Interactive effect of arbuscular mycorrhizal fungi and potassium on growth and yield in *Cyamopsis tetragonoloba* (L.) under water stress

Nisha Kadian, Kuldeep Yadav and Ashok Aggarwal

Department of Botany, Kurukshetra University, Haryana 136119, India
nishakadian4@gmail.com

Abstract: Water stress is one of the most important limiting factors affecting the growth and yield of field crops particularly in arid and semi-arid regions of the world. In the recent years, the use of soil microorganisms have generated great interests in agriculture, by reducing the damage from drought stress and providing sustainable solutions for crop production in such climates. A pot experiment was conducted to examine the consortium impact of two different Arbuscular mycorrhizal fungi (AMF) i.e. *Glomus* mosseae and *Acaulospora laevis* along with Potassium fertilizer on growth enhancement and drought tolerance of *Cyamopsis tetragonolobla* (L.) under two different water treatments. Different plant growth (including plant shoot and root weight, Pod number and pod weight) were higher for well-watered than for water-stressed plants. Results also showed that the imposed water stress and well watered conditions significantly improved the different physiological attributes like total chlorophyll content, acidic and alkaline phosphatase activity and protein content in mycorrhizal and Potassium treated plants in comparison. AM spore number and percent root colonization was higher in AM-treated plants than non-AM-treatment regardless of potassium concentration. This study confirms that AM colonization and Potassium fertilizer can mitigate the deleterious effect of water stress on growth and yield in cluster bean.
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1. Introduction

Throughout evolutionary time, plants have been confronted with changing environmental conditions, among which water stress is considered as the most important abiotic factors limiting plant growth and yield in many areas. The response of plants to water stress depends on several factors such as developmental stage of the crop, severity and duration of stress and the genotype.

The prominent effects of water stress on the crops include: reduction in growth, nodulation, and photosynthetic activity. If different physiological processes and yield potential are studied together in relation to water stress conditions, it could overcome most of the factors leading to yield barriers in grain legumes.

Some of the soil microorganisms living in rhizospheric soil of plants are known to alleviate the environmental stress conditions. One of the possible mechanisms to increase drought tolerance in plants is through the symbiotic association of environmentally friendly and potentially cost effective microbial biofertilizer i.e. Arbuscular Mycorrhizal (AM) fungi. Eighty percent studies on the effects of AM fungi on drought stress in plants have shown to enhance growth and vigor of plants by altering the plant water relations, which is the result of accumulative physical, nutritional, physiological and cellular effect (Auge 2001). The application of potassium also plays several physiological and biochemical roles, influencing the water economy and plant growth, affecting the water uptake, root growth, maintenance of turgor, transpiration and stomatal regulation (Nelson 1980). Potassium (K+) has substantial effect on enzyme activation, protein synthesis, photosynthesis, respiration, stomatal movement and osmoregulation enhancing the growth and yield in different crops under water stress conditions (Marschner 1995, Yadav *et al.* 1995, Egilla *et al.* 2001, Zaidi 1994).

Food legumes are important components of farming systems in the developing country both economically and nutritionally. *Cyamopsis tetragonoloba* (L.) Taub. commonly known as cluster bean, summer moong or "guar" is a potential vegetable and industrial leguminous crop occupying a major portion of moisture deficient areas. Being a rich source of gum and protein, it has become an important industrial crop in recent years. Guar gum is a polysaccharide known to have cholesterol lowering effect (Butt *et al.* 2007). Guar plants are also commonly used as fodder and green manure. Derivatives of guar gum have used in industrial applications, such as paper and textile industry, ore flotation, the manufacture of explosives and hydraulic fracturing of oil and gas formations.

Since cluster bean is an important legume, screening of different mycorrhizal strains with

potassium is necessary for the selection of best ones to get maximum benefits. Keeping the above in view, the present investigation was undertaken to evaluate the effects of dominant AM fungi alone and in different combination with potassium to find out the best combination having the maximum capability of increasing plant growth, nutrient uptake and better yield under water stress conditions.

2. Material and Methods

2.1Mass multiplication and inoculation of mycorrhizal fungus:

Two dominant AM fungi i.e. Glomus mosseae and Acaulospora laevis were isolated from the rhizospheric soil of cluster bean plants. The starter inoculum of each selected dominant AM fungus was raised by "funnel technique" of Menge and Timmer (1982) using maize as host for three months. Chopped AM colonized root pieces of maize having 80%-85% of colonization along with the soil having AM spores (620-650/ 100 g inoculum) were used as AM inoculum. To each pot 10% (w/w), i.e. 200g/pot inoculum of AM fungi alone and in combinations were added into the soil before plantation.

