Environmental and Experimental Biology (2017) 15: 15–24 *Original Paper*
DOI: 10.22364/eeb.15.03

Role of potassium and arbuscular mycorrhizal fungi in alleviation of water stress on *Vigna mungo*

Navnita Sharma, Kuldeep Yadav, Ashok Aggarwal*

Department of Botany, Kurukshetra University, Kurukshetra, Haryana 136119, India

*Corresponding author, E-mail: aggarwal_vibha@rediffmail.com

Abstract

A pot culture experiment was carried out under greenhouse conditions to study the effect of potassium fertilizer (70 mg kg^{-1}, K1, and 100 mg kg^{-1}, K2) and arbuscular mycorrhizal fungi (*Glomus mosseae* and *Acaulospora laevis*) alone and in different combinations on water stress amelioration of *Vigna mungo* during three growth stages. Treatment of the plants with mycorrhizal fungi and potassium improved physiological parameters including chlorophyll concentration, membrane stability, phosphatase activity, leaf protein concentration and mycorrhization over the untreated control regardless of water stress treatments. Imposition of water stress at reproductive stage caused maximum reduction in membrane stability, leaf protein concentration, phosphatase activity and mycorrhization as compared to stress during other stages. Treatment combinations of *G. mosseae* + *A. laevis* + K2 and *G. mosseae* + *A. laevis* + K1 proved to be the best for promoting physiological and morphological parameters studied both in stressed and unstressed plants. A combination of *G. mosseae* and K2 was the best among double inoculation treatments while *G. mosseae* was the best among single treatments of either dose of potassium and *A. laevis* for stress alleviation and growth amelioration. Imposition of water stress during vegetative stage had the least effect on the physiological parameters studied, except for leaf area, which was adversely affected. Chlorophyll concentration was the most affected parameter by water stress at the pod formation stage.

Key words: black gram, drought tolerance, electrolyte leakage, phosphatase activity, legume, mycorrhizal fungi, *Vigna mungo*.
Abbreviations: AL, *Acaulospora laevis*; AMF, arbuscular mycorrhizal fungi; DAS, days after sowing; GM, *Glomus mosseae*.

Introduction

Black gram [*Vigna mungo* (L.) Hepper], an important food legume, is one of the Asiatic species of pan-tropical genus *Vigna*. The pulse legume has its origin in the Indian subcontinent, where it has a long history of cultivation as carbonized grains (Vavilov 1926). The seeds have high nutritive value with about 26% protein, which is thrice that of cereals. Pulse legume is not only important as food and nutritive feed but it also plays a vital role in sustaining soil fertility as well as soil physical properties through symbiotic nitrogen fixation. It is the staple legume of semi-arid and sub-tropical areas of South East Asia. Due to its wider adaptability to semi-arid climates it is an ideal crop for dry land farming. India ranks first in production and consumption of black gram in the world. Over the last decades, there has been an increase in production of *V. mungo* due to better agricultural and breeding techniques but the increase is not enough to meet domestic consumption in the country (Gautam et al. 2016). One of the important reasons for decreased productivity is water stress experienced by the crop during different stages of growth, as nearly 87% of the area under cultivation of pulses is rain fed.

Under the context of climate change, water deficit is becoming a severe problem restraining plant growth and productivity of terrestrial ecosystems, chiefly in arid regions. It is estimated that water stress can potentially reduce world crop yield by up to 20% (Scheiermeier 2008). Water is required at every stage of plant growth from seed germination to maturation; any type of imbalance in water acquisition causes a serious threat to agriculture affecting ultimately the yield. Furthermore, the effect of water stress on growth is determined by genotypes, the duration and severity of water deficit, age and stage of plant growth during which stress has occurred (Barnabás et al. 2008).

In order to cope with environmental hazards and struggle against water stress, plants develop various defense approaches; mutualistic association of arbuscular mycorrhizal fungi (AMF) with plant in roots is an example (Wang, Qiu 2006). In this symbiotic association, the intra-radical mycelium propagates in the cortex layer of root and the extraradical mycelium of fungus penetrates through the contact regions of roots in the soil causing better exploration of soil volume for improving uptake of water and mineral nutrients (Wu et al. 2013). Besides this, AMF increase soil aggregate stability by releasing glomalin, a glycoprotein, into the soil (Bedini et al. 2010). Under water deficit conditions, mycorrhizal fungi ameliorate water status of plant by direct water uptake through

extraradical hyphae, regulation of stomatal conductance, improved phosphorus (P) nutrition due to higher activity of phosphatases, increased photosynthetic pigment concentration and antioxidant enzyme activity, as well as osmotic adjustment by different osmolytes (Sharma et al. 2015). In addition to mycorrhizal fungi, potassium enables the plants to overcome stress by efficient water utilization from soil, increased growth of roots and nutrient uptake. Potassium also regulates the opening of stomata, increases photosynthetic rate and thus yield (Umar 2006).

Considering the wide adaptability and drought tolerance of *V. mungo* it can be an ideal crop for sustainable agricultural systems in arid regions where crops are stricken by frequent drought stress. For cultivation in such conditions, the drought tolerance of crop needs to be further improved to obtain maximum productivity. To strengthen the cultivation of *V. mungo* in such drought affected areas of Haryana (India), the present study was aimed to investigate whether AMF enhances drought tolerance and to elucidate physiological responses of AMF colonized plants exposed to water stress at different growth stages.

Materials and methods

Study site and soil characteristics

The experiment was a laid in a randomized block design with eleven treatments replicated five times, during the kharif season of 2014 and 2015 in a cage house at the Botany Department, Kurukshetra University, Kurukshetra. Top soil used for the experiment consisted of sand (64.2%), silt (21.81%), clay (3.90%), with initial pH 6.8, electrical conducticvity 0.25 dS m^{-1}, total nitrogen 0.042%, organic carbon 0.06%, available K 0.022 kg m^{-2}, available P 0.0018 kg m^{-2} and available S 14.80 mg kg^{-1}.

Inoculum preparation

Dominant AMF spores were isolated from the rhizosphere of *V. mungo* by wet sieving and decanting technique of Gerdemann and Nicolson (1963) and identified using the key of Schenck and Perez (1990). *Glomus mosseae*, also known as *Funneliformis mosseae* (T. H. Nicolson and Gerd.), C. Walker and A. Schüßler, as well as *Acaulospora laevis* Gerdemann and Trappe, were found to be the dominant AMF. Starter inoculum of the AMF was raised using the funnel technique of Menge and Timmer (1982). The two AMF species were propagated with maize as a host for a period of three months under pot culture conditions. *Bradyrhizobium japonicum* culture (procured from Department of Microbiology, CCS Haryana Agricultural University, India) was multiplied by using nutrient broth medium.

