# The enhancement of drought tolerance for pigeon pea inoculated by arbuscular mycorrhizae fungi

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## ABSTRACT

Pigeon pea (*Cajanus cajan*) has been rapidly grown in the drought-striken Karst regions of southwest China. Present research aimed to investigate the effects of arbuscular mycorrhizae (AM) on the drought tolerance of pigeon pea, as well as to elucidate the physiological responses of AM-colonized seedlings to the water deficit. As subjected to drought stress, AM symbiosis (AMD) highly led to the positive effects on root system, plant height and stem diameter. AMD demonstrated a remarkably higher chlorophyll content, photosynthetic rate and stomatal conductance. The soluble sugar in AMD was significantly higher than that of the non-AM seedlings (NAMD), indicating the enhanced tolerance at least partially correlated with osmotic solute. Conversely, the proline (Pro) of AMD was lower, revealing the excessive Pro was not imperative for drought tolerance. After 30 days drought stress, AMD gave around a third less lipid peroxides than that of NAMD. Rather, the root activities of AMD were significantly higher than that of the latter after 10 days drought stress. Thereby, AM fungi might substantially elevate the tolerance to drought of pigeon pea, and the cumulative effects contributed to the enhanced tolerance. To date, this has been the first report concerning the enhancement of drought tolerance via AM colonization in this legume species.

**Keywords**: arbuscular mycorrhizal fungi; drought stress; osmotic solute; photosynthesis; pigeon pea (*Cajanus cajan*)

Arbuscular mycorrhizal (AM) fungi form a mutualistic association by colonizing roots of many land plants (Parniske 2008, Wu et al. 2010). The beneficial effects of AM symbioses on improving growth and water-stress tolerance are well documented (Augé 2004, Wu and Xia 2006, Liu et al. 2007, Parniske 2008). Under water deficit, the amelioration of water status in AM-colonized plants may be ascribed to direct water uptake and transport via external hyphae (Augé 2004), regulation of stomatal conductance (Goicoechea et al. 1997), as well as indirect effects of elevated P level (Fitter 1988, Xavier and Germida 2003), higher antioxidative status (Porcel and Ruiz-Lozano 2004), and osmotic adjustment (Ruiz-Lozano 2003, Porcel and Ruiz-Lozano 2004, Wu and Xia 2006).

Pigeon pea (*Cajanus cajan*) is a perennial member of the Fabaceae family, and one of the major grain legume crops of the tropics and subtropics (Vanaja et al. 2010). Compared with other grain legumes, pigeon pea ranks only the sixth in area and production, but it is used in more diverse ways than others (Wu et al. 2009, Domoguen et al. 2010). Pigeon pea is a hardy, widely adapted and drought tolerant crop. These traits allow its cultivation in a wide range of environments and cropping systems. In addition to its main use as de-hulled split peas, its immature green seeds and pods are also consumed fresh as a green vegetable. This crop may fix about 40 kg/ha N during a cropping season (Domoguen et al. 2010). Additionally, its roots help in releasing soil-bound P to make it available for plant growth (Ae et al. 1990, Saxena 2008). Therefore, pigeon pea has become an ideal crop for sustainable agriculture systems in south China (Li et al. 2001, Gong et al. 2005), especially

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for the ecological reconstruction in the Karst regions. Unfortunately, these Karst areas are severely striken by frequent drought stress, which require the further enhanced tolerance to water deficit.

To date, however, little has been known regarding the effects of AM fungi on growth and tolerance to water deficit or on the mechanisms of drought tolerance of pigeon pea. To extend and strengthen the cultivation of this species in Karst areas, the present research aimed to investigate whether AM further enhances drought tolerance of pigeon pea, as well as to elucidate the physiological responses of AM-colonized plants to the drought stress.