2.2 Experimental design:

The experiment was laid out in a randomized complete block design, with five replicates for each treatment. Top soil (0–30 cm) was collected from the Botanical garden of Botany Department, Kurukshetra University, India which consisted of 20.8% silt, 3.78% clay, and had a pH of 8.05, 0.0485 total N and 0.015% available P. Surface sterilized seeds of cluster beans were grown in experimental earthen pot (25.5 x 25 cm) filled with sterilized soil: sand mixture (3200:800 g). To each pot 10 % (w/w) of inoculum of each AM fungi (*G. mosseae* or *A. laevis*) was added alone and in combination with potassium fertilizer (i.e. 70 ppm or 100 ppm) under two water regimes i.e. Stressed and Unstressed (Well watered).

The experiment had seven treatments with different combinations:

(1) Control (autoclaved sterile sand: soil without AM inoculum and/or potassium)
(2) 70 ppm of Potassium (K1)
(3) 100ppm of Potassium (K2)
(4) *G. mosseae* (G)
(5) *A. laevis* (A)
(6) G+A+K1
(7) G+A+K2

Half of the pots received well watered throughout the experiment while the half were subjected to water stress conditions by skipping one irrigation at three stages i.e. vegetative stage, flowering stage and pod formation stage. After each stage, short term stress period was applied and the plants were permitted to recover by re-watering until harvesting (recovery period). The experiment was carried out at constant temperature (25 ± 2 °C) and humidity (50 – 70 %) under aseptic conditions of polyhouse.

2.3 Growth parameters, biochemical attributes and nutrient analysis:

Growth parameters were recorded after pod formation, morphological and physiological parameters were determined. After harvest, roots and shoots were weighed separately to determine fresh weight, and then placed in an oven to dry at 70°C until a constant dry weight was obtained.

The chlorophyll content in cluster bean plants was estimated by the method of Arnon (1949).The proline content in cluster bean plants was estimated by the method of Bates *et al*. (1973) pure proline was used as a standard. The total protein was estimated by the method of Bradford (1976).

Mycorrhizal colonization of roots was determined using the "Rapid Clearing and Staining Method" of Phillips and Hayman (1970). Percentage AM colonization of roots was calculated using: (Number of root segments colonized / number of root segments studied) × 100.

Phosphatase activity was assayed by using p-nitrophenyl phosphate (PNPP) as substrate, hydrolyzed by the enzyme to p-nitrophenol. For this, ice cold sodium acetate buffer (0.05 M with pH 4.8) for acid phosphatase and sodium carbonate-bicarbonate buffer (0.05 M with pH 10) for alkaline phosphatase activity was used and was measured in terms of IU/g FW (Tabatabai and Bremner (1969).

2.4 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).

3. Results and Discussion

Inoculation of cluster bean plant with arbuscular mycorrhizal fungi and potassium fertilizer improved growth and biomass, though the performance varied with host plant and AM fungal species.

3.1 Plant height and Biomass

Under well-watered conditions, mycorrhizal fungi and potassium significantly increased all the growth parameters (Table 1) in comparison over drought stress treatment. The maximum increment in plant height was observed in plant treated with GAK1 followed by *G. mosseae* alone. While maximum shoot and root weight was found in GAK1 followed by GAK2. Beltrano and Ronco (2008) also reported the importance of AM fungal inoculation on wheat plant under water-stress condition. This enhancement in growth and biomass in cluster bean may be due to improved water transport and better

uptake of nutrients, especially of available soil phosphorus (P) and other minerals like potassium by AM hyphae, resulting in the hydration of plant tissues. Baque *et al.* (2006) also noticed the role of potassium in increasing plant growth and biomass in *Triticum aestivum* under water-stress condition.

Table 1: Interactive effect of Potassium fertilizer (K) and AM fungi on morphological parameters of Cluster bean under well water and water stress conditions