Experimental setup

The experiment was laid in a randomized block design. Five replicates of each treatment were used. Soil and sand in ratio 3:1 was sterilized and added to each earthen pot (25 × 25 cm). Sand was added to increase porosity of the soil. Also, 10% selected AMF inoculum *G mosseae* (GM) or *A. laevis* (AL) with around 860 spores and nearly 80% colonized root segments of trap host maize was added. Each pot was filled with 1.8 kg soil sand mixture; 180 g of inoculum (ie 10% of the soil mass in the pot) was added for single mycorrhizal inoculation while 90 g inoculum of each mycorrhizal fungus was added for double inoculation treatment (AL + GM). Potassium was added to the soil after germination in the form of muriate of potash at 70 and 100 mg kg^{-1} concentration alone and in combinations with mycorrhizal treatments. Seeds of *V. mungo* (procured from Haryana Agriculture University Hisar, Haryana, India) after surface sterilization with 10% sodium hypochlorite solution were sown in the pots. To each pot, including an uninoculated pot, 10 mL *Bradyrhizobium* sp. culture suspension with density 10^8 cells mL^{-1} was applied. Pots were treated either with single inoculum, combined inoculum or no inoculum as summarized below.

There were four sets of identical treatments as listed above [no water stress, water stress at vegetative stage 25 days after sowing (DAS), reproductive stage 45 DAS and pod formation stage 80 DAS]. For every set, each of the eleven treatments was replicated five times. Water stress was applied only once for a particular stage (at vegetative stage 25 DAS, reproductive stage 45 DAS and pod formation stage 80 DAS) for 10 subsequent days (the plants began wilting), then the plants were watered for next 20 days. After 20 days of rewatering, the plants overcame stress and resumed normal growth and were analyzed for different parameters. The set of well watered plants was analysed at vegetative stage. During well watered treatment soil water content was kept at 80% of the field capacity. During water stress treatment, the soil water was kept at 40% field capacity by weighing the pots every day to control soil water content.

A preliminary experiment was carried out to fine tune the drought stress period (data not shown). After a 10-day period of water stres at 40% field capacity, the plants

Table 1. Treatments used in the present study

No.	Treatments
1	Control (uninoculated i.e. autoclaved sterile sand/soil with no inoculum)
2	K1 (potassium fertilizer in concentration 70 mg kg^{-1})
3	K2 (potassium fertilizer in concentration 100 mg kg^{-1})
4	*G. mosseae* (GM)
5	*A. laevis* (AL)
6	GM + K1
7	GM + K2
8	AL + K1
9	AL + K2
10	GM + AL + K1
11	GM + AL + K2

started wilting; defoliation appeared at the time when they received severer drought (at 30% of field capacity). Thus, we chose 40% of field capacity as drought stress for 10 days in order to allow the plants to fully recover after this stress period. Chlorophyll concentration, leaf area, electrolyte leakage, protein concentration of the fresh leaves were determined in the 4th leaf from the growing tip of the plant bythe following methods.

Measurement of physiological and morphological parameters

Chlorophyll and carotenoid concentration was estimated according to the method of Arnon (1949). Fresh leaf samples (0.1 g) were homogenised in a pre-chilled pestle and mortar with 80% acetone under dark conditions. To avoid photodestruction of chlorophyll, a pinch of $CaCO_3$ was added. The suspension was centrifuged at 2000 rpm for 15 min. The supernatant was collected and the volume was made up to 20 mL with 80% acetone. The absorbance of the solution was red at 645 nm and 663 nm against the solvent (acetone) blank was determined.

Leaf area (cm^2) was measured by using leaf area meter (Systronics 211, Ahmedabad, India).

The electrolyte leakage test was performed by cutting 200 mg of fresh leaf sample into small discs of 5 mm diameter. The discs were placed in test tubes containing 10 mL of deionized water. The tubes were covered with cotton plugs and placed in a water bath at temperature 32 ± 8 °C. After 2 h initial electrical conductivity of medium (EC1) was measured using an electrical conductivity meter. The samples were autoclaved at 121 ± 8 °C for 20 min to release all the electrolytes. Samples after cooling were analyzed for final electrical conductivity of medium (EC2). Electrolyte leakage percentage was estimated by using the formula of Dionisio-Sese and Tobita (1998):

$$\text{Electrolyte leakage} = EC1 / EC2 \times 100.$$

Total protein concentration was estimated by the method of Bradford (1976). In this experiment, 100 mg of the leaf sample was warmed in 10 mL 80% ethanol in a water bath for 2 to 5 min. Then it was cooled at room temperature and homogenized with the same ethanol. The homogenate was centrifuged at 5000 rpm for 10 min. The residue was reextracted with 10 mL of 5% perchloric acid with same speed and time. Supernatent was again discarded and the residue was reextracted with 5 mL of 1N NaOH by keeping it in warm water at temperature 40 to 50 °C for 30 min. After this, centrifugation was done at 5000 rpm for 10 min and the supernatent was used as protein source.

The protein source taken was 0.2 mL in each test tube to which 0.8 mL of double distilled water was added. It was followed by addition of 5 mL of Comassie Brilliant Blue (protein estimating reagent) to the test tube with immediate shaking at room temperature. Blank was made of 1 mL double distilled waterwith no plant extract along with 5 mL of dye. Absorbance was measured at 595 nm in UV-VIS spectrophotometer (Specord-205, Analytik Jena, Germany). The protein content of the samples was determined against a standard calibration curve of bovine serum albumin (Sigma , USA).

Phosphatase activity was estimated by using *p*-nitrophenyl phosphate as a substrate, which is hydrolyzed by the enzyme to *p*-nitrophenol (Tabatabi, Bremer 1969). Fresh roots were used for extraction of acid and alkaline phosphatases. For this, 1 g of fresh washed roots were homogenized in 5 mL of ice cold sodium acetate buffer (0.1 M with pH 4.8) for acid phosphatase and sodium carbonate/bicarbonate buffer (0.05N with pH 10) for alkaline phosphatase activity using a prechilled pestle and mortar. The resulting homogenate was centrifugrd at 10 000 rpm for 15 min and supernatant thus obtained; referred to as crude extract was used for assay of phosphatase activity which was expressed in terms of units per g fresh mass.

Identification and quantification of the number and colonization by AMF spores

AMF spores were isolated by wet seiving and decanting technique (Gerdemann, Nicolson 1963). Identification of AMF spores (*F. mosseae* and *A. laevis*) was done by using the identification manual of Scheneck and Perez (1990); Morton and Benny (1990) and Mukerji (1996). Quantification of AMF spores was done using the Adholeya and Gaur 'Grid Line Intersect Method' (1994). For assesment of mycorrhizal colonization of roots, the roots from each treatment were washed gently with tap water and rinsed with distilled water. A sub sample of 0.5 g was taken from each root which was then cut into 1cm long segments. These segments were bleached for 30 min in 10% KOH at 90 °C and then were acidified for 10 min in 1% HCl. The roots were then stained in trypan blue (Phillips, Hayman 1970) was used to estimate Mycorrhizal colonization of roots. Percent mycorrhizal colonization of roots was calculated by using the formula: (Number of root segments colonized / number of root segments studied) × 100. One hundred root segments were examined for each particular treatment to determine the root colonization.