## MATERIAL AND METHODS

**Seedling preparation and mycorrhizal inoculation**. Seeds of pigeon pea (cv. Guimu 1) were surface sterilized in a 0.1% $HgCl_2$ for 5 min, followed by washing several times in sterile distilled water, then the seeds were pre-cultured at 30°C for 24 h in incubator. The seeds were germinated in plastic pots (15 cm × 12 cm) containing either sterilized loam (ca. 1 000 g) collected from Karst area alone (non-AM) or sterilized loam inoculated with 50 g (ca. 750 spores) *Glomus mosseae* (GM) inoculum (AM-colonized), which was purchased from the Plant Nutrition and Resource Research Institute, Beijing Academy of Agriculture and Forestry (China). AM infection of root was detected by staining and assessing root segments for presence or absence of arbuscles and vesicles using the magnified intersections method (McGonigle et al. 1990).

**Stress treatment**. All seedlings were grown under 70–80% of field water capacity (FWC) in the greenhouse with a maximum photosynthetic active radiation of 1 200 $\mu mol/m^2/s$ and temperature of 25 ± 2°C before treatments. After 90 days of growth, both AM-colonized (AMD) and non-AM (NAMD) seedlings were randomly chosen to carry out drought stress, whose water content of soil was kept to around 50% FWC. The unstressed plants, both mycorrhizal (AMC) and non-mycorrhizal (NAMC), were watered daily to maintain 70–80% FWC based on the lose of soil weight.

**Growth and physiological measurements**. After every 5 days exposure to drought stress, measurements were made of growth and physiological parameters, including plant height (PH), stem diameter (SD), water content (WC), chlorophyll content (Chl), photosynthetic rate ($P_n$), stomatal conductance ($G_s$), soluble sugar content (SS), proline titer (Pro), malondialdehyde (MDA) accumulation, and root oxidase activity (RA). Leaf WC and Chl was quantified followed the methods by Fitter (1988) and Ni et al. (2009), respectively. $P_n$ and $G_s$ of leaves were measured out using the portable CI-301 $CO_2$ gas analyzer (CID Inc., Vancouver, USA). SS, Pro or MDA in leaves was quantified based on the method by Chandler and Thorpe (1987), Vasquez-Tello et al. (1990) or Wen et al. (2010), respectively. The RA were determined according to the description by Liu et al. (2007).

**Statistical analysis**. For morphological investigations, one treatment consisted of one seedling and measurements were repeated 10 times, while for other investigations, the experiment was a completely randomized 2 × 2 factorial design, with two mycorrhizal treatments, AM and non-AM, and two water treatments, drought (D) and control (C). Each of the four treatments consisted of three plants and measurements were repeated three times. Experimental data were statistically analyzed using the ANOVA with SAS software (SAS Institute, Inc., Cary, USA), taking $P < 0.05$ as significant.

## RESULTS AND DISCUSSION

**Mycorrhizal infection and its effect on plant growth under drought stress**. Under well-watered conditions, the 90 days old seedlings of pigeon pea inoculated with or without GM fungi were used to detect AM infection status. No infected case was investigated in the root cut from the non-GM seedlings (Figure 1A). Conversely, all the seedlings inoculated with GM demonstrated AM infection in their roots (Figure 1B). Further, more than 80% of root segments from the GM-inoculated seedlings were infected by AM, and the obvious arbuscule were observed (Figure 1B).

Morphologically, AM symbiosis (AMC) highly led to a positive effect on growth of the seedlings compared with non-AM (NAMC), including root system (Figure 1C), PH (Figure 2A) and SD (Figure 2B) under the drought-free (control). As exposed to drought stress, both AMD and NAMD seedlings demonstrated an obvious repression in growth compared with the stress-free, however, PH (Figure 2A) and SD (Figure 2B) of the former were greatly higher than the latter as the stress progression. Compared with treatment 0 day, PH or SD increase at treatment 30 day of AMD (5.89% or 8.18%, respectively) were considerably higher than that of NAMD (2.79% or 4.49%, respectively), further indicating that AM can overcome

