Amelioration of the Activities of Reactive Oxygen Species in Water Stressed *Sclerocarya Birrea* (A. Rich.) Hochst., Seedling in the Presence of Arbuscular Mycorrhal Fungi

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Abstract

Water scarcity is the main limiting factor facing tree planting in drylands. Plants growing under water stress often produce destructive oxygen species which in the absence of any protective mechanism, can damage different aspects of cell structure and function. The aim of this study was to determine the effect of arbuscular mycorrhal (AM) fungus inoculation of the activities of superoxide dismutase (SOD) in three subspecies of *Sclrocarya birrea* (A. Rich.) Hoscht. growing under water stress. A greenhouse experiment was set up involving seedlings of three subspecies of *S. birrea* (spp. *birrea, caffra* and *multifoliata*). *Gigaspora margarita* Baker and Hall was used for inoculatrion. Dry matter weight was taken and super oxide dismutase (SOD) enzyme and catalase activities, and total protein accumulation were analyzed in water stressed and non-water stressed seedlings, both inoculated and non-inoculated. AM fungi inoculation improved water stress tolerance as reflected in increased biomass production and reduction in stress related biochemical reactions. The findings of the study showed that SOD and catalase activities were low in mycorrhizal compared to non-mycorrhizal seedlings. On the contrary, non-mycorrhizal seedlings had lower total protein content compared to mycorhizal seedlings. Increased SOD activity is involved in defense of anti-oxidative stress.

Key words: Arbuscular mycorrhizae, proline, *Sclerocarya birrea*, superoxide dismutase (SOD), water stress.

Résumé

La carence hydrique constitue le facteur le plus restrictif à l'arboriculture dans des zones arides. La plantation d'arbres dans des conditions de stress hydrique conduit souvent à la production des espèces réactives à l'oxygène et qui en l'absence des mécanismes de protection, peuvent endommager différents aspects structurels et fonctionnels des cellules. Cette étude avait pour objectif de déterminer l'effet de l'inoculation du champignon arbusculaire mycorrhizien sur les activités du hyperoxyde de dismutase chez trois sous-espèces de Sclerocarya birrea (A. Rich.) Hoscht. poussant dans des conditions de stress hydrique. Un essai en serre a été mené sur de jeunes plants de trois sous-espèces de S. birrea (ssp birrea, caffra et multifoliata). Un champignon arbusculaire mycorhizien, Gigaspora margarita Baker et Hall a été utilisé pour l'inoculation. Le poids de la matière sèche a été calculé et les activités enzymatiques de l'hyperoxyde de dismutase et celles de la catalase, et l'accumulation des protéines totales ont été analysées chez les jeunes plants soumis soit au stress hydrique ou non, et chez ceux qui étaient inoculés ou non. L'inoculation avec des champignons arbusculaires mycorrhiziens a amélioré la tolérance au stress hydrique comme reflété dans l'augmentation de la production de la biomasse et la réduction des réactions biochimiques en relation avec le stress hydrique. Les résultats de l'étude ont prouvé que les activités enzymatiques de l'hyperoxyde de dismutase et celles de la catalase étaient faibles chez les jeunes plants avec les mycorhizes comparés aux jeunes plants sans traitement mycorhizien. En revanche, les jeunes plants sans mycorhizes avaient une basse teneur en protéines totales en comparaison des jeunes plants avec des mycorhizes. L'activité élevée de l'hyperoxyde de dismutase et de catalase est impliquée dans la défense contre le stress antioxydant.

Mots clés: Mycorhizes arbusculaires, proline, Sclerocarya birrea, hyperoxyde de dismutase, stress hydrique

Introduction

The drylands of Kenya has abundant wild plants with great agronomic and commercial potential as food crops, but many of these species, particularly the indigenous fruits have not been promoted or researched and therefore remain underutilized (Muok 2002). Studies have shown that the products from these fruits are as nutritious as exotic fruits and some times better. In the past, indigenous plants played an integral role in the diet of many communities, especially, in the nutritional quality and diversification of the food base in Africa (Arum, 1989). Plant species with edible fruits that also posses nutritious oil seeds

are of special interest to humans, especially in dryland areas, which experience frequent drought and food scarcity. One such species is *Sclrocarya birrea* (A. Rich.) Hoscht. (marula). Thiongo and Jaennicke (2000) reported that *S. birrea* has four times (200mg/g) more vitamins compared to citrus fruits.

S. birrea (Anacardiaceae, the mango family) is native to the semi-arid, deciduous savannas of much of sub-Saharan Africa. It is common in wooded grasslands, riverine woodland and bush land, being frequently associated with rocky hills (Muok et al 2007; Peters, 1988). Marula is widely used by rural populations in most countries in which it is found (Palmer and Pitman, 1972; Shackleton et al., 2000; Shone, 1979). There is an increasing awareness on the importance of marula as a new crop both locally and internationally (Muok et al., 2000; Nerd and Mizrahi, 1993). Unfortunately, due to the growing popultation, expansion of agricultural activities and land degradtion in the drylands, these important natural resources are facing a serious conservation threat (Muok 2002). Developing technologies for their cultivation will increase fruit production and ease pressure on the wild trees.