Statistical analysis

Data were subjected to analysis of variance and means separated using the least significant difference test in the Statistical Package for Social Sciences (ver.11.5, Chicago, IL, USA).

Results

Chlorophyll concentration

In the present experiment, a reduction in the concentration of photosynthetic pigments in water stressed plants, in contrast to unstressed plants regardless of the various treatments. All the untreated and treated plants in well watered conditions had higher chlorophyll concentration

Table 2. Effect of AM fungi and potassium on chlorophyll content, leaf area and electrolyte leakage of *Vigna mungo* grown under well watered conditions. GM, *Glomus mosseae*; AL, *Acaulospora laevis*; K1, 70 mg kg^{-1} K fertilizer; K2, 100 mg kg^{-1} K fertilizer; FM, fresh mass. Each value is a mean of five replicates ± SD. Values in column followed by same letter are not significantly different ($P \leq 0.05$)

Treatment	Chlorophyll concentration (mg g^{-1} FM)			Leaf area (cm^2)	Electrolyte leakage
	Chl *a*	Chl *b*	Total Chl		(%)
Control	0.973 ± 0.003k	0.322 ± 0.003j	1.296 ± 0.006k	14.10 ± 2.626f	28.17 ± 0.351a
K1	1.263 ± 0.003j	0.379 ± 0.003i	1.643 ± 0.006j	17.01 ± 3.261f	27.70 ± 0.370b
K2	1.327 ± 0.002i	0.395 ± 0.005h	1.723 ± 0.005i	21.41 ± 3.020e	27.05 ± 0.388c
GM	1.734 ± 0.002g	0.530 ± 0.004f	2.265 ± 0.004g	26.60 ± 3.106d	26.13 ± 0.306e
AL	1.533 ± 0.004h	0.451 ± 0.004g	1.984 ± 0.007h	22.15 ± 2.569e	26.55 ± 0.370d
GM + K1	2.133 ± 0.016d	0.703 ± 0.002d	2.837 ± 0.016d	31.41 ± 2.964c	25.14 ± 0.288g
GM + K2	2.615 ± 0.003b	1.040 ± 0.003b	3.656 ± 0.004b	34.12 ± 1.880c	24.20 ± 0.140i
AL + K1	1.746 ± 0.003f	0.531 ± 0.004f	2.278 ± 0.006f	26.27 ± 2.328d	25.53 ± 0.243f
AL + K2	2.028 ± 0.002e	0.628 ± 0.003e	2.657 ± 0.005e	26.20 ± 5.813d	24.70 ± 0.167h
GM + AL + K1	2.496 ± 0.003c	1.001 ± 0.003c	3.498 ± 0.005c	48.61 ± 1.639a	23.53 ± 0.118j
GM + AL + K2	2.684 ± 0.002a	1.155 ± 0.003a	3.840 ± 0.006a	44.28 ± 1.976b	21.93 ± 0.149k
L.S.D ($P \leq 0.05$)	0.0073	0.0051	0.0095	3.8632	0.3575
ANOVA $F_{(10, 23)}$	502.04	266.44	664.34	61.900	222.869

than that in stress conditions (Table 2 to 5). Among the stressed and unstressed plants, well-watered plants with triple inoculation with GM + AL + K2 had the highest chlorophyll *a* concentration followed by GM + K2 (Table 2). The same treatments had the highest chlorophyll a concentration in plants stressed at reproductive as well as pod formation stages (Table 4 and 5). The plants exposed to stress during vegetative stage had maximum chlorophyll *a* concentration when treated with GM + AL + K2 followed by GM + AL + K1 (Table 2). Treatment GM + AL + K2 was the best in respect to chlorophyll *b* and total chlorophyll concentration for all stressed and unstressed plants. However, plants stressed during vegetative stage had comparatively higher chlorophyll concentration than those stressed during reproductive stage (Table 3 and 4), but treatments GM + K2 and AL + K1 resulted in higher chlorophyll concentration in the plants stressed at reproductive stage than those stressed during vegetative stage.

Leaf area

Among treatments, maximum leaf area was recorded in treatment GM + AL + K1 under well watered conditions (Table 2). When stress was given at vegetative stage, maximum leaf area occurred in treatment GM and GM + K2 (Table 3). *G. mosseae* when used alone and in combination with potassium resulted in higher leaf area than in the *A. laevis* treatment. At reproductive stage,

Table 3. Effect of AM fungi and potassium on chlorophyll concentration, leaf area and electrolyte leakage of *Vigna mungo* grown under water stress during vegetative stage. GM, *Glomus mosseae*; AL, *Acaulospora laevis*; K1, 70 mg kg^{-1} K fertilizer; K2, 100 mg kg^{-1} K fertilizer; FM, fresh mass. Each value is a mean of five replicates ± SD. Values in column followed by same letter are not significantly different ($P \leq 0.05$)

Treatment	Chlorophyll concentration (mg g^{-1} FM)			Leaf area (cm^2)	Electrolyte leakage
	Chl *a*	Chl *b*	Total Chl		(%)
Control	0.765 ± 0.002k	0.214 ± 0.004j	0.981 ± 0.006k	6.54 ± 1.80i	32.72 ± 0.177a
K1	1.150 ± 0.003j	0.319 ± 0.005i	1.469 ± 0.008j	9.25 ± 2.25h	30.01 ± 0.453b
K2	1.302 ± 0.003i	0.379 ± 0.003h	1.682 ± 0.006i	12.11 ± 1.56g	28.63 ± 0.236c
GM	1.531 ± 0.002g	0.490 ± 0.004f	2.022 ± 0.007g	39.09 ± 2.41a	26.71 ± 0.343e
AL	1.387 ± 0.005h	0.418 ± 0.004g	1.805 ± 0.006h	13.78 ± 1.64fg	27.41 ± 0.119d
GM + K1	2.127 ± 0.002d	0.690 ± 0.005d	2.817 ± 0.007d	21.12 ± 2.69de	25.84 ± 0.148f
GM + K2	2.393 ± 0.004c	0.876 ± 0.003c	3.269 ± 0.006c	36.19± 2.01b	24.30 ± 0.168g
AL + K1	1.618 ± 0.014f	0.488 ± 0.007f	2.089 ± 0.047f	18.60 ± 1.95e	26.09 ± 0.332f
AL + K2	2.004 ± 0.002e	0.593 ± 0.009e	2.602 ± 0.007e	21.61 ± 2.08cd	24.31 ± 0.247g
GM + AL + K1	2.538 ± 0.003b	0.964 ± 0.004b	3.503 ± 0.005b	23.97 ± 1.62c	23.50 ± 0.139h
GM + AL + K2	2.614 ± 0.002a	1.043 ± 0.004a	3.658 ± 0.005a	15.44 ± 1.96f	22.94 ± 0.171i
L.S.D ($P \leq 0.05$)	0.0066	0.0065	0.0202	2.5878	0.3408
ANOVA $F_{(10, 23)}$	690.94	144.14	154.64	128.942	631.272