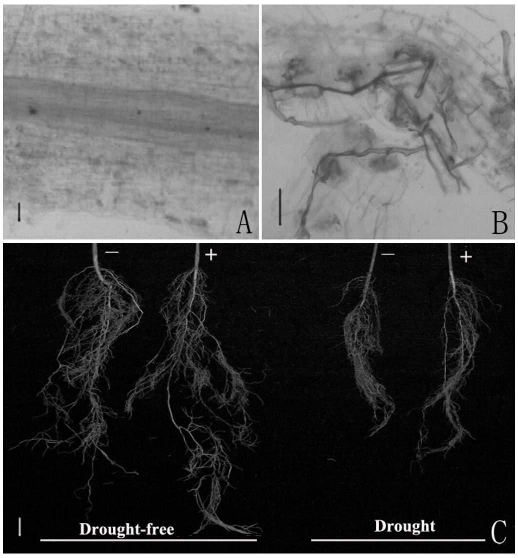

Figure 1. The arbuscular mycorrhizae (AM) and its host root system of pigeon pea inoculated with *Glomus mosseae* (GM) strain

A – anatomic structure of uninfected root; B – arbuscules in cortical cell; C – root system of non-AM (NAM, –), and non-AM-drought (NAMD, +) seedling. Vertical bars in A–B and C indicate 20 μm and 1 cm, respectively

the negative effects of stress. Interestingly, leaf WC of AM seedlings were significantly superior to that of NAM after 10 days stress (Figure 2C), which might directly justify that AM can elevate the drought tolerance via improving water uptake.

Under drought stress, AM may highly promote the growth of host plant, which is well described (Liu et al. 2007, Parniske 2008). To AM-colonized plants, the improvement of the root surface and/or the external hyphae *per se* may highly elevate the uptake of water and nutrients (Augé 2004, Porcel and Ruiz-Lozano 2004, Liu et al. 2007), consequently resulting in the increased tolerance to drought. In the current case, AM seedlings possessed an obviously greater root system (Figure 1C) besides the extraradical AM fungal mycelia, giving rise to an obvious superiority in PH (Figure 2A), SD (Figure 2B), leaf WC (Figure 2C), as well as leaf number and weight (available upon query) compared with the non-AM plant under both presence and absence of water deficit, verifying the earlier findings in soybean (Porcel and Ruiz-Lozano 2004), citrus (Wu et al. 2010), and licorice (Liu et al. 2007).

**The effect on photosynthesis**. Physiologically, the AMC seedlings showed a remarkable elevation in Chl (Figure 3A), $P_n$ (Figure 3B) and $G_s$ (Figure 3C) in comparison with that of the NAMC. As subjected to drought stress, these parameters gradually went down, however, the AMD seedlings demonstrated significantly higher level compared