However, tree planting in drylands is hampered by the problem of water scarcity. Plants exposed to water stress undergo changes in their metabolism in order to adapt to the changes in their environment. These have endogenous protective mechanisms to scavenge the toxic oxygen species released as a result of stress conditions. The protective mechanism includes carotenoids, glutathione, ascorbate, _-tocopherol and several enzymes such as SOD, catalase, ascorbate peroxidase (APOX) and glutathione reductase (GR) (Elstner, 1982; Asada and Takahashi, 1987; Salin, 1988).

The beneficial effects of arbuscular mycorrhizae in improving tolerance to water stress in *S. birrea* have been reported (Muok and Ishii, 2006). The mechanisms of the improved tolerance includes increased nutrient uptake, especially for P and Zn (Muok and Ishii, 2006, Al-Karaki, 2000; Osonubi et al., 1991; Rutto et al., 2002; Solaiman and Hirata, 1995). Formation of mycorrhizal roots enables plants to obtain more moisture from the surrounding soil than non-mycorrhizal plants (Stahl et al., 1998). Ishii (2000) reported that *S. birrea* forms association with AM fungi in its natural habitat. In this survey, *Gigaspora margarita* was one of the mycorrhizae species found to occur naturally in Kenyan soils.

Though it is known that mycorrhizal symbiosis causes important changes in metabolism, there is little information in biochemical activities in response to mycorrhizal symbiosis under water stress conditions. The aim of this study was to determine the effect of arbuscular mycorrhal (AM) fungus inoculation on the activities of superoxide dismutase (SOD) in *S. birrea* under water stress.

Materials and Methods

Plant Material and Inoculation

Fruits of the three marula subspecies were collected separately using random sampling techniques. Freshly collected seeds were extracted after which equal samples of seeds were combined to give the bulk population from which sub samples were taken for germination. Before sowing, the seeds were surface sterilized in 10% sodium hypochlorite solution for 30 minutes and the operculum, which covers the embryo removed before sowing. Seed were germinated in pots containing sterile vermiculite in a growth chamber. A month later, healthy looking seedlings of uniform height (average 12 cm) were transferred to a green house and transplanted according to the pre-determined experimental design into growth pots (18 cm in diameter, 15 cm in depth) containing vermiculite, perlite and zeolite (2:1:1 by volume). The greenhouse was maintained under natural conditions with normal day length and no air conditioner. A week after transplanting, each seedling was inoculated with 5g of inoculum (approximately 250 spores of *G. margarita*) (Central Glass Co. Ltd, Tokyo). The number of spores was determined according to Ishii et al. (1996). Inoculation was done by spreading a thin layer of the inoculum on the sand surface and watered lightly.

Effect of AM Inoculation on Growth of Marula Seedlings Under Water Stress Conditions

The experiment was layed-out in a randomized 2 x 2 factorial design consisting of water stressed seedlings inoculated with *G. margarita* or un-inoculated, and well-watered seedlings (control) inoculated with *G. margarita* or un-inoculated. Twenty plants were used per treatment. Control seedlings were watered daily in the morning while water stressed seedlings were watered once a week. The growth pots were shuffled weekly at random to reduce bias which could be caused by effects of the angle of the sun light reaching the seedlings. The experiment was maintained for four months. Pre-dawn leaf water potentials were taken once using pressure chamber (Meiwa Shoji Co. Ltd, Tokyo), just before harvesting, after the seedlings were denied water for a week.

Harvesting and Determination of Root Colonization, Catalase and SOD Activities, and Total Protein

At the termination of the experiments, shoots were severed from the roots. The roots were rinsed and

samples taken for estimation of root colonization according to Ishii and Kadoya (1994). Root catalase and SOD activities were analyzed by the methods of Aebi (1974) and McCord and Fridorich (1969), respectively. Total protein content in the roots was measured according to the procedure of Lowry et al. (1951).

Collected data was subjected to statistical analysis using the analysis of variance (ANOVA) procedure and differences between the mean determined by Duncan's multiple range test (DMRT) at 95% significant level.

Results

All inoculated seedlings (both water stressed and control) became mycorrhizal (Fig. 1). There was no significant difference in the rate of AM fungi colonization between water stressed and control seedlings which were 40.4±1.6% and 48.5±1.6%, respectively.

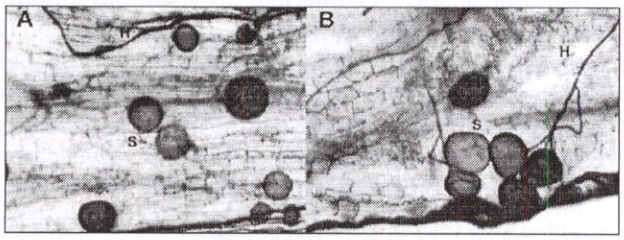

Figure 1: Arbuscular mycorrhizae in control (A, x 150) and water stressed roots (B, x150) of *S. birrea.* S: spore, H: hypha.

