level. The nitrate ion can disrupt penetration of *Rhizobium* into the root hair. So, all increased nitrate content could interrupt nodule development. This interruption is explained by a limitation of the biochemical nitrogen fixation mechanism following a very rapid closure of the nodule's oxygen diffusion barriers. This reduces or even eliminates the supply of energy substrates to the bacteroids.

Water stress is the first environmental stress causing a drastic reduction in nitrogen fixation [22]. The reduction in nitrogen fixation following water deficit can result from low photosynthetic activity and consequently low availability of carbohydrates in the nodules, insufficient transpiratory flux to ensure the transport of nitrogen products from the nodules or certain direct effects on the metabolic activity of the nodules [23]. In short, in the *Rhizobium*-legume symbiosis, both partners and all the stages in the establishment of the symbiosis are sensitive to stress [24]. The dry weight of nodules at the vegetative stage of plants treated with potassium nitrate on the ground is significantly lower than that of plants treated with water. On the other hand, plants having received the KNO_3 solution at leaf level had a higher nodule dry weight than other plants. Nitrates at the ground level inhibit not only nodule formation but also nodule growth. Nitrogenase activity is inhibited when the nodules are exposed to nitrate. In word, nitrate inhibits nodule formation, growth and activity when KNO_3 is applied to the plant's root system. Plants treated with KH_2PO_4 have a higher dry weight of nodules when the treatment is carried out at ground. Phosphorus at the soil improves symbiotic fixation of atmospheric nitrogen.

Leaf water potential is the most commonly used parameters to determine the water status of plants. Water deficit occurs in the plant when water losses exceed root absorption capacity. The variety's sensitivity to stress water increases as its water potential decreases. Our result from basic water potential show that the plants having receive the treatment at soil level experienced low to moderate water stress. However, when the treatment is brought to the level of the leaves, the water stress is moderate to severe. Plants having received with KH_2PO_4 had a significantly higher basic leaf water potential than the other plants having received with water and KNO_3. This could be explained by the fact that these substances have physical properties allow the little water available to be retained in the soil for the plant during water stress. This allows these plants to develop less force to acquire water than other plants. On the other hand, the measurement of minimum water potential shows that plants treated with water have a significantly high leaf water potential compared to other plants treated with KH_2PO_4 and KNO_3. Our results could be explained by the phenomenon of osmotic adjustment, characterized by the reduction in water potential, thus making it possible to maintain the movement towards the leaves and consequently their turgor. Indeed, when potassium is supplied, it accumulates in plant the cells due to its high mobility. This accumulation leads to an increase in the absorption of the water by the plant to restore osmotic balance following the establishment of the pressure gradient osmotic.

Measuring the chlorophyll content provides information on the operation of the photosynthetic apparatus. It can also be used to identify the target of stress at the level of photosynthetic apparatus. The results obtained for chlorophyll A content show that, at the vegetative stage, the plants treated with nutrient solutions have a significantly different content from that of the plant that received water. This difference could be explained on the one hand by the fact that potassium is necessary to maintain the opening of the stomata, the movement of carbon in the leaves and the formation of photosynthetic pigments [25,26]. When the provision of the nutrient solution is done at the level of the apical leaves, we note a favourable influence of potassium on photosynthesis shown that potassium stimulates the assimilation of carbon dioxide. When the various solutions were applied at ground level and at the level of the fifth leaf from the collar (leaf A), plants treated with KNO_3 had a low chlorophyll A content. This situation shows that nitrate accumulation would reduce the realization of photosynthesis.

CONCLUSION

The main objective of this study was to contribute to a better understanding of the comparative effect of potassium on the physiological responses of the cowpea variety KN-1 in water stress situation. The action of potassium on cowpea varies depending on the level of administration of the potassium solution and also depending on the solution used. During the water deficit, plants having received nutrient solutions at ground level had a significant root biomass compared to those treated with water. However, when the nutrient solutions are brought to the level of the leaves, the quantity of biomass produced by the plants having received the KNO_3 is much greater. The supply of potassium had a beneficial effect on root biomass. It should be noted that for above-ground biomass, supplying plants with different potassium solutions had little effect on cowpea response to water deficit. Stressed plants and having received a nutrient solution had a minimum water potential than control plants (plants having received only water). While, we observed that at the time when transpiration is almost zero, the basic leaf water potential of the control plants was lower. Remember that potassium allows maintenance cellular turgor. Plants treated with nutrient solutions have a content of chlorophyll A different from that of plants having received water. For cowpea cultivation in emergency situation limiting water conditions, we recommend the use potassium solutions to water KN-1 plants in soil-less cultivation.

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