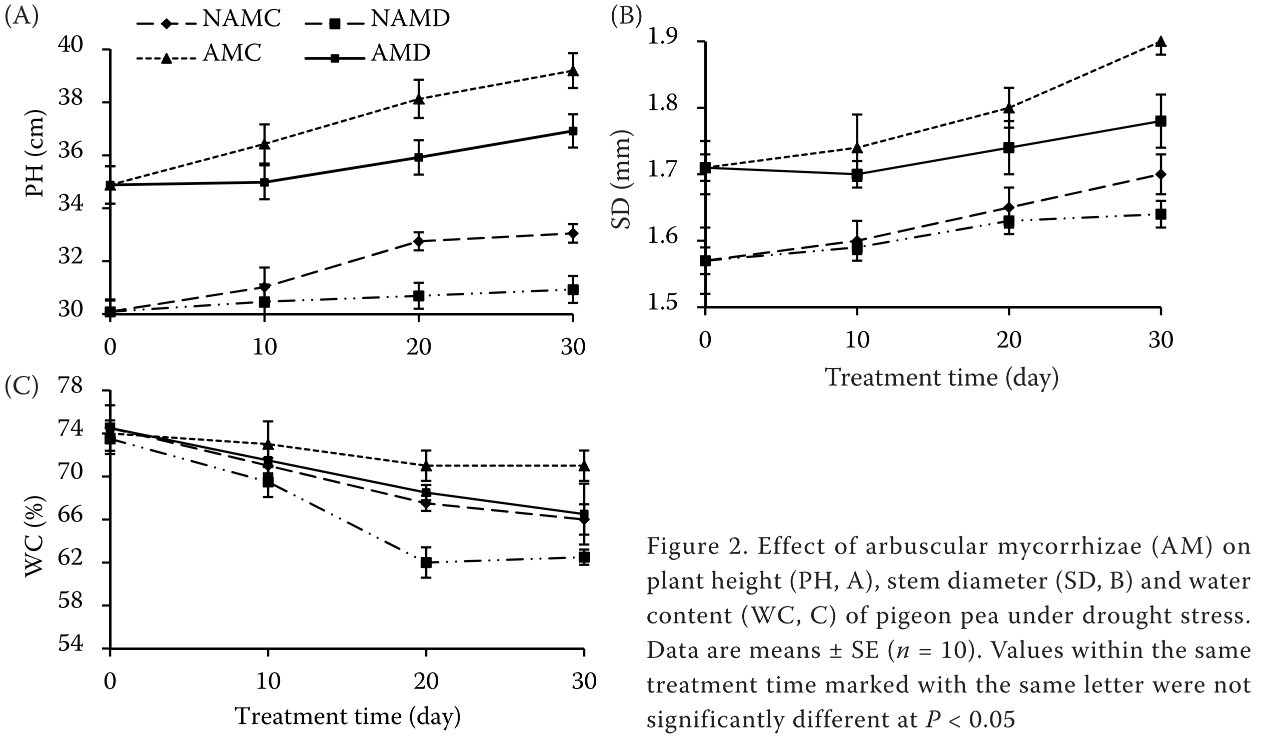

Figure 2. Effect of arbuscular mycorrhizae (AM) on plant height (PH, A), stem diameter (SD, B) and water content (WC, C) of pigeon pea under drought stress. Data are means ± SE ($n$ = 10). Values within the same treatment time marked with the same letter were not significantly different at $P < 0.05$

