



## Early effects of water stress on some biochemical and mineral parameters of mycorrhizal *Vigna subterranea* (L.) Verdc. (Fabaceae) cultivated in Cameroon

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### Abstract

Regarding effects of water stress on plant, many research reports have been release during recent years, but, studies on effects of this stress on biochemical parameters and mineral content at an early stage are scarce. This study investigated drought stress effects on some biochemical parameters and mineral content of mycorrhizal *Vigna subterranea* plant, in a randomized block design. The microbial material comprised of a mixture of locally selected arbuscular fungi. The four levels of watering expressed in % of field capacity were: 90, 60, 30 and 15; with or without mycorrhizal inoculation. Experiment was carried out on a sterilize substrate during 31days of water stress. Results showed that with increasing level of water stress, mycorrhization increased: mineral content, both soluble sugars and acid phosphatase, but lessened proline content. Arbuscular mycorrhizal fungi (AMF) could thus be an effective tool in the alleviation of harmful effects of drought stress on plants, by improving their tolerance to this abiotic stress and consequently contributing to a better growth of *V.subterranea*.

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Voandzou [*Vigna subterranea* (L.) Verdc.], commonly called Bambara groundnut, belongs to Fabaceae family which in term of importance come after that of Poaceae. It is grown within the limits of sub-Saharan Africa, where it is adapted to various climatic and ecological conditions (steppe, savanna and forest) (Yao Djè *et al.*, 2005). Leguminous plants ameliorate agricultural practice worldwide due to their high content in nitrogen and their ability to fix molecular nitrogen in symbiosis with *Rhizobia*, 20-100kg.ha<sup>-1</sup> / year (Giller, 2001, Ncube *et al.*, 2007). Furthermore, they can improve fertility of agricultural soils and thus reduce amount of mineral fertilizer used. This property is highly exploited in sustainable agriculture. Voandzou can give an average output production of 350-800 kg/ha in areas where soil is not fertile and rain fall is low (Linnemann, 1994). This production could be thanks to its remarkable capacity to adapt to tropical climate and the genetic diversity of seeds sown (Azam-Ali *et al.*, 2001). Leguminous plants contribute highly in human nutrition, particularly through their seeds, which are very rich in good quality protein containing essential amino-acids. For this reason their seeds are often used in complementing diet based on cereals which are poor in nitrogenous compounds. The seeds of *V. subterranea* contain 63 % carbohydrate, 19 % proteins and 6.5 % fat. Voandzou is a good source of fiber, calcium, iron and potassium (Hillocks *et al.*, 2012).

In their natural environment leguminous plants in general and voandzou in particular are regularly subjected to biotic and abiotic constraints which interfere with their growth and development, amount them drought stress is most frequent because of scarcity of water unpredicted rain fall and global warming. Water is one of the factors that plants need in sufficient quantities to manufacture carbohydrates, to transport mineral elements (Mg, K, P, N), moreover, several biological processes such as cell division and cell elongation depend on it. The reduction of water availability for a plant subjects it to a condition of drought stress, which according to Mamoudou Dicko (2005) is an environmental

constraint to which the crop plants in arid and semi-arid regions are subjected. Maggio *et al.*, (2000) showed that in these arid and semi-arid regions, water deficit limits the productivity of plants. Water stress is the major cause of the loss of more than 50 % of plants productivity in the world (Wang *et al.*, 2003). *V. subterranea* is mainly cultivated from sahelian region of the far north to the peri-forest savanna of Center Region of Cameroon, where water scarcity is an important limiting factor for its plants growth. It was shown that the microbial community and particularly mycorrhizal fungi may play a significant role in water and mineral uptake by plants (Nwaga *et al.*, 2010).

It is however noted that mycorrhiza constitutes an effective widespread symbiosis with more than 80 % of mycorrhizal terrestrial plants (Oehl *et al.*, 2011). This association may contribute to alleviate drought or salinity stress effects on plants (Juniper and Abott, 1993) by enhancing plant water relations (Allen and Allen, 1986, Nelsen, 1987), increasing nutrients uptake (Abdelmoneim *et al.*, 2014). Moreover it enhanced plant disease control (Linderman, 1994; Song *et al.*, 2011) and makes osmotic adjustment fast in mycorrhizal plants compared to unmycorrhizal plants (Porcel and Ruiz-Lozano, 2004).

Although the physiological behavior of the plants inoculated with AMF in a situation of water stress has been studied for a long time that of *V. subterranea* remains less explored, particularly those targeting drought tolerance and how to alleviate water stress effects on their early growth phase. The aim of this study was to evaluate the effect of drought stress on mycorrhizal *V. subterranea*, mycorrhization using a mixture AMF (*Glomus hoï* + *Glomus intraradices* + *Gigaspora margarita* + *Scutellospora gregaria*) strains. In order to achieve this objective we evaluated some biochemical and mineral parameters such as: total amino acids, proline, sugars, soluble proteins, N, P, K, Mg content; and specific activity of phosphatase acid.

## Material and methods

### *Plant material and conditions of growth*

Healthy seeds of *V. subterranea* were sorted and surface sterilized with sodium hypochloride containing 2.4° chlorine for 10 mn and then washed thoroughly 3 times with distilled water to remove all traces of chemical products. Afterwards, they were allowed to germinate in sterilized plastic pots between two layers of Whatmann filter paper N°1 regularly watered with distilled water, in the dark and under laboratory temperature for 7 days. Germinated seeds were transferred to culture plastic pots containing a sterilized substrate, the test pots contained mycorrhizal inoculated germinated seeds, while the control pots without mycorrhizal inoculum received bacterial filtrate of the inoculum. Watering was sustained every morning and evening to maintain the substrate at field capacity (FC). This phase that was carried out for 14 days permitted us to obtain seedlings that were then transferred to development pots and different levels of stress applied immediately. The FC of the substrate was calculated according to Guissou *et al.* (2001) method, the various levels of water stress were applied according to Tobar *et al.* (1994) method. The substrate consisted of field soil taken at 0-15 cm depth where the previous cultures included *Manihot esculenta* and *Zea mays* and coarse river sand, in proportions 1/2 ground, 1/2 sand and 3/4 ground, 1/4 sand respectively for culture substrate and development substrate. The substrate was autoclaved at 120°C during 1hour (Oyun *et al.*, 2010) with a pressure of 1.5 bar and was oven dried at 105°C for 24 hours (Petard, 1993) in order to obtain a substrate with 0% moisture. The characteristics of the soil used consisted of: pH (H<sub>2</sub>O) 5.79; available phosphorus 0.34 µg/g; available potassium, sodium, magnesium and calcium in order 0.26 cmol(+).kg<sup>-1</sup>, 0.37 cmol(+).kg<sup>-1</sup>, 1.06 cmol(+).kg<sup>-1</sup> and 3.06 cmol(+).kg<sup>-1</sup>; nitrogen, carbon, ratio carbon/nitrogen and organic matter: of 0.34 %, 2.71 %, 7.98 % and 4.63 % respectively. The experiment was carried out in a transparent plastic shelter (length:3 m, width 2.5 m and height: 2.20 m) built in the University of Yaoundé I, with an average minimum temperature of 22°C and maximum

temperature of 42°C; sun light 12 hours at 65.74 - 89.82µmol m<sup>-2</sup> s<sup>-1</sup>. The experiment was carried out on a sterile substrate containing only known micro-organisms. .