Table 4. Effect of AM fungi and potassium on chlorophyll content, leaf area and electrolyte leakage of *Vigna mungo* grown under water stress during reproductive stage. GM, *Glomus mosseae*; AL, *Acaulospora laevis*; K1, 70 mg kg^{-1} K fertilizer; K2, 100 mg kg^{-1} K fertilizer; FM, fresh mass. Each value is a mean of five replicates ± SD. Values in column followed by same letter are not significantly different ($P \leq 0.05$)

Treatment	Chlorophyll content (mg g^{-1} FM)			Leaf area (cm^2)	Electrolyte leakage
	Chl *a*	Chl *b*	Total Chl		(%)
Control	0.699 ± 0.003k	0.166 ± 0.005i	0.865 ± 0.004k	8.92 ± 1.412h	45.03 ± 0.820a
K1	1.118 ± 0.003j	0.283 ± 0.009h	1.402 ± 0.011j	11.79 ± 2.056g	38.26 ± 0.366b
K2	1.228 ± 0.007i	0.359 ± 0.014g	1.586 ± 0.012i	14.32 ± 1.711fg	44.04 ± 0.689a
GM	1.492 ± 0.006g	0.430 ± 0.008f	1.923 ± 0.006g	18.52 ± 1.752de	33.10 ± 0.260d
AL	1.362 ± 0.002h	0.357 ± 0.007g	1.720 ± 0.010h	16.41 ± 1.547ef	35.06 ± 0.112c
GM + K1	1.891 ± 0.002d	0.444 ± 0.007e	2.336 ± 0.009d	22.87 ± 1.972c	29.91 ± 0.232e
GM + K2	2.400 ± 0.003b	0.724 ± 0.006c	3.126 ± 0.005c	40.61 ± 2.044a	28.26 ± 0.210f
AL + K1	1.661 ± 0.003f	0.430 ± 0.008f	2.092 ± 0.005f	20.55 ± 2.284cd	33.70 ± 0.374d
AL + K2	1.718 ± 0.001e	0.461 ± 0.009d	2.181 ± 0.009e	22.50 ± 3.243c	33.16 ± 0.207d
GM + AL + K1	2.366 ± 0.002c	0.789 ± 0.007b	3.151 ± 0.010b	28.24 ± 1.639b	24.80 ± 0.662g
GM + AL + K2	2.494 ± 0.004a	0.898 ± 0.005a	3.413 ± 0.041a	38.84 ± 1.917a	25.31 ± 0.478g
L.S.D ($P \leq 0.05$)	0.0042	0.011	0.0194	2.5721	1.0164
ANOVA $F_{(10, 23)}$	156.05	333.83	137.24	128.844	378.176

imposition of water stress had less adverse effect on leaf area of the plants, compared to those stressed during vegetative stage; treatment of plants with GM + AL + K1 and GM + AL + K2 resulted in maximum leaf area (Table 4). Among the three stages, plants subjected to water stress at pod formation stage had largest leaf area (Table 5). Water stress at pod formation stage caused the least effect on the leaf area because, during this stage, plant leaves could expand to the maximum as no water stress was imposed.

Electrolyte leakage

In the present experiment, maximum rate of cell membrane damage (as electrolyte leakage) was recorded when the plants were stressed during reproductive stage in untreated control plants. Treatment of plants with GM + AL + K1 at this stage resulted in minimum membrane damage (Table 4). Of the three stages of stress imposition, highest membrane stability was observed in plants treated with GM + AL + K2 during vegetative stage (Table 3). Among stressed and unstressed plants, maximum stability of membrane was reported in well watered plants with the same treatment (Table 2). Combination of mycorrhizal fungi with potassium was quite effective as compared to single inoculation treatment with either of the AMF used in the experiment.

Table 5. Effect of AM fungi and potassium on chlorophyll content, leaf area and electrolyte leakage of *Vigna mungo* grown under water stress during pod formation stage. GM, *Glomus mosseae*; AL, *Acaulospora laevis*; K1, 70 mg kg^{-1} K fertilizer; K2, 100 mg kg^{-1} K fertilizer; FM, fresh mass. Each value is a mean of five replicates ± SD. Values in column followed by same letter are not significantly different ($P \leq 0.05$)

Treatment	Chlorophyll content (mg g^{-1} FM)			Leaf area (cm^2)	Electrolyte leakage
	Chl *a*	Chl *b*	Total Chl		(%)
Control	0.551 ± 0.003k	0.163 ± 0.016k	0.818 ± 0.023j	10.37 ± 1.909h	37.37 ± 0.040a
K1	0.698 ± 0.003i	0.209 ± 0.004j	0.906 ± 0.008h	14.85 ± 2.112g	34.38 ± 0.018b
K2	0.785 ± 0.002h	0.301 ± 0.015h	1.086 ± 0.013g	18.68 ± 1.993f	33.23 ± 0.278c
GM	1.056 ± 0.002g	0.285 ± 0.005i	1.341 ± 0.007f	21.47 ± 3.012ef	31.59 ± 0.403f
AL	0.649 ± 0.031j	0.313 ± 0.003g	0.865 ± 0.006i	19.60 ± 1.732f	32.69 ± 0.193d
GM + K1	1.706 ± 0.003d	0.512 ± 0.004d	2.219 ± 0.007c	23.25 ± 2.514e	28.27 ± 0.153h
GM + K2	1.995 ± 0.003b	0.633 ± 0.003b	2.552 ± 0.060b	30.91 ± 2.401c	27.78 ± 0.138i
AL + K1	1.172 ± 0.017f	0.326 ± 0.002f	1.499 ± 0.017e	22.69 ± 1.774e	32.12 ± 0.199e
AL + K2	1.539 ± 0.003e	0.441 ± 0.003e	1.981 ± 0.007d	26.34 ± 1.549d	30.02 ± 0.194g
GM + AL + K1	1.931 ± 0.002c	0.556 ± 0.002c	2.563 ± 0.007b	46.67 ± 1.879a	24.63 ± 0.703k
GM + AL + K2	2.015 ± 0.004a	0.747 ± 0.005a	2.762 ± 0.007a	40.85 ± 1.753b	25.87 ± 0.074j
L.S.D ($P \leq 0.05$)	0.0145	0.0100	0.0149	2.6737	0.2784
ANOVA $F_{(10, 23)}$	127.44	274.73	206.34	132.327	151.83

Table 6. Effect of AM fungi and potassium on some biochemical parameters and mycorrhization of *Vigna mungo* grown under well watered conditions. GM, *Glomus mosseae*; AL, *Acaulospora laevis*; K1, 70 mg kg^{-1} K fertilizer; K2, 100 mg kg^{-1} K fertilizer; FM, fresh mass. Each value is a mean of five replicates ± SD. Values in column followed by same letter are not significantly different ($P \leq 0.05$)