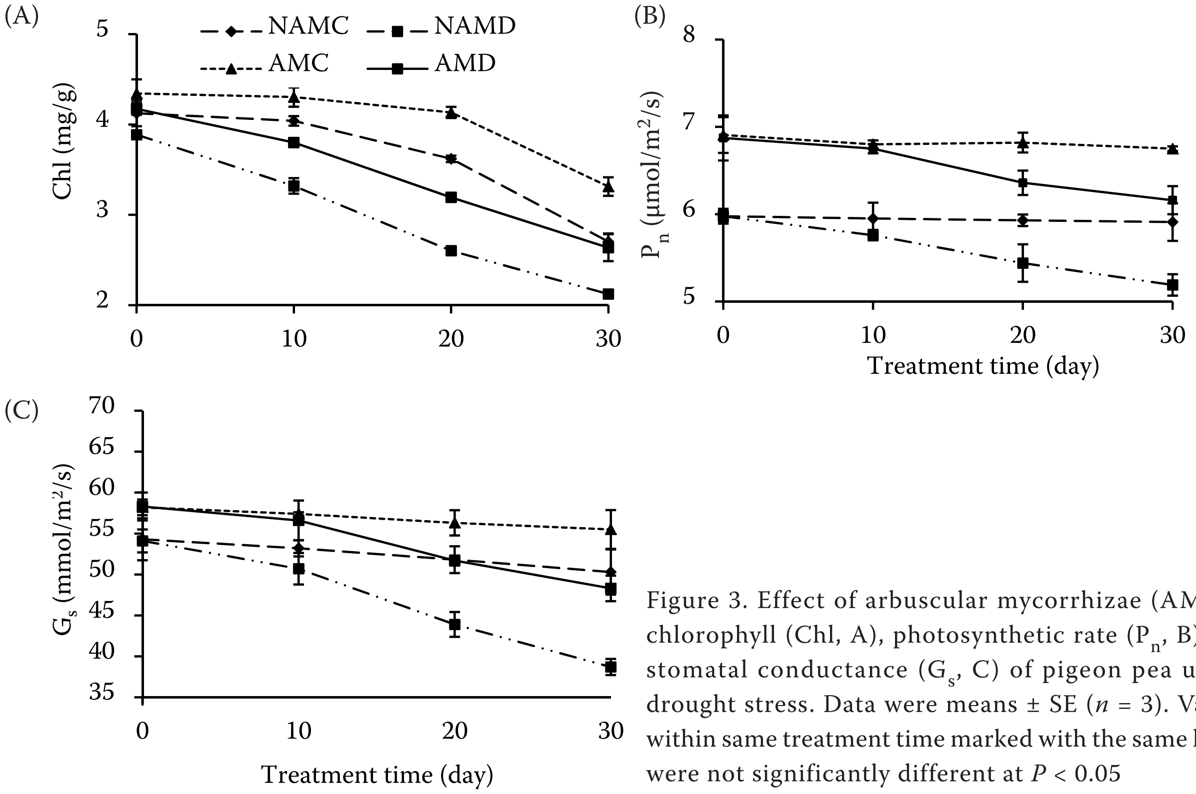

Figure 3. Effect of arbuscular mycorrhizae (AM) on chlorophyll (Chl, A), photosynthetic rate ($P_n$, B) and stomatal conductance ($G_s$, C) of pigeon pea under drought stress. Data were means ± SE ($n = 3$). Values within same treatment time marked with the same letter were not significantly different at $P < 0.05$

with the NAMD, suggesting that AM fungi could alleviate the depression of the biomass accumulation in response to the water deficit. These data agreed with the previous evidence obtained from *Vicia faba* (Jia et al. 2004), citrus (Wu and Xia 2006) and *Ipomoea carnea* (Amaya-Carpio et al. 2009). Taking photosynthetic data herein and the above morphological data together, AM fungi substantially improved the tolerances of pigeon pea to drought stress.

**Osmotic solute response**. Soluble sugar is a kind of important osmotic solute, whose role relates to the stability of cell turgor pressure. Exposed to drought stress, the leaf SS were enhanced in both AMD and NAMD seedlings. Interestingly, the content in AMD seedlings was significantly higher than the NAMD (Figure 4A), which corroborated the findings from citrus (Wu et al. 2010). Therefore, it might be concluded that the enhanced drought tolerance of AMD at least partially contributed to their better osmotic adjustment.

It is well known that proline is a non-protein amino acid, and is believed to protect plant tissues against stress by acting as an N-storage compound, osmosolute, and hydrophobic protectant for enzymes and cellular structures (Madan et al. 1995). In the present case, the Pro of both AMC and NAMC leaves were comparatively low under stress-free. Drought stress increased Pro, however, the contents in AMD was remarkably lower than that in NAMD after 10 days water deficit (Figure 4B). The similar trends were also documented in citrus (Wu and Xia 2006) and soybean (Porcel and Ruiz-Lozano 2004). This circumstance might be attributed to the more successful avoidance to drought stress of AM seedlings (Augé 2004) since they demonstrated the superior root system (Figure 1C), and excessive Pro was not imperative for serving as an osmoprotectant, a hydroxyl radical scavenger, as well as a macromolecule-denaturing protector in AM seedlings when subjected to water deficit.

**MDA accumulation**. The oxidation of membrane lipids is a reliable indicator of unbalanced free-radical production, which may be mirrored by MDA, a parameter of lipid peroxidation (Wen et al. 2010). In the present case, MDA level of both AMC and NAMC seedling were considerably stable under drought-free conditions. Compared with the NAMC (35–38 nmol/g FW), the MDA accumulation of the AMC (33–35 nmol/g FW) was somewhat lower under stress-free conditons (Figure 4C). When subjected to drought stress, the MDA levels of both AMD and NAMD were progressively elevated with the elongation of stress (Figure 4C), revealing that the lower oxidative damage to lipids was a consistent effect of AM symbiosis in the former. The similar results were also observed in soybean (Porcel and Ruiz-Lozano 2004). Strikingly, remarkably lower MDA was investigated from the AM seedling after 10 days