### *Microbial material*

The microbial inoculum from selected mycorrhizal fungi stocks obtained from cultures of sorghum and groundnut (as host plants) was provided by the Soil Microbiology laboratory of the Biotechnology Center of the University of Yaoundé I. The microbial material was a mixture of spores of *Glomus hoï* (4 spores/g of soil), *Glomus intraradices* (5 spores/g of soil), *Gigaspora gregaria* (7 spores/g of soil) and *Scutellospora gregaria* (9 spores/g of soil); soil and roots fragments. The bacterial filtrate was elaborated from the inoculum: 25 g of AMF inoculum in one liter of sterile distilled water, homogenized for 30 mn before sieving through sieve of 10 µm. The inoculation of the AMF was carried out in 2 phases, according to the recommendations of Soil Microbiology laboratory (Anonyme, 2007): phase 1 was conducted in culture pots with 5 g of mycorrhizal inoculum /germinated seed; Phase 2 was conducted in development pots with an inoculation of 10 g of AMF in the rhizosphere for each treated seedling while the control pots did not have the mycorrhizal inoculum, but received 5 ml and 10 ml/germinated seed of bacterial filtrate respectively in culture pots and development pots.

### *Experimental design*

The experiment was conducted in transparent plastic shelter, on a flat: 40 cm high from the ground level under controlled conditions, in completely randomized blocks design (2 blocks) with 3 factors:

1. leguminous plant specie (*V. subterranea*);
2. treatments (mycorrhizal plant and nonmycorrhizal control);
3. watering regime: control (90% of FC), low stress (60% of FC), average stress (30% of FC) and severe stress (15% of FC).

The experimental design was composed of 8 treatments and 5 replication for each, 5 plants/pots

that makes 25 plants/treatment and 200 plants in the entire design. The quantity of water corresponding to the various level of water stress expected were 90 % for the control, 60 % for low stress, 30 % for average stress and 15 % for severe stress, which in terms of FC corresponded to 774 ml, 516 ml, 258 ml and 129 ml respectively. Drought stress lasted 31 days and the various levels of water stress were maintained by a daily control of the weight of the pot in order to adjust the suitable quantity of water in each pot.

#### *Evaluation of total amino acid and proline content*

To do that, 1 g of fresh leaves was crushed in 5 ml of 80° ethanol and centrifuged at 5000 rpm at 4 °C during 15 mn. The supernatant was used for titration. The total amount of amino acids and proline were determined using ninhydrin reaction according to Yemm and Cocking, (1955). The absorbance of the complex formed was read at 440 nm for the proline and at 570 nm for the total amino acids using a spectrophotometer Jenway model.

#### *Evaluation of the total soluble sugars*

The extraction of total soluble sugars was carried out by crushing 1 g of fresh leaves in 10 ml of ethanol-water mixture: 80-20 (v/v) and then centrifuged at 5000 rpm at 4°C during 15 mn. The supernatant containing sugars was recovered by titration of total sugars according to the Anthron method (Yemm and Willis, 1954).The absorbance of the green solution of furfural complex obtained was read at 620 nm by using a spectrophotometer Jenway model.

#### *Evaluation of soluble proteins content*

Extraction of soluble proteins was made by crushing 0.5 g plant samples (leaves and fresh roots) in a mortar in the presence of Fontainebleau sand, 5 ml of tris-HCl 0.4M (pH = 6,8 ) buffer, containing 1.5 M NaCl followed by centrifugation at 5000 rpm at 4 °C during 10 mn. The supernatants was recovered and the titration of proteins by the colorimetric method of Bradford (1976).The optical density of the blue complex formed was read at 595 nm with a spectrophotometer Jenway model.

#### *Evaluation of the activity of acid phosphatases*

The determination of the activity of acid phosphatases was carried out according to Hooley (1984). Fresh root (1 g) was crushed at 4°C in a mortar with 10 ml of extraction buffer (0.4 M acetate-HCl, pH = 5) and 1 g of Fontainebleau sand. The crude extract was centrifuged at 5000 rpm for 15 mn at 4°C. The supernatant obtained was used for the determination of the specific activity of acid phosphatases. The optical density was read at 410 nm with a spectrophotometer Jenway model.

#### *Evaluation of mineral content of leaves*

The extraction and proportioning of mineral elements were conducted at the soil laboratory of IITA (*International Institute of Tropical Agriculture*).The cationic bases of magnesium and potassium were extracted from dried leaves using a mixture of diluted HCl / HNO<sub>3</sub> acid. This hot extraction was conducted in a muffle furnace at 500°C; after mineral content was performed using atomic absorption spectrophotometer model Buck Scientific (Benton and Vernon, 1990). Extraction of phosphorus (P) was performed in a similar way like above in the presence of Murphy Riley reagent (Murphy and Riley, 1992), and titration was conducted with the same equipment. Total nitrogen (N) was extracted using a two-step digestion with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), boiling sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) (Buondonno *et al.*, 1995) and titration done with a colorimeter Lovibond.