Treatments	Plant height (cm)		Fresh shoot weight (g)		Dry shoot weight (g)		Root length (cm)		Fresh root weight (g)		Dry root weight (g)	
	W.W	W.S	W.W	W.S	W.W	W.S	W.W	W.S	W.W	W.S	W.W	W.S
Control	34.2±1.92^{f}	30.6±2.07^{g}	3.54±0.002^{g}	3.51±0.029^{f}	0.29±0.003^{g}	0.24±0.003^{g}	4.48±0.13^{g}	3.98±0.19^{g}	0.37±0.020^{f}	0.31±0.010^{f}	0.04±0.002	0.02±0.002^{f}
K1	53.4±2.51^{d}	50.6±4.61^{e}	11.53±0.029^{c}	10.88±0.027^{b}	2.83±0.029^{c}	2.45±0.031^{c}	9.57±0.01^{b}	9.03±0.03^{a}	0.50±0.002^{b}	0.48±0.002^{b}	0.09±0.001	0.07±0.001^{a}
K2	46.6±2.07^{e}	39.6±2.07^{f}	06.83±0.029^{f}	05.51±0.029^{e}	1.19±0.002^{f}	1.12±0.003^{f}	8.01±0.01^{c}	7.88±0.02^{c}	0.45±0.001^{d}	0.40±0.002^{d}	0.12±0.002^{c}	0.09±0.002^{b}
GAK1	74.6±2.30^{a}	70.8±2.77^{a}	13.32±0.033^{a}	12.30±0.027^{a}	5.10±0.025^{a}	4.73±0.040^{a}	9.93±0.02^{a}	9.15±0.01^{a}	2.03±0.020^{a}	1.81±0.010^{a}	0.17±0.002^{a}	0.14±0.002^{a}
GAK2	57.4±2.07^{c}	54.4±2.70^{c}	11.66±0.028^{b}	10.86±0.026^{b}	4.02±0.021^{b}	3.92±0.033^{b}	9.00±0.15^{b}	8.54±0.27^{b}	0.50±0.002^{b}	0.48±0.002^{b}	0.10±0.002^{d}	0.08±0.002^{c}
G	62.0±2.12^{b}	61.6±2.07^{b}	08.32±0.019^{d}	07.68±0.027^{c}	2.03±0.027^{d}	1.83±0.029^{d}	8.52±0.27^{c}	8.00±0.15^{c}	0.48±0.001^{c}	0.45±0.002^{c}	0.12±0.002^{b}	0.09±0.002
A	47.4±1.51^{c}	45.2±2.86^{c}	07.26±0.021^{e}	06.26±0.033^{d}	1.89±0.002^{e}	1.50±0.002^{e}	7.14±0.23^{d}	6.44±0.15^{d}	0.46±0.002^{d}	0.31±0.003^{e}	0.09±0.001^{f}	0.06±0.003

K1: 70 ppm, K2: 100ppm, G: *Glomus mosseae*, A: *Acaulospora laevis*
‡: each value is a mean of five replicates. ±: standard deviation, AM: Arbuscular mycorrhizal.
Values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test

Table 2: Interactive effect of Potassium and AM fungi on mycorrhization, yield and protein content of Cluster bean under well water and water stress conditions

Treatments	AM spore number		% Root colonization		No. of pods		Wt. of pods		Protein content (%)	
	W.W	W.S	W.W	W.S	W.W	W.S	W.W	W.S	W.W	W.S
Control	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00 ^{c}	0.00±0.00^{d}	7.34±0.28^{f}	06.18±0.18^{f}	2.142±0.021^{f}	3.64±0.023^{g}	11.38±0.025^{g}	10.33±0.026^{g}
K1	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00 ^{c}	0.00±0.00^{d}	16.57±0.38^{d}	12.34±0.10^{d}	11.00±0.003^{d}	9.09±0.013^{d}	14.87±0.003^{f}	12.87±0.003^{f}
K2	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00 ^{c}	0.00±0.00^{d}	11.48±0.34^{e}	09.88±0.02^{e}	07.81±0.002^{e}	7.37±0.020^{f}	16.87±0.003^{e}	14.87±0.003^{e}
GAK1	81.8±1.78^{c}	68.4±2.07^{c}	59.0±4.06^{b}	50.6±2.40^{c}	30.12±0.65^{a}	25.56±0.18^{a}	25.46±0.001^{a}	22.82±0.015^{a}	24.42±0.030^{a}	22.34±0.032^{a}
GAK2	64.6±3.64^{d}	54.6±3.36^{d}	56.0±3.39^{b}	49.2±1.92^{c}	21.34±0.40^{c}	20.68±0.10^{c}	17.76±0.002^{c}	11.89±0.013^{c}	19.14±0.032^{c}	18.72±0.024^{c}
G	86.0±2.00^{b}	81.0±1.00^{b}	73.2±3.27^{a}	63.4±2.70^{b}	26.48±0.24^{b}	21.42±0.19^{b}	14.70±0.002^{b}	12.42±0.033^{b}	17.87±0.001^{d}	16.43±0.026^{d}
A	90.8±2.16^{a}	83.6±2.40^{a}	75.8±2.58^{a}	68.4±1.14^{a}	20.88±0.17^{c}	19.56±0.15^{c}	18.37±0.004^{c}	08.95±0.028^{e}	21.56±0.024^{b}	20.55±0.024^{b}