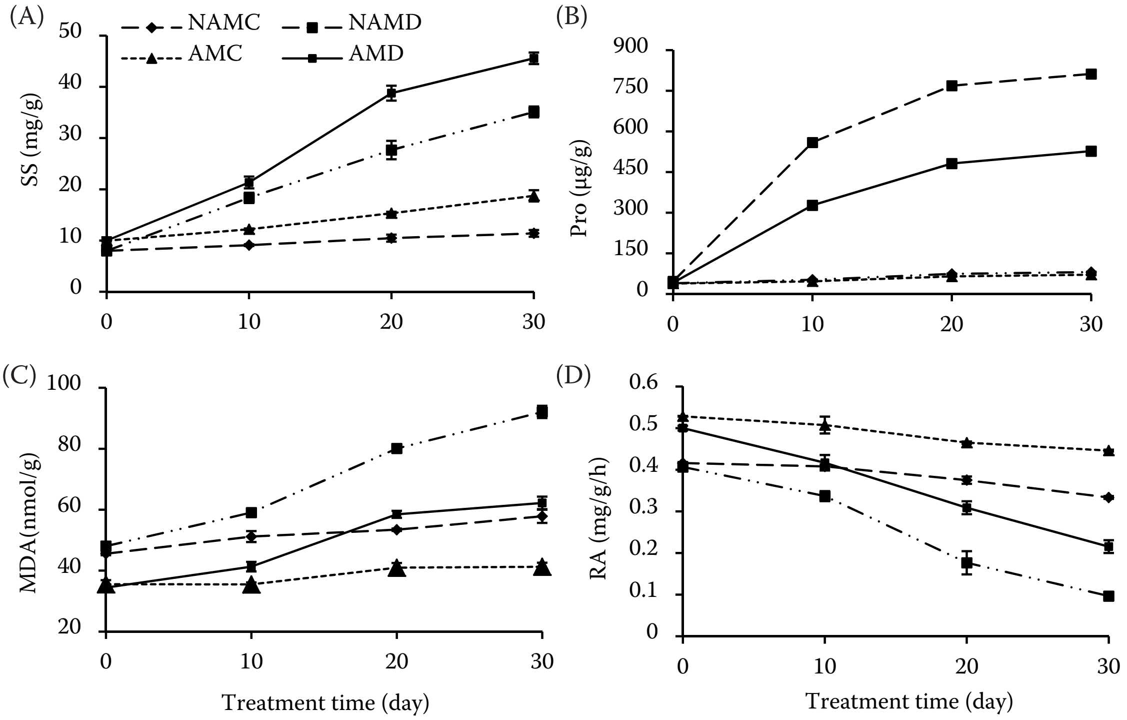

Figure 4. Effect of arbuscular mycorrhizae (AM) on the contents of soluble sugar (SS, A), proline (Pro, B), malondialdehyde (MDA, C), and root oxidase activity (RA, D) of pigeon pea under drought stress. Data were means ± SE ($n = 3$). Values within same treatment time marked with the same letter were not significantly different at $P < 0.05$

drought stress in comparison with the non-AM. After 30 days drought stress, the former gave 33% less lipid peroxides than that of the latter. Therefore, the elevation of drought tolerance of AM seedlings was also partially contributed to their remarkable mitigation of oxidative status.

**Root oxidase activity**. RA was used as an indicator of root vigor, and was also considered as an index of the peroxidases catalysis. Pigeon pea is a kind of legume crop, and AM colonization may lead to the establishment of tripartite symbiotic association of *Rhizobium*-AMF-legume. With the colonization of AM fungi herein, the RA was remarkably enhanced (Figure 4D). In comparison with the stress-free conditions, drought led to gradual decreases in RA of both AMD and NAMD seedlings, however, the status of the former was significantly higher than the latter after 10 days water deficit (Figure 4D), implying that AM might considerably improve root metabolism, and could effectively alleviate oxidative stress, consequently strengthened the tolerance potential to water deficit. The higher RA herein might be an adaptive mechanism to moderate drought stress, which could facilitate drought tolerance by scavenging oxidative radicals (Liu et al. 2007).

Collectively, the overall evidence proved that AM symbiosis substantially increased the drought tolerance of pigeon pea, and the mechanisms underlying the enhancement were quite comprehensive. AM symbiosis extended the root system and elevated the $P_n$ as exposure to water deficit, leading, therefore, to improving water and nutrient uptake, as well as superior growth of the host plant. Also, AM remarkably enhanced the RA, which might consequently improve the absorption ability, resulting in an elevation of tolerance to water deficit. Further, the higher SS in AM plants could contribute to maintaining a water potential gradient favorable to the water passing into the roots, which enabled higher leaf water status in AM plants during the drought, and keeps the plants protected against oxidative stress. These cumulative effects elevated the drought tolerance of AM-infested pigeon pea.

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