#### *Statistical Analysis*

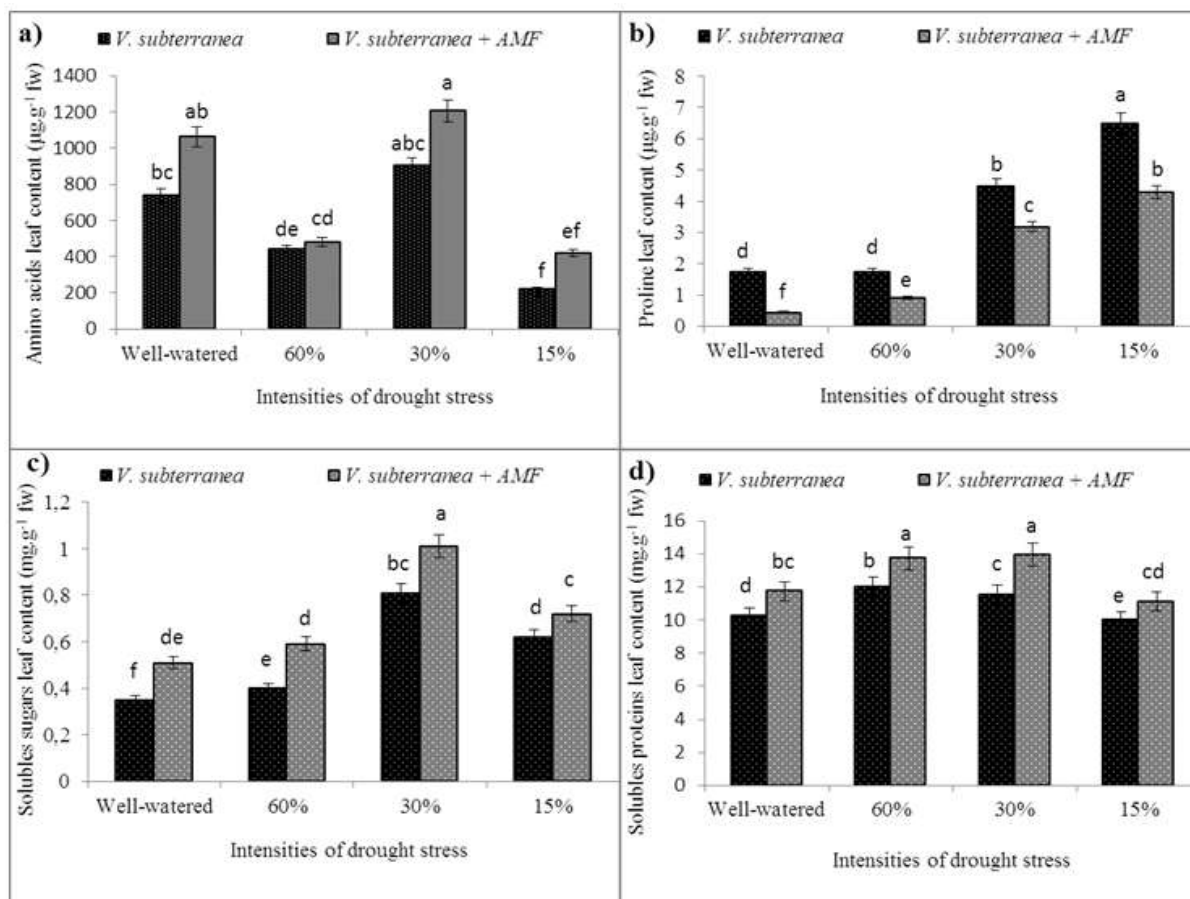
The data obtained in a completely randomized design with five replications was analyzed using ANOVA and SPSS 18.0 software. The means was then separated using DUNCAN's test with an experimental error of 5 %. Means obtained were compared according to water stress level (60, 30 and 15% of FC) within each treatment (mycorrhizal and nonmycorrhizal), then between the treatments having received mycorrhizal inoculum and control according to the level of water supply.

#### **Results**

The total amino acid content (Fig. 1a) of well watered

plants was significantly low for nonmycorrhizal plants compared to mycorrhizal ones. For stressed plants it drops with the increase in the level of the water stress for low (60% of FC) and severe (15% of FC) water stress. For average (30% of FC) water

stress a significant improvement of total amino acid content of 19 and 12% for the nonmycorrhizal and mycorrhizal plants respectively was observed; the mycorrhization alone having allowed an increase of 25%.



**Fig. 1.** Amino acids leaf content (a), Proline leaf content (b), Solubles sugars leaf content (c), Solubles proteins leaf content (d) in mycorrhizal and non-mycorrhizal *V. subterranea* plants under severe (15%), moderate (30%), mild (60%) and no drought stress (Well-watered = 90%) conditions.

For well watered plants (Fig. 1b), proline was present in leave of both plants, but it content was significantly high in nonmycorrhizal then in mycorrhizal plants. Under water stress the proline content was significantly high for nonmycorrhizal plants compared to mycorrhizal ones and increases with the level of water stress except for non mycorrhizal plants where it remain unchanged for low water stress. The proline content of nonmycorrhizal plants increases of 155 and 269% for average and severe water stress respectively, for mycorrhizal ones, it increases of 107, 623 and 875% for low, average and severe water stress respectively. The increment of

proline content is always high in mycorrhizal plants then nonmycorrhizal ones and increases with the stress level.

In well watered plants, soluble sugars content (Fig. 1c) was present in leaves of both plants, but in significantly high amount in mycorrhizal plant than in nonmycorrhizal ones. Under water stress soluble sugars content increased in leaves of both plants with the level of stress; the increment was 14, 131 and 77% for nonmycorrhizal plant, 16, 98 and 41% for mycorrhizal plants respectively for low, average and severe water stress. The increase was significantly

high for average water stress. In well watered plants leave soluble proteins was present (Fig.1d). The foliar content was significantly high for mycorrhized plants compared to nonmycorrhized ones. Under stress, for all plants soluble proteins increased for low and average stress and dropped for the severe stress. The increment was 17 and 13% for mycorrhized plants, 19 and 15% for mycorrhized ones, for low and average stress respectively. The decrement was 3% for nonmycorrhized plants and 5% for mycorrhized ones.

For well watered plants the activity of acid phosphatases (Fig.2a) was present and was significantly high for mycorrhizal plants compared to nonmycorrhizal ones. Under water stress acid phosphatases activity increased with the level of stress for all plants. The increment was 40, 87 and 345% for nonmycorrhizal plants, 22, 81 and 293% for mycorrhizal plants for low, average and severe water stress respectively. Acid phosphatases activity increment was significantly high in nonmycorrhizal plants than in mycorrhizal ones.

The mineral element content (Fig. 2b) showed that the concentration of P and N was significantly lower with increase in the level of water stress for nonmycorrhized plants; while for mycorrhized plants the drop is significant only for the average (30% of FC) and severe (15% of FC) water stress. The Mg and K content increased significantly with the level of water stress in the absence of mycorrhization. For the mycorrhized plants the Mg concentrations dropped with increase in water stress level, whereas K was relatively constant and increased significantly only for severe stress. However for the low (60% of FC), average (30% of FC) and severe (15% of FC) stresses, the mycorrhization increases significantly by 14 %, 24 % and 25 % respectively for Mg content. In addition, an increase of 50 % of P for the low and average stress, 39 % for the severe stress was recorded. An improvement of nitrogen content by 17 % for low stress, 30 % for average stress and 22 % for severe stress, was noted for mycorrhized plants. In the case of K, a significant increase in its content was observed only on the level of the low and severe

stress.

### Discussion

In conditions of water stress, plants accumulate organic and inorganic osmolytes (Wu and Xia, 2006), it is in accordance with results of this experiment. Among organic osmolytes we have total amino acids (TAA); proline; total soluble sugars (TSS); the leaves total soluble proteins (LTSP) and for inorganic ones we have  $K^+$  and  $Mg^{2+}$  ions. Low-molecular weight nitrogenous compounds such as proline, increased probably because, plants under water stress, showed enhanced activities of proteases (Mukherjee and Choudhuri, 1985). The increase of synthesis of TAA by inoculated plant and in particular on the level of the average water stress, is in accordance with previously reported findings of Hanson and Hitz (1982) on stressed maize; these amino acids may play a significant role in osmotic adjustment which is an important mechanism of drought tolerance. The osmotic adjustment observed in this study may minimize harmful effects of drought (Morgan, 1990), delay dehydrative damage in drought stressed plants by continued maintenance of cell turgor and physiological processes (Taiz and Zeiger, 2006). High turgor maintenance may also lead to higher photosynthetic rate and growth (Ludlow and Muchow, 1990). In contrast, it has been shown that the amino-acid content in plants under water stress increased (Subramanian and Charest, 1995) or decreased (Augé *et al.*, 1992) according to the state of the symbiosis carried out with AMF.