Treatment	Phosphatase activity (IU g^{-1} FM)		AMF spore number per 10 g of soil	AMF root colonization (%)	Protein concentration (mg g^{-1} FM)
	Acidic	Alkaline			
Control	0.069 ± 0.005f	0.087 ± 0.006h	8.4 ± 4.393h	0.4 ± 0.288h	0.205 ± 0.002j
K1	0.096 ± 0.006e	0.135 ± 0.005g	24.4 ± 3.209g	14.2 ± 3.193g	0.194 ± 0.003j
K2	0.101 ± 0.011e	0.141 ± 0.005fg	29.6 ± 3.847f	22.8 ± 4.147f	0.292 ± 0.003i
GM	0.141 ± 0.005b	0.186 ± 0.007c	76.0 ± 3.674d	65.4 ± 3.714d	0.415 ± 0.002g
AL	0.111 ± 0.004d	0.145 ± 0.008ef	69.0 ± 4.472e	53.4 ± 3.847e	0.345 ± 0.003h
GM + K1	0.125 ± 0.004c	0.149 ± 0.007ef	88.6 ± 4.615bc	76.6 ± 4.277b	0.627 ± 0.004d
GM + K2	0.139 ± 0.009b	0.178 ± 0.005d	92.6 ± 3.974ab	83.8 ± 4.024a	0.815 ± 0.005a
AL + K1	0.113 ± 0.007d	0.147 ± 0.006ef	83.6 ± 4.615c	71.0 ± 4.301c	0.465 ± 0.002f
AL + K2	0.132 ± 0.004bc	0.152 ± 0.008e	85.6 ± 3.435c	73.6 ± 4.615bc	0.550 ± 0.028e
GM + AL + K1	0.151 ± 0.008a	0.214 ± 0.009b	94.8 ± 3.114a	85.6 ± 3.209a	0.739 ± 0.003c
GM + AL + K2	0.160 ± 0.005a	0.239 ± 0.004a	94.8 ± 3.563a	88.0 ± 4.301a	0.781 ± 0.002b
L.S.D ($P \leq 0.05$)	0.009	0.008	5.0182	4.8488	0.0228
ANOVA $F_{(10, 23)}$	71.650	193.910	322.960	332.777	317.63

Protein concentration

Among the stressed plants, minimum protein content was found in plants subjected to water stress at reproductive stage and the treatments GM + AL + K2 followed by GM + AL + K1 resulted in maximum protein content in the plants stressed during reproductive and pod formation stage (Table 8 and 9) . The treatment GM + AL + K2 followed by GM + K2 proved to be better treatment for increasing leaf protein concentration in well watered plants and in plants stressed during vegetative stage (Table 6 and 7).

Phosphatase activity

Imposition of water stress during reproductive stage caused maximum reduction in acid and alkaline phosphatase enzyme activity (Table 8). However, stress during vegetative stage had the least effect on root phosphatase activity (Table 7). Alkaline phosphatase activity was found to be larger than acidic phosphatase activity, and the treatment of plants with GM + AL + K2 and GM + AL + K1 resulted in the highest phosphatase activity (both acid and alkaline) in plants stressed during pod formation stage along with well watered plants (Table 6 and 9), while the plants stressed during reproductive and vegetative stage had maximum phosphatase activity in treatments with GM + AL + K2 and GM + K2 (Table 7 and 8).

Table 7. Effect of AM fungi and potassium on some biochemical parameters and mycorrhization of *Vigna mungo* grown under water stress during vegetative stage. GM, *Glomus mosseae*; AL, *Acaulospora laevis*; K1, 70 mg kg^{-1} K fertilizer; K2, 100 mg kg^{-1} K fertilizer; FM, fresh mass. Each value is a mean of five replicates ± SD. Values in column followed by same letter are not significantly different ($P \leq 0.05$)

Treatment	Phosphatase activity (IU g^{-1} FM)		AMF spore number per 10 g of soil	AMF root colonization (%)	Protein concentration (mg g^{-1} FM)
	Acidic	Alkaline			
Control	0.622 ± 0.009g	0.083 ± 0.007g	0.0 ± 0.000g	0.0 ± 0.000g	0.193 ± 0.003k
K1	0.089 ± 0.005f	0.128 ± 0.004f	0.0 ± 0.000g	0.0 ± 0.000g	0.200 ± 0.004j
K2	0.094 ± 0.005f	0.135 ± 0.007ef	0.0 ± 0.000g	0.0 ± 0.000g	0.255 ± 0.002i
GM	0.107 ± 0.004e	0.143 ± 0.005cd	71.6 ± 4.393e	55.6 ± 4.277e	0.334 ± 0.003g
AL	0.104 ± 0.007e	0.139 ± 0.005de	54.8 ± 3.492f	46.0 ± 4.062f	0.295 ± 0.002h
GM + K1	0.128 ± 0.009cd	0.150 ± 0.007c	83.6 ± 4.393cd	71.6 ± 3.049c	0.507 ± 0.003d
GM + K2	0.150 ± 0.006ab	0.234 ± 0.006a	87.0 ± 3.535bc	75.2 ± 3.701bc	0.717 ± 0.002a
AL + K1	0.121 ± 0.005d	0.146 ± 0.006cd	74.8 ± 3.701e	66.4 ± 4.929d	0.417 ± 0.002f
AL + K2	0.142 ± 0.005b	0.175 ± 0.006b	81.6 ± 4.615d	71.6 ± 5.319c	0.474 ± 0.002e
GM + AL + K1	0.133 ± 0.003c	0.172 ± 0.005b	90.8 ± 4.147ab	81.4 ± 4.037a	0.602 ± 0.001c
GM + AL + K2	0.154 ± 0.003a	0.235 ± 0.003a	93.0 ± 3.872a	79.2 ± 3.492ab	0.672 ± 0.002b
L.S.D ($P \leq 0.05$)	0.0081	0.0080	4.3903	4.5294	0.0039
ANOVA $F_{(10, 23)}$	94.584	253.262	628.962	444.366	184.14

Table 8. Effect of AM fungi and potassium on some biochemical parameters and mycorrhization of *Vigna mungo* grown under water stress during reproductive stage. GM, *Glomus mosseae*; AL, *Acaulospora laevis*; K1, 70 mg kg^{-1} K fertilizer; K2, 100 mg kg^{-1} K fertilizer; FM, fresh mass. Each value is a mean of five replicates ± SD. Values in column followed by same letter are not significantly different ($P \leq 0.05$)