K1: 70 ppm, K2: 100ppm, G: *Glomus mosseae*, A: *Acaulospora laevis*
‡: each value is a mean of five replicates. ±: standard deviation, AM: Arbuscular mycorrhizal.
Values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test

3.2 Root length

The study revealed a prominent increment in root length in water stressed treated plants over well water (Table 1). However, the best results were observed in plants inoculated with GAK1 followed by *G. mosseae*. AMF colonization enhances soil aggregation through extraradical hyphae, external to plant root and exuding the glycoprotein and glomalin from extraradical hyphae that cements soil microaggregates into large soil aggregate structure, ultimately improving the root system to assess soil water and nutrients. Udaiyan *et al.* (1997) also reported an improvement in root system in *Vigna unguiculata* and *Leucacena latisiliqua* inoculated with AM fungi under drought condition. It might be due to the fact that mycorrhizal inoculation assists the plants to counter photo inhibition and photo destruction of pigments under stressed conditions by increasing the content of carotenoids. It is well known that carotenoids are involved in protecting photosynthetic apparatus against the photo inhibitory damage by the single oxygen. Therefore, carotenoids can not only directly deactivate but can also quench the excited triple state of chlorophyll. Many researchers had reported the different effects of AM fungi on photosynthetic activity of different crops under drought stress conditions (Ruiz-Lozano 1995).

3.3 AM colonization and spore number

Table 3: Interactive effect of Potassium and AM fungi on chlorophyll content and phosphatase activity of Cluster bean under well water and water stress conditions

Treatments	Chlorophyll a		Chlorophyll b		Acidic phosphatase		Alkaline phosphatase	
	W.W	W.S	W.W	W.S	W.W	W.S	W.W	W.S
Control	0.65±0.004^{g}	0.57±0.005^{g}	0.29±0.004^{g}	0.06±0.007^{g}	0.78±0.004^{g}	0.76±0.003^{g}	1.09±0.001^{g}	1.08±0.001^{g}
K1	1.02±0.003^{e}	0.93±0.025^{e}	0.73±0.004^{e}	0.50±0.011^{f}	0.93±0.002^{f}	0.92±0.002^{f}	1.17±0.002^{f}	1.16±0.003^{f}
K2	0.92±0.005^{f}	0.63±0.020^{f}	0.56±0.014^{f}	0.58±0.020^{d}	1.14±0.003^{e}	1.13±0.003^{e}	1.26±0.003^{e}	1.25±0.002^{e}
GAK1	1.39±0.003^{c}	1.85±0.027^{b}	1.41±0.004^{a}	0.51±0.003^{e}	1.30±0.002^{c}	1.25±0.021^{c}	1.82±0.003^{c}	1.80±0.002^{c}
GAK2	1.23±0.003^{d}	1.27±0.023^{c}	1.08±0.004^{d}	0.62±0.040^{c}	1.21±0.003^{d}	1.20±0.007^{d}	1.35±0.003^{d}	1.34±0.002^{d}
G	2.37±0.005^{a}	2.55±0.009^{a}	1.36±0.008^{b}	1.08±0.018^{a}	1.43±0.036^{b}	1.33±0.024^{b}	1.97±0.002^{a}	1.95±0.003^{a}
A	1.50±0.004^{b}	1.23±0.004^{d}	1.14±0.003^{c}	0.94±0.004^{b}	1.52±0.020^{a}	1.42±0.020^{a}	1.95±0.002^{b}	1.94±0.003^{b}

K1: 70 ppm, K2: 100ppm, G: *Glomus mosseae*, A: *Acaulospora laevis*
‡: each value is a mean of five replicates. ±: standard deviation, AM: Arbuscular mycorrhizal.
Values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test

Table 4: Interactive effect of Potassium and AM fungi on proline content of Cluster bean under well water and water stress conditions

Treatments	Proline (Root)		Proline (Shoot)	
	W.W	W.S	W.W	W.S
Control	0.022±0.007[g]	0.30±0.002 [g]	0.237±0.002[g]	2.04±0.001[g]
K1	0.027±0.001[f]	0.40±0.001[f]	0.314±0.001[f]	3.41±0.001[f]
K2	0.035±0.002[e]	0.44±0.002[e]	0.409±0.002[e]	4.09±0.001[e]
GAK1	0.047±0.002[a]	1.704±0.002[a]	0.649±0.001[b]	6.19±0.00[b]
GAK2	0.034±0.003[b]	1.023±0.002[b]	0.613±0.002[c]	5.79±0.002[c]
G	0.041±0.001[c]	0.683±0.002[c]	0.683±0.001[a]	6.82±0.003[a]
A	0.042±0.001[d]	0.614±0.002[d]	0.401±0.001[d]	5.11±0.001[d]