The increase in proline on leaves is a good indicator of the exposure of plant to water stress (Abdelmoneim *et al.*, 2014). Proline is an important compatible osmolyte normally produced in higher plants in response to environmental stresses (Rhodes *et al.*, 1999, Ozturk and Demir, 2002). This osmolyte is maximum in soybean plants under drought stress and is altered owing to mycorrhizal symbiosis (Porcel and Ruiz-Lozano, 2004). This amino acid has roles as: energy, carbon and nitrogen source, and enhances tissues recovery in the relive of *stress in sorghum* (Blum and Ebercon,1976) and *barley* (Singh *et al.*,

1973a); photodamage reducing in thylakoid membranes by scavenging and/or reducing the production of O<sub>2</sub> (Reddy *et al.*, 2004); a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte (Reddy *et al.*, 2004); protector of membranes and protein structures when relative water content decreases (Lawlor and Cornic, 2002). Also, its accumulation contributes to play an adaptive role in the tolerance of water stress (Ashraf and Iram, 2005, Mafakheri *et al.*, 2010, Din *et al.*, 2011, Karimi *et al.*, 2012). Thus, the increase in the foliar proline content of uninoculated plants according to the severity of water stress shows that harmful effect of water stress is severe on them compared to mycorrhizal plant. On the other hand within mycorrhizal plant, the content of proline is significantly low meaning that mycorrhization impairs negative effects of drought stress. This result was allotted to a great resistance conferred by symbiosis between plant and AMF (Ruiz-Lozano and Azcón, 1997). The low content of proline is an indication of the best tolerance to water constraint (Ruiz-Lozano *et al.*, 1995). These results are similar to those of Abdelmoneim *et al.* (2014) on *Zea mays* which showed that after 7 weeks of water stress (33% of FC) and for an inoculum containing a spores density of *Glomus mosseae* of 300 spores.pot<sup>-1</sup>, the foliar content of proline of mycorrhized plants was reduced by 29%. Mycorrhiza by ameliorating the uptake of water and nutrients by roots through extra radical fungus mycelia, which extend the root surface area (Bethlenfalvay *et al.*, 1988) allowed drought stress plants to avoid its negative effects and maintain their normal metabolism. This may explain the drop of proline content in mycorrhizal plants compared to nonmycorrhizal ones as it was observed for *Zea mays* (Abdelmoneim *et al.*, 2014) and for *V. subterranea* in the present work. The induction of drought stress in plants decrease organs water content induced rapid stomatal closure followed by reduction of transpiration, accumulation of proline and drop in internal carbon dioxide content (Campos *et al.*, 1999, Scotti *et al.*, 1999). As consequence of stomatal closure there is drop in internal carbon dioxide content, decreased photosynthesis activity in

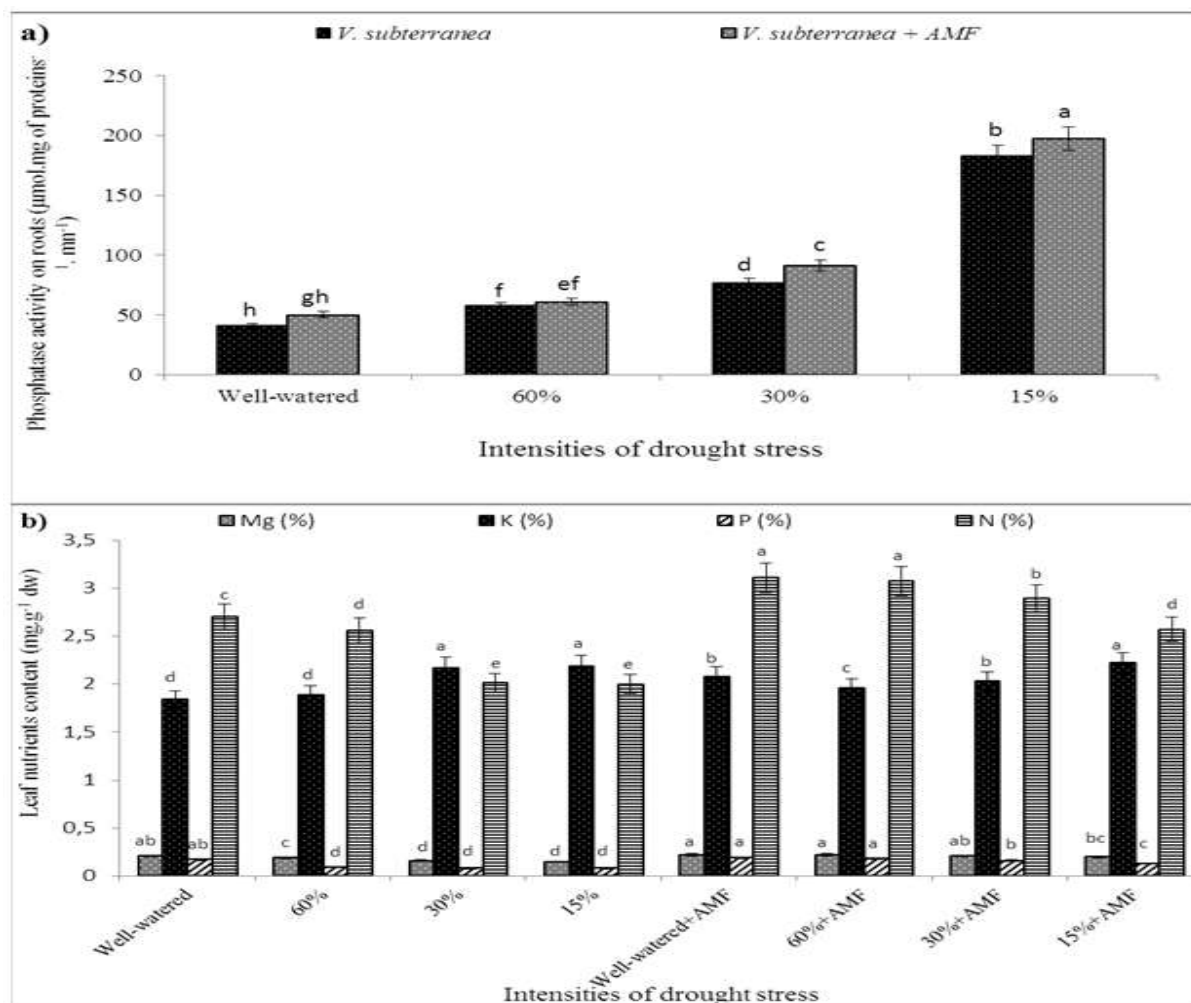
leaves due to inhibition of photochemical activities of Calvin cycle enzymes (Monakhova and Chernyadèv, 2002). The reduction in photosynthesis arises by a decrease in leaf expansion, impairing photosynthetic machinery, premature leaf senescence (Vahid and Rasul, 2005). The impairing of transpiration and photosynthetic machinery by drought stress may be sufficient to affect biochemical and nutrients parameters of *V. subterranea* in this study.