Arbuscular mycorrhizal fungi inoculation significantly (p _ 0.05) increased total dry weight of seedlings under control and water stress conditions (Table 1). The increment in total dry weight due to AM fungi inoculation was more prominent in water stressed seedlings (59%) compared to control seedlings (36%). Non-mycorrhizal water stressed seedlings had significantly (p _ 0.05) lower total protein content compared to water stressed mycorrhizal seedlings and all control seedlings (inoculated and un-inoculated) (Table 1).

Table 1: Effect of AM fungi on total dry weight and total protein content in *S. birrea* seedlings growing underwater stress conditions

Treatment	Total DW (g)	Total protein (mg/plant) (%)
C-AM	62.7±1.6b[z]	0.428 ± 0.027a
C+AM	85.5±2.8c	0.604 ± 0.087a
WS-AM	38.1±1.5a	0.163 ± 0.061b
WS+AM	60.6±1.4b	0.578 ± 0.034a

[z] Means± standard error (SE) (n=20). Means within each column followed by different letters are significantly different at p _ 0.05. C-AM: un-inoculated control, C+AM: inoculated control, WS-AM: un-inoculated and wa ter stressed, WS+AM: inoculated and water stressed, NC: no root colonization.

Non-mycorrhizal water stressed *S. birrea* seedlings had higher SOD and catalase activities compared to water stressed mycorrhizal seedlings and all control seedlings (inoculated and un-inoculated). However, no significant difference in SOD and catalase activities was recorded between control seedlings (mycorrhizal or non-mycorrhizal) and water stressed mycorrhizal seedlings. AM fungi inoculation reduced catalase and SOD activities by 38% and 40%, respectively (Table 2).

Table 2: Effect of AM fungi on activities of catalase and superoxide dismutase in *S. birrea* seedlings growing underwater stress conditions

Treatments	CAT (mmol.H_2O_2 destroyed/ min 1gFW)	SOD unit/ gFW[y]
C-AM	164.1 ± 10.7a[z]	7092.8 ± 688.7a
C+AM	161.5 ± 5.2a	6209.3 ± 178.6a
WS-AM	273.4 ± 11.5b	9934.2 ± 293.6b
WS+AM	169.3 ± 11.5a	5956.2 ± 324.6a

[z] Mean±SE (n=4), means within each column followed by different letters are significantly different at p _ 0.05. [y] fresh weight (g), CAT: catalase, SOD: superoxide dismutase, C-AM: un-inoculated control, C+AM: inoculated control, WS-AM: un-inoculated and water stressed, WS+AM: inoculated and water stressed.

Discussion

The present study has demonstrated that inoculating marula seedlings with *G. margarita* improved the seedlings tolerance to water stress as shown in both dry matter production and stress related biochemical activities. In the present study, mycorrhizal marula seedlings under water stress recorded lower levels of catalase and SOD compared non-mycorrhizal seedlings while higher total protein was recorded in mycorrhizal seedlings under water stress compared to non-mycorrhizal seedlings.

Catalase and SOD are among constituents of important primary defense of cells against toxic free oxygen radicals generated under stress conditions (Tsang et al., 1991). Plants under stress produce excess

free oxygen radicals which are destructive to the plant cells. Leaves are known to close their stomata under water stress. This is a temporary adaptive change that prevents further water loss from the plants, but the influx of CO_2 is also lowered at the same time resulting in net reduction of photosynthetic capacity (Lawlor and Uprety, 1993). Photosysnthetic electron transport is, however, maintained at relatively higher rate in the stressed leaves as compared to large decrease in the rate of CO_2 fixation. This imbalance between electron transport and CO_2 fixation rates may result in the over-reduction of the electron transport chain components and facilitate the transfer of electron to O_2. This univalent reduction of O_2 gives rise to formation of $O_2^{\dot{Y}-}$ and then other reactive oxygen species such as H_2O_2, $^{\dot{Y}}OH$ and $^1O_2$ which are highly reactive. To remove excess oxygen radical plants produce catalase and SOD. Catalase and SOD may therefore act as an important and indirect indicator of the level of stress in plants (Salin, 1988).

The fact that catalase and SOD activities were low in mycorrhizal compared to non-mycorrhizal seedlings could be an indication that mycorrhizal seedlings were less stressed than non-mycorrhizal seedlings. It can be concluded thus; AM fungi inoculation ameliorated water stress in mycorrhizal seedlings resulting in less production of reactive oxygen species thus lower SOD and catalase activities. Higher total protein observed among the mycorrhizal seedlings under water stress could have been due to more photosynthetic reaction in the mycorrhizal seedlings. This supports Muok and Ishii (2006) who indicated that mycorrhizal *S. birrea* seedlings had higher chlorophyll concentration under water stress than non-mycorrhizal seedlings.

In conclusion, water stress activates different components of the reactive oxygen species which may destroy different cell components and the degree of the destruction depends on the degree of the imposed water stress. Enhancing the decomposition of these reactive oxygen species can be a strategy for impacting drought resistance in a plant. AM fungi incoculation thus can play an important role in enhancing plant water stress tolerance.

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