Treatment	Phosphatase activity (IU g^{-1} FM)		AMF spore number per 10 g of soil	AMF root colonization (%)	Protein concentration (mg g^{-1} FM)
	Acidic	Alkaline			
Control	0.048 ± 0.009f	0.064 ± 0.005g	0.0 ± 0.000h	0.0 ± 0.000h	0.175 ± 0.003k
K1	0.064 ± 0.004e	0.107 ± 0.004f	0.0 ± 0.000h	0.0 ± 0.000h	0.184 ± 0.003j
K2	0.071 ± 0.005e	0.120 ± 0.005e	0.0 ± 0.000h	0.0 ± 0.000h	0.190 ± 0.002i
GM	0.100 ± 0.011cd	0.129 ± 0.006d	52.8 ± 4.147f	37.0 ± 3.162f	0.265 ± 0.002g
AL	0.092 ± 0.005d	0.126 ± 0.008de	48.0 ± 3.674g	29.4 ± 3.361g	0.209 ± 0.004h
GM + K1	0.117 ± 0.006b	0.150 ± 0.010b	65.2 ± 4.549cd	61.2 ± 3.033c	0.395 ± 0.003d
GM + K2	0.143 ± 0.006a	0.156 ± 0.005ab	69.0 ± 3.535c	67.8 ± 3.420b	0.464 ± 0.003c
AL + K1	0.107 ± 0.004c	0.133 ± 0.004cd	59.6 ± 4.277e	43.0 ± 3.674e	0.300 ± 0.002f
AL + K2	0.118 ± 0.005b	0.138 ± 0.005c	63.8 ± 4.324de	55.6 ± 3.209d	0.345 ± 0.003e
GM + AL + K1	0.126 ± 0.006b	0.157 ± 0.006ab	74.8 ± 3.492b	67.6 ± 0.714b	0.507 ± 0.002b
GM + AL + K2	0.148 ± 0.008a	0.160 ± 0.006a	84.6 ± 4.615a	74.6 ± 4.037a	0.524 ± 0.002a
L.S.D ($P \leq 0.05$)	0.0089	0.0081	4.4554	3.7675	0.0037
ANOVA $F_{(10, 23)}$	105.460	96.605	413.499	478.094	104.14

Mycorrhization

Among the three stress stages, imposition of water stress during reproductive stage most affected mycorrhization (Table 8). The vegetative stage was least affected by drought stress followed by pod formation stage (Table 7 and 9). During reproductive stage, a large amount of carbon resources is allocated to flowering, and as a result availability of photosynthates to the mycorrhizal symbiont is lower which might have resulted in decreased colonization at this stage. Among the different treatments used, maximum spore number and root colonization were observed on triple inoculation of plants with GM + AL + K2 and GM + AL + K1 indicating stimulatory role of potassium in enhancement of mycorrhization. Mycorrhizal colonization increased when both species were supplemented with potassium in concentration 100 mg kg^{-1}.

Discussion

In this experiment, decrease in photosynthetic pigments due to water stress could be attributed to drought induced deterioration of thylakoid membranes causing substantial

Table 9. Effect of AM fungi and potassium on some biochemical parameters and mycorrhization of *Vigna mungo* grown under water stress during pod formation stage. GM, *Glomus mosseae*; AL, *Acaulospora laevis*; K1, 70 mg kg^{-1} K fertilizer; K2, 100 mg kg^{-1} K fertilizer; FM, fresh mass. Each value is a mean of five replicates ± SD. Values in column followed by same letter are not significantly different ($P \leq 0.05$)

Treatment	Phosphatase activity (IU g^{-1} FM)		AMF spore number per 10 g of soil	AMF root colonization (%)	Protein concentration (mg g^{-1} FM)
	Acidic	Alkaline			
Control	0.064 ± 0.012g	0.079 ± 0.005g	0.0 ± 0.000g	0.0 ± 0.000f	0.181 ± 0.002k
K1	0.083 ± 0.011f	0.124 ± 0.005f	0.0 ± 0.000g	0.0 ± 0.000f	0.189 ± 0.002j
K2	0.078 ± 0.022f	0.129 ± 0.007ef	0.0 ± 0.000g	0.0 ± 0.000f	0.215 ± 0.003i
GM	0.102 ± 0.008de	0.135 ± 0.006de	68.2 ± 3.898e	44.0 ± 4.358d	0.306 ± 0.003g
AL	0.097 ± 0.010e	0.130 ± 0.003def	49.6 ± 2.701f	34.6 ± 3.911e	0.264 ± 0.004h
GM + K1	0.123 ± 0.005bc	0.167 ± 0.007b	77.2 ± 4.147c	67.4 ± 4.827b	0.427 ± 0.003e
GM + K2	0.133 ± 0.004b	0.167 ± 0.007b	83.2 ± 4.764b	73.6 ± 3.361a	0.525 ± 0.007c
AL + K1	0.111 ± 0.005cd	0.138 ± 0.005d	69.6 ± 4.449de	56.4 ± 3.974c	0.386 ± 0.003f
AL + K2	0.122 ± 0.005bc	0.145 ± 0.004c	73.4 ± 4.037cd	65.6 ± 3.847b	0.475 ± 0.002d
GM + AL + K1	0.147 ± 0.005a	0.191 ± 0.006a	87.6 ± 3.847a	76.8 ± 4.919a	0.585 ± 0.006b
GM + AL + K2	0.149 ± 0.006a	0.196 ± 0.004a	91.2 ± 3.701a	74.2 ± 3.701a	0. 611 ± 0.002a
L.S.D ($P \leq 0.05$)	0.0131	0.0076	4.331	4.5049	0.0036
ANOVA $F_{(10, 23)}$	37.148	155.896	584.175	397.151	15870.294

damage to photosynthetic pigments (Anjum et al. 2011). Decreased chlorophyll concentration could be due to higher activities of chlorophyllase causing increased breakdown of chlorophyll (Kaewsuksaeng 2011). Reduction in photosynthetic pigments due to water stress has also been confirmed by Mafakheri et al. (2010) and Din et al. (2011). Mycorrhizal treatment to the plants along with potassium proved beneficial for increasing chlorophyll concentration in the plants despite of water stress treatments. An increase in photosynthetic pigment concentration as a result of mycorrhizal inoculation under drought stressed conditions has been confirmed by Asrar and Elhindi (2011) in *Tagetus erecta*. The minimum chlorophyll concentration observed in the plants stressed during pod formation stage, among the three stress stages, was because at later growth stages, chlorophyll content decreases due to increasing senescence and the source sink relationship, as observed by Nursu'aidah et al. (2014). In the present experiment, water stress affected leaf area of the plants, and well watered plants had greater leaf area than stressed plants. Reduction in leaf area under water stressed conditions is a mechanism for minimizing higher transpiration rates. Among the three stages of water stress imposition, minimum leaf area has been reported in the plants stressed at vegetative stage. Water stress during vegetative stage caused much greater reduction in leaf area than in the other two stress stages, due to reduction in cell division, and leaf expansion to maximum attainable size. Our results agree with the findings of Mohamed et al. (2010). Mycorrhizal treatment increased leaf area of plants regardless of water stress. Similar results have been noted by Asrar and Elhindi (2011) in mycorrhizal *Tagetus erecta*. Mycorrhizal fungi increase potassium nutrition of the plant (Gracia, Zimmermann 2014). Dual treatment of AMF fungi and potassium increased leaf area to the maximum because of increased potassium nutrition to the plants, as potassium plays an important role in increasing leaf area of the plants under water stress conditions (Parvez et al. 2006). Increased photosynthetic pigment concentration and leaf area as a result of inoculation with mycorrhizae and potassium may increase photosynthesis, because more light energy will be absorbed by chlorophyll molecules in plant leaves having large surface area to drive photosynthesis (Sheng et al. 2008) in plants. Increase in photosynthesis thus results in enhancement in the yield under stressfull conditions (Parry et al. 2011).