According to the severity of water stress, cellular soluble sugars content recorded were significantly high with a maximum accumulation at the level of the average water stress. However for inoculated plants, the increase in sugars content was more significant compared to uninoculated plants. These results are in accordance with previously reported finding of Qiao *et al.* (2011), who showed that, 90 days after sowing, the mycorrhization of *Cajanus cajan* with *Glomus mosseae* enhanced its soluble sugars content by 44% for control (80% of FC) and 24% when watering with 50% of FC. The increase of this soluble sugars in water deficient plants could be attributed to the stimulation of conversion of starch in sucrose at the carbon dioxide compensation concentration (Fox and Geiger, 1986) presumably for osmotic adjustment (Morgan, 1984, Shao *et al.*, 2009) thereby helping the movement of water (Goicoechea *et al.*, 2004, Mahajan and Tuteja, 2005) and may also contribute to maintain the size of metabolic pools of the photosynthetic carbon reduction cycle. The water deficit could be the main reason for accumulation of soluble sugars observed in the present work. High accumulation of sugars in mycorrhizal plants may be due to the amelioration of water and mineral uptake. The drop of soluble sugars observed for severe drought stress (15% of FC) may be explained by intensive inhibition of photosynthetic activity which is the main source of carbohydrates. In contrast stress and unstressed grapevine plants showed insignificant differences in the sugars content (Patakas *et al.*, 2002).

The significant increase in the content of total leaves soluble proteins (TLSP) of inoculated plants observed

in the present experiment can be justified by the role of the AMF in the improvement of inoculated plants mineral-water nutrition. In fact AMF can substantially enhance the uptake of different nutrients under different conditions, because of their extensive network of hypha (Miransari *et al.*, 2009). The findings of this experiment showed that the TLSP increased significantly for low and average water stress, decreased for severe level. This result could be due to the experimental conditions and according to Tardieu (2005) due to the fact that at the beginning of water stress, stressed plants react dynamically to restore their water status and their metabolism is not significantly affected. When water deficit becomes severe, metabolic changes in response to water stress include decreased of soluble proteins (Irigoyen *et al.*,

1992, Guehl *et al.*, 1993, Keller and Ludlow, 1993). This decrement may be explained by a reduction in bio availability of some essential mineral element such as nitrogen (Costa and Lobato, 2011); the enhancement of proteases activities (Mukherjee and Choudhuri, 1985) and/or the inhibition of protein synthesis by oxidative stress (Feng *et al.*, 2003). Rodriguez *et al.* (2002) reported a decrease in leaf soluble proteins in sunflower due to water stress. In contrast, Ashraf and Mehmood (1990) reported that higher degree of drought resistance was associated with higher proteins content. However, the nature of plant species and the type of tissue modulate the concentration of soluble proteins under water stress (Irogoyen *et al.*, 1992).



**Fig. 2.** Roots phosphatase activity (a), leaf mineral content (b) in mycorrhizal and non-mycorrhizal *V. subterranea* plants under severe (15%), moderate (30%), mild (60%) and no drought stress (Well-watered = 90%) conditions.



Mineralization of organic P occurs through the activity of acid phosphatase enzymes (Duff *et al.*, 1994, Chen *et al.*, 2002, Georges *et al.*, 2002), by means of these enzymes, AMF are able to transform organic P, which is not directly mobilize by plants, into useful form such as ortho- and polyphosphates (Richardson *et al.*, 2009). In the present study, the activity of acid phosphatases was weak in uninoculated plants and increased significantly when water deficit rose. For *V. subterranea* the mycorrhization increased the activity of acid phosphatases by 18% (for 90% of FC), 6% (for 60% of FC), 16 % (for 30% of FC) and 7% (for 15% of FC). These results are in accordance with those of Kinfack Dongmo (2006) and Nwaga *et al.*, (2011) on plantlets of *Musa* sp. (Musaceae) mycorrhizal with *Scutellospora gregaria*; their results showed that 40 days after the setting in the ground plantlets of banana from tissue culture (Elat variety), the activity of acid phosphatases of the inoculated plantlets increased by 42% (for 90% of FC), by 53% (for 60% of FC) and by 52% (for 30% of FC). The increase in the activity of acid phosphatases for stressed plants may improve their tolerance to water stress (Sharma *et al.*, 2005). In mycorrhized plant under water stress, such as *V. subterranea* in this experiment, AMF enhanced production of different enzymes, among them acid phosphatases that enhanced the solubility of nutrients including P and the less mobile microelements and thus substantially enhanced the uptake of different nutrients under different conditions owing to their extensive network of hypha (Miransari *et al.*, 2009).

Water deficit generally results in limited nutrient uptake and their diminished tissue concentrations in crop plants. In order to optimize their hydro-mineral nutrition, most crop plants, may associate themselves with fungi, like AMF, which ensure efficient soil prospectation. AMF improves plant growth by increasing absorption of inorganic nutrients which enhance their tolerance to water stress (Screenivasa and Bagyaraj, 1989); that is probably why mycorrhizal plants of *V. subterranea* are rich in mineral nutrients (Mg, P, N, K).

Potassium (K) is essential for many physiological processes such as transpiration and photosynthesis (Marschner, 1995, Mengel and Kirkby, 1987). K has substantial effect on stomatal movement and water relation (osmotic potential and turgor regulation of the cell, osmotic adjustment) in plants (Marschner, 1995, Lindhauer, 1995) and regulates the stomatal functioning under water stress conditions (Kant and Kafkafi, 2002). Stomatal function is to control water loss from the plant through transpiration. When K<sup>+</sup> is deficient, the stomata cannot function properly and transpiration may reach damaging levels (Gethings, 1990). Lowered absorption of the inorganic nutrients can reduce transpiration flow (Garg, 2003, Mc Williams, 2003). Under water deficit conditions, K nutrition increases crop tolerance to water stress by utilizing the soil moisture more efficiently than in K-deficient plants (Lindhauer, 1995). The increase in the potassium content recorded with the severity of the stress could be an effective tolerance response of *V. subterranea*. Potassium enhances photosynthetic rate under stress conditions (Egilla *et al.*, 2001); without this element no photosynthetic activity can take place. Alleviation of detrimental effects of drought stress, especially on photosynthesis, by sufficient K supply has been shown in legumes (Sangakkara *et al.*, 2000). K has important effect on enzyme activation, proteins synthesis and photosynthesis in plants (Marschner, 1995). Under water stress, the photosynthetic efficiency of plants is reduced drastically as a consequence of chloroplast dehydration (Sen Gupta and Berkowitz, 1987, Berkowitz and Kroll, 1988). The chloroplasts lose large amounts of K<sup>+</sup> with a simultaneous decrease in photosynthesis. The reason for the enhanced need for K by plant suffering from environmental stresses appears to be related to the fact that K is required for maintenance of photosynthetic carbon dioxide fixation (Waraich *et al.*, 2011). It may be the case for *V. subterranea* used in the present study. The pH of leaf sap, which can promote abscisic acid accumulation and concomitantly diminish stomatal conductance, is increased by environmental conditions that enhance the transpiration rate (Farooq *et al.*, 2009). In plants suffering from

drought stress K plays protective role by maintenance of high pH in stroma and against the photooxidative damage to chloroplast (Cakmak, 1997). This may be one of the reasons of enhancement of K content *V. subterranea* observed in the present work. The amelioration of mineral absorption observed in this study has already been noted for phosphorus in mycorrhized maize with *Glomus fasciculatum* (Subramanian *et al.*, 2006), the improvement of nitrogen assimilation was also noted in mycorrhized soybean with *Glomus fasciculatum* (Patreze and Cordeiro, 2004). Phosphorus and nitrogen are both essential macronutrient, increased uptake of these element by drought stress plants can improve their drought tolerance (Waraich *et al.*, 2011).