K1: 70 ppm, K2: 100ppm, G: *Glomus mosseae*, A: *Acaulospora laevis*
‡: each value is a mean of five replicates. ±: standard deviation, AM: Arbuscular mycorrhizal.
Values in columns followed by the same letter are not significantly different, $P \leq 0.05$, least significant difference test

Mycorrhization and spore number was found to be more in GAK2 followed by GAK1 under both the conditions while zero percent colonization and spore number was observed in the plants not provided with AM inoculum (Table 2). The AM colonization under well watered was 10% higher than under water stressed conditions. The results are in accordance with the finding of Kaya *et al.* (2003), who also reported that water stress significantly decreased the AM colonization in watermelon plants. The positive effects may be due to an increment in root length density resulting in higher phosphorus absorption and water uptake by AM hyphae (Stevens *et al.* 2011, Al-Qarawi 2010).

3.4 Chlorophyll content

Chlorophyll content was found to be increased in all treated plants under both conditions. The highest increase in total chlorophyll content was observed in single inoculation of *G. mosseae* followed by triple inoculation of GAK1 (Table 3).

3.5 Phosphatase Activity

It was observed that phosphatase activity was greatly enhanced in inoculated plants in comparison to control. It was found that plants with higher mycorrhizal root colonization had maximum phosphatase activity (alkaline and acidic). The maximum phosphatase activity was found in *G. mosseae* followed by *A. laevis* (Table 2). Soil Phosphorus is usually present in the form of orthophosphate which may be directly absorbed at the soil-root interface through root epidermis and hairs and indirectly at the fungal root interface through external AM hyphae (Garg and Manchanda 2009, Requena *et al.* 2007). Our results indicated that the major phosphatase form in cluster bean rhizosphere was acid phosphatase, which was significantly higher in the mycorrhizal colonized plants under drought stress. Phosphatase activity was positively correlated to soil water content and was also increased by the sole mycorrhizal inoculation (Kumar *et al.* 2008, Sardans *et al.* 2008). As a consequence, more soil available Phosphorus could be released with an increase in AMF mediated acid phosphatase resulting in partial alleviation of drought stress (Stancheva *et al.* 2008). Wu *et al.* (2011) also observed the similar effect in trifoliate orange inoculated with mycorrhizal fungi under water stressed condition.

3.6 Proline content

Proline is an important amino acid in plant under drought stress that prevents oxidation of cells from inside. It also regularizes osmotic pressure of plant under drought stress for absorbing water. Drought stressed plants have been shown to accumulate organic osmolytes such as sugars and amino acids, known to have contribution in host-plant tolerance under water deficit conditions (Schellembaum *et al.* 1998, Trotel-Aziz *et al.* 2000). Proline is a non-protein amino acid that forms in most tissues subjected to water stress and, together with sugar, it is readily metabolized upon recovery from drought. Proline content was found to be more in stressed plants as compared to well water plants. The accumulation of proline was found to be maximum in GAK1 followed by GAK2 (Table 4). The results of our study are in accordance with Porcel and Ruiz-Lozno (2003) in Soybean plant inoculated with AM fungi under drought stress. Similar results were recorded by Aliabadi Farahani *et al.* (2008) in *Coriandrum sativum*.

3.7 Yield and Protein content

The drought stress had an undesirable effect on growth, yield and protein content in inoculated plants in comparison to well watered plants. Data on yield (Table 3) showed that bioinoculation of cluster bean plant with AMF and potassium fertilizer significantly increased pod weight and consequently higher yields over control. Treatment with mix consortium GAK1

followed by *G. mosseae* resulted in maximal number and weight of pods and ultimately resulted in higher protein content. It might be due to decrement in photosynthetic activity under drought stress, leading to inhabitation of some essential material for protein synthesis resulting in dramatically reduction or even inhibition of protein synthesis (Mohammadkhani and Heidari 2008, Karimi *et al.* 2012). The gradual decrease in protein content during water deficiency was induced by proteolysis or decline in some essential mineral for protein synthesis which uptake with water as nitrogen compounds (Iqbal *et al.* 2009, Costa *et al.* 2011). Similar results are reported by Abdelmoneim *et al.* (2014) in maize plant inoculated with Arbuscular Mycorrhizal fungi under water stress condition.

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Corresponding Author:
Prof. Ashok Aggarwal
Department of Botany
Kurukshetra University
Kurukshetra, Haryana 136119, India
E-mail: aggarwal_vibha@rediffmail.com

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