In plant cells, electrolyte leakage is mainly associated with efflux of potassium ions, which is intervened by plasma membrane cation conductances (Demidchik et al. 2014). Potassium nutrition to plants reduces water stress induced membrane damage by deeper penetration of roots, improving root architecture and water retention by the plant tissues (Wang et al. 2013). Compared to the plants stressed at vegetative stage, relatively more membrane damage due to water stress was observed in the plants stressed during reproductive stage. These findings of our study are in agreement with those of Farjam et al. (2014). Mycorrhizal treatment to the water stressed and well watered plants decreased electrolyte leakage, which has also been confirmed by Fouad et al. (2014). Water stress had undesirable effect on leaf protein concentration regardless of potassium and AMF treatments. Well watered plants had maximum leaf protein concentration. Reduction in plant leaf protein concentration during water deficiency may be due to proteolysis or non availability of some vital minerals like nitrogen required for protein synthesis, which are taken up by plants with water. In the present experiment, drought stress affected the concentration of photosynthetic pigments, which might have resulted in decreased photosynthesis by the plants. This reduction in photosynthesis could have affected the availability of compounds required for protein synthesis (Karimi et al. 2012). Similar results have been reported in maize by Abdelmoneim et al. (2013). Increase in leaf protein concentration on mycorrhizal inoculation has also been confirmed (Abdelmoneim et al. 2013). Plants performed better under mycorrhizal treatment with potassium than under mycorrhizal fungi alone, as potassium also improves water relations of drought stressed plants, which increases uptake of essential minerals required for protein formation. Phosphatase enzymes are found in the vacuoles of AMF hyphae, which aid in phosphorus metabolism of plants. They help in mineralization of organic P into useful forms such as ortho- and polyphosphates, which can be easily utilized by plants (Richardson et al. 2009). In the present experiment, the activity of acid and alkaline phosphatase was weak in uninoculated plants, which increased significantly with mycorrhization.

Combination of arbuscular mycorrhizal fungi and potassium was also found to be helpful in increasing phosphatase activity over that in single treatment of AMF. Higher phosphatase activity has been recorded in mycorrhizal plants by Kumar et al. (2008) and Wu et al. (2011) under water stressed conditions. Role of potassium in improving phosphatase activity has been investigated by Ali and Meihy (2015). Increased phosphatase activity in mycorrhizal plants under drought stress helps in increasing tolerance of plants to water stress by increasing the availability of phosphorus (Stancheva et al. 2008). Increased photosynthetic pigments and leaf area help in increasing photosynthesis membrane stability; phosphorus uptake of mycorrhizal plants increases growth and productivity under water stress conditions. Mycorrhizal root colonization was found to be positively related with leaf area, protein concentration, membrane stability and phosphatase activity, suggesting reduction in electrolyte leakage, improvement in phosphorus metabolism and growth of mycorrhizal plants under well watered and water stressed conditions. Water stress affected mycorrhization, as number of AMF spores and percentage of root colonization in well watered plants was found to be higher than in stressed plants (Table

5). The reason for this may be slow spread of fungal hyphae after germination of AMF spores due to water deficit. Our results agree with the findings of Gong et al. (2014), who observed inhibition in AMF colonization in foxtail millet roots as a result of water stress, which could be attributed to reduced availability of carbon to the stressed plants (Subramanian, Charest 1995). Our results confirm the findings of Kadian et al. (2013). As different AMF species have different distribution of hyphae in the soil, hyphal density of AMF mycelium surrounding the roots due to colonization by two species will be greater than for single species. Moreover, increase in mycorrhization by two AMF species might be attributed to decreased competition for the form of carbon required by individual AMF species, as the fungi may require different forms of carbon. Crop productivity is affected by stress induced physiological changes. The results of the present investigation suggest the amelioration of physiological parameters of *V. mungo* under water stress conditions on application of *G. mosseae, A. laevis* and K2 i.e. 100 mg kg^{-1} during all the stages of growth.

In conclusion, results of the present experiment could be employed to avoid the wasteful irrigation of crops during any phase of growth. For cultivation of *V. mungo*, reproductive and pod formation stages were found to be most sensitive to water stress. Thus, irrigation of fields at these two stages may help in obtaining high yield, comparable to when irrigating agricultural land throughout the growing season, which causes inefficient utilization of the natural resource. Water stress during vegetative stage caused minimum damage to the plants while stress during reproductive stage caused maximum damage to the plants in terms of photosynthetic pigments, membrane stability, leaf protein concentration and mycorrhization. *G. mosseae* proved to be a better bioinoculant as compared to *A. laevis*, when used in combination or alone, indicating biological specificity of host for a particular microbial strain.

Acknowledgements

The authors are thankful to Kurukshetra University, Kurukshetra for providing laboratory and infrastructural facilities to carry out the research work.

References

Abdelmoneim T.S., Moussa T.A.A., Almaghrabi O.A., Hassan S.A., Abdelbagi I. 2013. Increasing plant tolerance to drought stress by inoculation with arbuscular mycorrhizal fungi. *Life Sci. J.* 10: 255–263.

Adholeya A., Gaur A. 1994. Estimation of VAM fungal spores in soil. *Mycorrhiza News* 6: 10–11.

Ali M.M.S., Meihy R.E. 2015. Microbiological indicators of a clayey soil planted with wheat (*Triticum aestivum* L.) as affected by potassium fertilization and different water regimes. *Res. J. Soil Biol.* 7: 72–83.

Anjum S.A., Xie X. L., Wang M.F., Saleem C.M., Lei W. 2011. Morphological physiological and biochemical responses of plants to drought stress. *Afr. J. Agric. Res.* 6: 2026–2032.

Arnon D.I. 1949. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. *Plant Physiol.* 24: 1–15.

Asrar A.W.A., Elhindi K.M. 2011. Alleviation of drought stress of marigold (*Tagetes erecta*) plants by using arbuscular mycorrhizal fungi. *Saudi J. Biol. Sci.* 18: 93–98.

Barnabás B., Jäger K., Fehér A. 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ.* 31: 11–38.

Bedini S., Pellegrino E., Avio L., Pellegrini S., Bazzoffi P., Argese E., Giovannetti M. 2010. Changes in soil aggregation and glomalin-related soil protein content as affected by arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*. *Soil Biol. Biochem.* 41: 1491–1496.

Bradford M.M. 1976. A rapid and sensitive for the quantization of microgram quantitites of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254.

Demidchik V., Straltsova D., Medvedev S.S., Pozhvanov G.A., Sokolik A., Yurin V. 2014. Stress-induced electrolyte leakage: the role of K^+-permeable channels and involvement in programmed cell death and metabolic adjustment. *J. Exp. Bot.* 65: 1259-1270.

Din J., Khan U., Ali I., Gurmani R.A. 2011. Physiological and agronomic response of canola varieties to drought stress. *J. Anim. Plant Sci.* 21: 78–82.