### Conclusion

The aim of this study was to evaluate the early effects of AMF species mixture (*Glomus hoï* + *Glomus intraradices* + *Gigaspora margarita* + *Scutellospora gregaria*) on *Vigna subterranea* grown in various water stress regimes. Mycorrhiza symbiosis alleviates detrimental drought stress effects on *Vigna subterranea* at early growth stage; by means of osmotic adjustment, thus enabling more synthesis of sugar, acid phosphatases. Thus, the use of mycorrhizal biofertilizer can be considered as one effective mean to alleviate unfavorable environmental factors such as drought stress common in sahelian zones or dry season in humid zones.

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### References

**Abdelmoneim TS, Tarek Moussa AA, Almaghrabi OA, Hassan Alzahrani S, Ismail Abdelbag.** 2014. Increasing Plant Tolerance to Drought Stress by Inoculation with arbuscular mycorrhizal fungi. *Life Science Journal* **11(1)**, 10-17.

**Allen EB, Allen MF.** 1986. Water relations of xeric grasses in the field: interactions of mycorrhizas and competition. *New Phytologist* **104**, 559-571.

**Anonyme.** 2007. Biotechnologie et maîtrise des intrants agricoles en Afrique central. CRESA. Ed. CRESA forêt-bois, Yaoundé-Cameroun, p. 7-54.

**Ashraf M, Mahmood S.** 1990. Response of four *Brassica* species to drought stress. *Environmental Experimental Biology* **30**, 93-100.

**Ashraf M, Iram A.** 2005. Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. *Flora* **200**, 535-546.

**Augé RM, Foster JG, Loesche WH, Stodola AJ.** 1992. Symplastic molality of free amino acids and sugars in *Rosa* roots with 34 regard to VA mycorrhizae and drought symbiosis. *Symbiosis* **12**, 1-17.

**Azam-ali SN, Sesay A, Karikari SK, Massawe FJ, Aguilar-manjarrez J, Bannayan M, Hampson KJ.** 2001. Assessing the potential of an underutilized crop - A case study using Bambara groundnut. *Experimental Agriculture* **37**, 433-472.

**Benton Jones J, Vernon Case W.** 1990. Sampling, handling and analyzing plant tissue samples. In: Westerman Kluwer RL, Eds. *Soil testing and plant Analysis*, Academic Publishers, Amsterdam, p. 1-76.

**Berkowitz GA, Kroll KS.** 1988. Acclimation of photosynthesis in *Zea mays* to low water potentials involves altered degree of protoplast volume reduction. *Planta* **175**, 374-379.

**Bethlenfalvay GJ, Brown MS, Ames RN, Thomas RE.** 1988. Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphate uptake. *Physiologia Plantarum* **72**, 565-571.

- Blum A, Ebercon A.** 1976. Genotypic responses in Sorghum to drought stress III. Free proline accumulation and drought resistance. *Crop Science* **16**, 428-431.
- Bradford M.** 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248-254.
- Buondonno A, Rashad AA, Coppola E.** 1995. Comparing tests for soil fertility. 11. The hydrogen peroxide/sulfuric acid treatment as an alternative to the copper/selenium catalyzed digestion process for routine determination of soil nitrogen-Kjeldahl. *Communications in Soil Science and Plant Analysis* **26**, 1607-1619.
- Cakmak I.** 1997. Role of potassium in protecting higher plants against photo-oxidative damage. In: Johnston, AE, Ed. Food security in the WANA region, the essential need for balanced fertilization, International Potash Institute, Basel Switzerland, p. 345-352.
- Campos PS, Ramalho JC, Lauriano JA, Silva MJ.** Do céu Matos M. 1999. Effects of drought on photosynthetic performance and water relations of four *Vigna* genotypes. *Photosynthetica* **36**, 79-87.
- Chen THH, Murata N.** 2002. Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Current Opinion Plant Biology* **5**, 250-257.
- Costa DLCR, Lobato SDKA, Silvera DG AJ, Laughinghousevi DH.** 2011. ABA mediated proline synthesis in cowpea leaves exposed to water deficiency and rehydration. *Turkish Journal Agricultural Forestry* **35**, 309-317.
- Din J, Khan U, Ali I, Gurmani RA.** 2011. Physiological and agronomic response of Canola varieties to drought stress. *The Journal of Animal and Plant Sciences* **21(1)**, 78-82.
- Duff SMG, Sarath G, Plaxton WC. 1994. The Role of acids phosphatase in plant phosphorus metabolism. *Physiologia Plantarum* **90**, 791-800.
- EgilLa JN, Davies FTJ, Drew MC.** 2001. Effect of potassium on drought resistance of *Hibiscus rosa-sinensis* cv. Leprechaun: plant growth, leaf macro and micronutrient content and root longevity. *Plant Soil* **229**, 213-224.
- Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA.** 2009. Plant drought stress effects, mechanisms and management. *Agronomy for Sustainable Development* **29**, 185-212.
- Feng G, Song YC, Li XL, Christie P.** 2003. Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in calcareous soil. *Apply Soil Ecology* **22**, 139-148.
- Fox TC, Geige DR.** 1986. Osmotic response of sugar beet source leaves at CO<sub>2</sub> compensation point. *Plant Physiology* **80**, 239-241.
- Garg BK.** 2003. Nutrients uptake and management under drought: nutrient-moisture interaction, *Current Agriculture* **27**, 1-8.
- George TS, Gregory PJ, Wood M, Read D, Buresh RJ.** 2002. Phosphatase activity and organic acids in the rhizosphere of potential agroforestry species and maize. *Soil Biology and Biochemistry* **34(10)**, 1487-1494.
- Gething PA.** 1990. Potassium and water relationships. In: Potash facts. IPI, Bern, 123 p.
- Giller KE.** 2001. Nitrogen fixation in tropical cropping systems. CABI Publishing series Wallingford UK, 423 p.
- Goicoechea N, Merino S, Sanchez-Diaz.** 2004. Contribution of mycorrhizal fungi (AMF) to the adaptations exhibited by the deciduous shrub

*Anthyllis cytisoides* under water deficit. *Physiologia Plantarum* **122**, 453-464.

**Guehl JM, Clement A, Kaushal P, Aussenac G.** 1993. Planting stress, water status and non structural carbohydrate concentrations in Corsicon pine seedlings. *Tree Physiology* **12**, 173-183.

**Guissou T, Ba AM, Plenchette C, Guinko S, Duponnois R.** 2001. Effet des mycorhizes à arbuscules sur la tolérance à un stress hydrique de quatre arbres fruitiers : *Balanite aegyptiaca* (L.) Del., *Parkia biglobosa* (Jacq.) Benth., *Tamarindus indica* L. et *Zyziphus mauritania* Lam. *Sécheresse* **12(2)**, 121-127.

**Hanson AD, Hitz WD.** 1982. Metabolic responses of mesophytes to plant water deficits. *Annual Review Plant Physiology* **33**, 163-203.

**Hillocks RJ, Bennett C, Mponda MO.** 2012. Bambara nut: A review of utilization market potential and crop improvement. *African Crop Science Journal* **20(1)**, 1-16.

**Hooley R.** 1984. Gibberelic acid controls the secretion of acid phosphatase in aleurone layers and isolated aleurone protoplasts of *Avena fatua*. *Journal Experimental Botany* **35**, 822-828.

**Irigoyen JJ, Emerich DW, Sanchez-Diaz M.** 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia Plantarum* **84**, 55-60.

**Juniper S, Abott L.** 1993. Vesicular arbuscular mycorrhizal and soil salinity. *Mycorrhiza* **4**, 45-57.

**Kant S, Kafkafi U.** 2002. Potassium and abiotic stresses in plants. Pasricha NS, Bansal SK, Eds. Role of potassium in nutrient management for sustainable crop production in India, Potash Research Institute of India, Gurgaon, Haryana, p. 249-304.

**Karimi S, Abbaspour H, Sinaki JM, Makarian H.** 2012. Effects of water deficit and chitosan spraying on osmotic adjustment and soluble protein of cultivars castor bean (*Ricinus communis* L.). *Journal of stress Physiology and Biochemistry* **8(3)**, 160-169.

**Keller F, Ludlow MM.** 1993. Carbohydrate metabolism in drought-stressed leaves of Pigeonpea (*Cajanus cajan*). *Journal Experimental Botany* **44**, 1351-1359.

**Kinfack Dongmo MM.** 2006. Incidence de la mycorhization des *in vitro* plants de bananier plantain (*Musa sp.*) sur la tolérance au stress hydrique. Mémoire de DEA en Biotechnologies végétales, Université de Yaoundé I, 48 p.

**Lawlor DW, Cornic G.** 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell & Environment* **25**, 275-294.

**Linderman RG.** 1994. Role of VAM fungi in biocontrol, In: Pflieger FL, Linderman RG, Ed. Mycorrhizae and plant health. APS Press, St Paul, Minn, p. 1-26.

**Lindhauer MG.** 1995. Influence of K nutrition and drought and water stressed sunflower plants differing in K nutrition. *Journal of Plant Nutrition* **10**, 1965-1973.

**Linnemann AR.** 1994. Phenological development in Bambara groundnut (*Vigna subterranea*) at alternate exposure to 12 and 14h photoperiod. *Journal of Agricultural Science* **123**, 333-340.

**Ludlow MM, Muchow RC.** 1990. A critical evaluation of traits for improving crop yields in water-limited environments. *Advances in Agronomy* **4**, 107-153.

**Mafakheri A, Siosemardeh A, Bahramnejad B, Straik PC, Sohrabi E.** 2010. Effect of drought

stress on yield, proline and chlorophyll contents in three chickpea cultivars. African Journal of Crop Science **4(8)**, 580-585.

**Maggio A, Reddy MP, Joly RJ.** 2000. Leaf gas exchange and soluble accumulation in the halophyte *Salvadora persia* grown at moderate salinity. Environmental and Experimental Botany **44**, 31-38.

**Mahajan S, Tuteja N.** 2005. Cold, Salinity and drought stresses. An overview. Archives of Biochemistry and Biophysics **444**, 139-158.

**Mamoudou Dicko H.** 2005. Réponses adaptatives de deux variétés de niébé à un stress hydrique. Cahiers Agricultures **14(6)**, 561-566.

**Marschner H.** 1995. Mineral nutrition of higher plants, 2<sup>nd</sup> Ed. , Academic Press, London, U.K., 889 p.

**Mc Williams D.** 2003. Identifying nutrient deficiencies for efficient plant growth and water use. New Mexico State University NMSU and the U.S. Department of Agriculture, 4 p.

**Mengel K, Kirck EA.** 1987. Principles of plant nutrition, 4<sup>th</sup> Ed. International Potash Institute, Switzerland, 687 p.

**Miransari M, Bahrami HA, Rejali F, Malakouti MJ.** 2009. Using arbuscular mycorrhizal to reduce the stressful effects of soil compaction on wheat (*Triticum aestivum*) growth. Soil Biology and Biochemistry **40**, 1197-1206.

**Monakhova OF, Chernyadèv II.** 2002. Protective role of karvolin-4 in wheat plants exposed to soil drought. Apply Biochemistry and Microbiology **38**, 373-380.

**Morgan JM.** 1984. Osmoregulation and water stress in higher plants. Annual Review of Plant Physiology **35**, 299-319.

**Morgan PW.** 1990. Effects of abiotic stresses on plant hormone systems, In: Stress responses in plants: adaptation and acclimation mechanisms, Wiley-Liss, Inc, p. 113-146.

**Mukherjee SP, Choudhuri MA.** 1985. Implication of hydrogen peroxide-ascorbate system on membrane permeability of water stressed *Vigna* seedlings. New Phytologist **99**, 335-360.

**Murphy J, Riley JP.** 1992. A modified single solution method for determination of phosphate in natural waters. Analytica Chimica Acta **27**, 31-36.

**Ncube B, Twomlow SJ.** 2007. Productivity and residual benefit of grain legumes to sorghum under semi-arid conditions in southwestern Zimbabwe. Plant and Soil **299**, 1-15.

**Nelsen CE.** 1987. The water relations of vesicular-arbuscular mycorrhizal systems, In: Safir GR, Ed. Ecophysiology of VA mycorrhizal plants. CRC Press, Boca Raton, Fla, p. 71-79.

**Nwaga D, Jansa J, Angue Abossolo AM, Frossard E.** 2010. The potential of soil beneficial microorganisms for slash-and-burn agricultural in the humid forest zone of sub-Saharan Africa. In: Soil biology and agriculture in the tropics, soil biology, Dion P, Springer-Verlag Eds. Berlin Heidelberg, p. 85-105.

**Nwaga D, Tenkouano A, Tomekpe K, Fogain R, Kinack DM, Tsané G, Yombo O.** 2011. Multi-functional properties of mycorrhizal fungi for crop production: the case study of banana development and drought tolerance. In: Bationo A, Boaz W, Jeremiah M, Okeyo, Fredah M, Job Kihara J, Eds. Innovations as key to green revolution in Africa. Vol.1. Springer Heidelberg Dordrecht. London, New York, p. 523-531.