Dionisio-Sese M.L., Tobita S. 1998. Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.* 135: 1–9.

Emam Y., Shekoofa A., Salehi F., Jalali A.H. 2010. Water stress effects on two common bean cultivars with contrasting growth habits. *Am. Eurasian J. Agric. Environ. Sci.* 9: 495–499.

Farjam S., Siosemardeh A., Kazemi-Arbat H., Yarnia M., Rokhzadi A. 2014. Response of chickpea (*Cicer arietinum* L.) to exogenous salicylic acid and ascorbic acid under vegetative and reproductive drought stress conditions. *J. Appl. Bot. Food Qual.* 87: 80–86.

Fouad M. O., Essahibi A., Benhiba L., Qaddoury A. 2014. Effectiveness of arbuscular mycorrhizal fungi in the protection of olive plants against oxidative stress induced by drought. *Spanish J. Agric. Res.* 12: 763–771.

Gautam N.K., Kumar K., Prasad M. 2016. Leaf crinkle disease in urdbean (*Vigna mungo* L. Hepper): An overview on causal agent, vector and host. *Protoplasma* 253: 729–746.

Gerdemann J.W., Nicolson Y.H. 1963. Spores of mycorrhizae endogone species extracted from soil by wet sieving and decanting. *Transact. Brit. Mycol. Soc.* 46: 235–244.

Girigowda K., Prashanth S. J., Mulimani V. H. 2005. Oligosaccharides of black gram (*Vigna mungo*.L.) as affected by processing methods. *Plant Food Human Nutr.* 60: 173–180.

Gong M., You X., Zhang Q. 2014. Effects of Glomus intraradices on growth and reactive oxygen metabolism of foxtail millet under drought. *Ann. Microbiol.* 65: 595–602.

Kadian N., Yadav K., Aggarwal A. 2013. Significance of bioinoculants in promoting growth, nutrient uptake and yield of *Cyamopsis tetragonoloba* (L.) "Taub." *Eur. J. Soil Biol.* 58: 66–72.

Kaewsuksaeng S. 2011. Chlorophyll degradation in horticultural crops. *Walailak J. Sci. Techn.* 8: 9–19.

Karimi S., Abbaspour H., Sinaki J.M., Makarian H. 2012. Effects of water deficit and chitosan spraying on osmotic adjustment and soluble protein of cultivars castor bean (*Ricinus communis* L.). *J. Stress Physiol. Biochem.* 8: 160–169.

Kumar A., Bernier J., Verulkar S., Lafitte H.R., Atlin G.N. 2008. Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. *Field Crops Res.* 107: 221–231.

Mafakheri A., Siosemardeh A., Bahramnejad B., Straik P.C., Sohrabi E. 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Afr. J. Crop Sci.* 4: 580–585.

Menge J.A., Timmer L.W. 1982. Procedure for inoculation of plants with VAM in the laboratory, greenhouse and field. In: Schenck N.C. (ed) *Methods and Principles of Mycorrhizal Research*. American Phytopathology Society, St Paul, pp. 59–67.

Morton J.B., Benny G.L. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): A new order, Glomales, two new suborders, Glomineae and Gigasporineae, with an remendation of Glomaceae. *Mycotaxon* 37: 471–491.

Mukerji K.G. 1996. Taxonomy of endomycorrhizal fungi. In: Mathur K.G., Chamola B., Chitralekha P. (eds) *Advances in Botany*. APH Pubishing Company, New Delhi, pp. 211–221.

Nursu'aidah H., Motior M.R., Nazia A.M., Islam M.A. 2014. Growth and photosynthetic responses of long bean (Vigna unguiculata) and mung bean (*Vigna radiata*) response to fertilization. *J. Anim. Plant Sci.* 24: 573–578.

Phillips J.M., Hayman D.S. 1970. Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. *Transact. Brit. Mycol. Soc.* 55: 158–161.

Richardson A.E., Barea J.M., McNeill A.M., Prigent-Combaret C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganism. *Plant Soil* 321: 305–339.

Sakova L.R., Paclik Curn V. 2002. The drought tolerance of four Brassica species. *Sbornik Jihoceska Univerzita Zemedelska Fakulta Ceske Budejovice Fytotechnicka Rada* 1: 77–86.

Schenck N.C., Perez Y. 1990. *Manual for the Identification of VA Mycorrhizal Fungi.* University of Florida Press, Florida, USA.

Schiermeier Q. 2008. Water: a long dry summer. Nature 452: 270–273.

Sharma N., Yadav K., Cheema J., Badda N., Aggarwal A. 2015. Arbuscular mycorrhizal symbiosis and water stress: a critical review. *Pertanika J. Trop. Agric. Sci.* 38: 427–453.

Stancheva M., Geneva E., Djonova N., Kaloyanova M., Sichanova M., Boychinova G., Geogiev. 2008. Response of alfalfa (*Medicago sativa* L.) growth at low accessible phosphorous source to the dual inoculation with mycorrhizal fungi and nitrogen fixing bacteria. *Gen. Appl. Plant Physiol.* 34: 319–326.

Subramanian K.S., Charest C. 1995. Influence of arbuscular mycorrhizae on the metabolism of maize under drought stress. *Mycorrhiza* 5: 273–278.

Tabatabai M. A., Bremner J. M. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1: 301–307.

Thalooth A.T., Tawfik M.M., Magda Mohamed H. 2006. A comparative study on the effect of foliar application of zinc, potassium and magnesium on growth, yield and some chemical constituents of mung bean plants grown under water stress conditions. *World J. Agric. Sci.* 2: 37–46.

Umar S. 2006. Alleviating adverse effects of water stress on yield of sorghum, mustard and groundnut by potassium application. *Pak. J. Bot.* 38: 1373–1380.

Vavilov N. I. 1926. Studies on the origin of cultivated plants. *Bulletin of Applied Botany and Plant Breeding* 14: 1–245. /in Rusian/

Wang B., Qiu Y.L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16: 299–363.

Wang M., Zheng Q., Shen Q., Guo S. 2013. The critical role of potassium in plant stress response. *Int. J. Mol. Sci.* 14: 7370–7390.

Wu Q.S., Srivastava A.K., Zoua Y. N. 2013. AMF-induced tolerance to drought stress in citrus: A review. *Sci. Hort.* 164: 77–87.

Wu Q.S., Zou Y.N., He X.H. 2011. Difference of hyphal and soil phosphatase activities in drought-stressed mycorrhizal trifoliate orange (*Poncirus trifoliata*) seedlings. *Sci. Hort.* 129: 294–298.

Garcia K., Zimmermann S.D. 2014. The role of mycorrhizal associations in plant potassium nutrition. *Front. Plant Sci.* 5: 337.

Pervez H.M., Makhdum I., Ashraf M., Shabab-Ud-Din 2006. Influence of potassium nutrition on leaf area index in cotton (*Gossypium hirsutum* L.) under an arid environment. *Pak. J. Bot.* 38: 1085–1092.

Received 5 August 2016; received in revised form 15 December 2016; accepted 5 March 2017