**Oehl F, Jan Jansa, Kurt IPM, Heijden MVD.** 2011. Champignons mycorrhiziens arbusculaires, bioindicateurs dans les sols agricoles Suisse.

Recherche Agronomique Suisse **2(7-8)**, 304-311.

**Oyun MB, Adeduntan SA, Suberu SA.** 2010. Influence of watering regime and mycorrhizae inoculations on the physiology early growth of *Acacia senegal* (L.) Wild. African Journal of Plant Science **4(7)**, 210-216.

**Ozturk L, Demir Y.** 2002. In vivo and in vitro protective role of proline. Plant growth Regulation **38**, 259-264.

**Patakas A, Nikolaou N, Zioziou E, Radoglou K, Nortsakis B.** 2002. The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. Plant Science **163**, 361-367.

**Patreze CM, Cordeiro L.** 2004. Nitrogen fixing and vesicular arbuscular mycorrhizal symbioses in some tropical legumes trees of tribe Mimosaceae. Forest Ecology and Management **196**, 275-285.

**Petard J.** 1993. Méthodes d'analyse des sols. Noumié. ORSTOM, 200 p.

**Porcel R, Ruiz-Lozano JM.** 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. Journal of Experimental Botany **55**, 1743-1750.

**Qiao G, Wen XP, Yu LF, Ji XB.** 2011. The enhancement of drought tolerance for pigeon pea inoculated by arbuscular mycorrhizae fungi. Plant Soil Environment **57(12)**, 541-546.

**Reddy AR, Chiatanya KV, Vivekanandan M.** 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology **161(11)**, 1189-1202.

**Rhodes D, Verslues PE, Sharp RE.** 1999. Role of amino acids in abiotic stress resistance. In Plant Amino Acids: Biochemistry and Biotechnology, Singh Marcel Dekker BK, Eds. NY, p. 319-356.

**Richardson AE, Barea J-M, McNeill AM, Prigent-Combaret C.** 2009. Acquisition of phosphorus and nitrogen in rhizosphere and plant growth promotion by microorganisms. Plant Soil **321**, 305-339.

**Rodriguez DJ, Romero-Garcia J, Rodriguez-Garcia R, Angulo-Sanchez JL.** 2002. Characterization of protein from sunflower leaves and seeds: Relationship of biomass and seed yield. In: Trends in new crops and new uses Janick J, Whipkey A, Eds. ASHS Press, Alexandria V, p. 143-149.

**Ruiz-Lozano JM, Azcon R.** 1997. Effect of calcium application on tolerance of mycorrhizal lettuce plants to polyethylene glycol-induced water stress. Symbiosis **23**, 9-21.

**Ruiz-Lozano JM, Azcon R, Gomez M.** 1995. Effects of arbuscular-mycorrhizal *Glomus* Species on drought tolerance: Physiological and nutritional plant responses. Applied and Environmental Microbiology **61(2)**, 456-460.

**Sangakkara UR, Frehner M, Nosberger.** 2000. Effect of soil moisture and potassium fertilizer on shoot water potential, photosynthesis and partitioning of carbon in mung bean and cowpea. Journal of Agronomy and Crop Science **185**, 201-207.

**Scotti CP, Ramalho JC, Lauriano JA, Silva LJ, Céu-Matos DM.** 1999. Effects of drought on photosynthetic performance and water relations of four *Vigna* genotypes. Photosynthetica **36**, 79-87.

**Screenivasa MN, Bagyaraj DJ.** 1989. Use of pesticide for mass production of vesicular-arbuscular mycorrhizal inoculum. Plant and Soil **119**, 127-132.

**Sen Gupta A, Berkowitz GA.** 1987. Osmotic adjustment, symplast volume and non-stomatally mediated water stress inhibition of photosynthesis in wheat. Plant Physiology **85**, 1040-1047.

- Shao HB, Chu L-Y, Abdul Jaleel Manivannan CP, Panneerselvam R, Shao MN.** 2009. Understanding water deficit stress-induced changes in the basic metabolism of higher plant-biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. *Critical Review in Biotechnology* **29(2)**, 131-151.
- Sharma AD, Singh N, Kang JK.** 2005. Short-term water logging-induced changes in phosphatase activity in shoot and roots of sorghum seedlings: Role of phosphatases during water logging in relation to phosphorus during water logging in relation to phosphorus. *General and Applied Plant Physiology* **31(2)**, 71-79.
- Singh TN, Aspinall D, Paleg L, Boggess SF.** 1973a. Changes in proline concentration in excised plant tissues. *Australian Journal of Biological Science* **26**, 57-63.
- Song F, Song G, Dong A, Kong X.** 2011. Regulatory mechanisms of host plant defense responses to arbuscular mycorrhiza. *Acta Ecologica Sinica* **31**, 322-327.
- Subramanian KS, Charest C.** 1995. Influence of arbuscular mycorrhizal on the metabolism of maize under drought stress. *Mycorrhiza* **5**, 273-278.
- Subramanian KS, Santhanakrishnan P, Balasubramanian P.** 2006. Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. *Scientia Horticulturae* **107**, 245-253.
- Taiz L, Zeiger E.** 2006. *Plant Physiology*, 4<sup>th</sup> Ed., Sinauer Associates Inc. Publishers, Massachusetts, p. 235-310.
- Tardieu F.** 2005. Plant tolerance to water deficit: Physical limits and possibilities for progress. *Geological Science* **337**, 57-67.
- Tobar R, Azcün R, Barea JM.** 1994. The improvement of plants N nutrition from an ammonium-treated, drought stressed soil by the fungal symbiotic in arbuscular mycorrhizal. *Mycorrhiza* **4**, 105-108.
- Wahid A, Rasul E.** 2005. Photosynthesis in leaf, stem, flower and fruit, in: Pessaraki M Ed. *Handbook of Photosynthesis*, 2<sup>nd</sup> ed., CRC Press, Florida, p. 479-497.
- Wang W, Vinocur B, Altman A.** 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* **218**, 1-14.
- Waraich EA, Ahmad R, Saifullah Y, Ashraf M, Ehsanullah.** 2011. Role of mineral nutrition in alleviation of drought stress in plants. *Australian Journal of Crop Science* **5(6)**, 764-777.
- Wu QS, Xia RX.** 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology* **163**, 417-425.
- Yao Djè, Béket SB, Zoro Bi IA.** 2005. Observation préliminaires de la variabilité entre quelques morphotypes de voandzou (*Vigna subterranea* L. Verdc.,(Fabaceae) de côte d'Ivoire. *Biotechnology, Agronomy, Society and Environment* **9(4)**, 249-258.
- Yemm EM, Cocking EC.** 1955. The determination of amino acids with ninhydrin. *The Analyst* **80**, 209-213.
- Yemm EM, Willis AJ.** 1954. The estimation of carbohydrates in plants extracts by Anthron. *Biochemistry Journal* **9(2)**, 27-36.