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EFFECTS OF MYCORRHIZAL INOCULATION ON GROWTH AND QUALITY OF ROSELLE (*Hibiscus sabdariffa* L.) GROWN IN SOILLESS CULTURE SYSTEM

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ABSTRACT

The effect of arbuscular mycorrhizal fungi (AM) and organic plant waste on the growth and calyx quality of roselle cultivated in a soilless system was investigated. Three treatments were compared in the study, viz. (i) control, (ii) AM, comprising a mixture of *Glomus* sp., *Gigaspora* sp. and *Scutellospora* sp. and (iii) AM supplemented with organic plant waste. Inoculation of soilless grown roselle resulted in the proliferation of fungal spores in the culture medium (varying from 55 to 61 spores per 10 gram substrate), while successful root infection ranged from 59% to 64%. The most significant observation in this study was the doubling of root volume and dry weight in the inoculated plants as compared with the control plants. Thus, AM inoculated plants showed significantly increased vegetative growth mainly below the ground level. In addition, total anthocyanin concentration in the calyces was also significantly improved. However, roselle yield and other quality attributes such as soluble solids concentration (SSC), titratable acidity (TA) and ascorbic acid were unchanged with the introduction of AM. It is concluded that while the AM treatments did not affect the growth and yield of soilless-grown roselle, AM infection of roselle roots increased their growth substantially. AM increased significantly the anthocyanin content of the calyces in cultures supplemented with organic plant waste.

Key words: mycorrhizal fungi, roselle, fertigation, postharvest quality

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is an annual shrub crop which is relatively a new plant in Malaysia. The plant is planted for its calyces, leaves, and young shoots which are eaten either raw or as cooked vegetables. Roselle was once widely cultivated in Malaysian plantations around the 1990s. Owing to low market demand, however, its planting declined, with most farmers switching over to other crops. The state of Terengganu was once the top roselle producer in Malaysia with a planted area of around 12.8 hectares (ha) in 1993, but this increased drastically to 506 ha in 2000 and to less than 150 ha in 2003. Data from the Ministry of Agriculture and Agro-based Industry Malaysia (2013) showed roselle production declining steadily from 714 metric tons (mt) in 2006 to 367 mt in 2012. Roselle had earlier been considered a suitable crop for Beach Ridges Interspersed with Swales (BRIS) soils, commonly found in the east coast states of Peninsular Malaysia. However, the low fertility of BRIS soil due to its poor physico-chemical properties such as low water holding capacity, high leach of nutrients and high surface temperature (Shamshuddin, 1990) was a serious constraint to roselle cultivation. To increase the yield and postharvest qualities of roselle, the adoption of a soilless culture system in combination with beneficial microbes such as arbuscular mycorrhizal (AM) fungi has been proposed as a replacement for BRIS soil.

The inoculation of different AM fungi formulations in a soilless system is a new approach to managing the problem of nutrient deficiency and improving the growth of the roselle plant and its crop. Mycorrhizal fungus hyphae are considered to function primarily by increasing the effective soil volume which facilitates the absorption of available forms of phosphorus by the roots. Thus, AM fungi may have access to forms of nutrients which are not

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directly available to plants, in addition to acquiring nutrients which are spatially or chronologically separated from roots. Currently, no research work has been done on the effects of AM fungi on growth, yield and quality of roselle grown in soilless culture system. Therefore, this warrants further investigation. Hence, with the inoculation of different AM formulations in addition with the soilless technology systems it is expected to provide latest and beneficial information to roselle suppliers and growers.

MATERIALS AND METHODS

Thirty-six roselle plants variety Terengganu (UMKL-1) were grown in a greenhouse at the School of Food Science and Technology, Universiti Malaysia Terengganu. The roselle seedlings were initially sown in a peat moss medium before transplantation after two weeks into the soilless culture medium (coco peat) equipped with a fertigation system. Water was supplied using a dripping method that delivered 1800-2000 part per thousand (ppt) per plant every 10 minutes. The planting was arranged in a randomized complete block design (RCBD), with three experimental treatments, *viz.* i) uninoculated (control, without AM), ii) AM alone (Pure) and iii) AM + organic plant waste (Enriched). The mixed species of AM fungi (*Glomus* sp., *Gigaspora* sp., and *Scutellospora* sp) were obtained from Malayisan Agri Hi-Tech Sdn. Bhd. The duration of this experiment was about 130 days, with roselle calyces harvested 85, 94 and 103 days after transplanting (DAT). Fungal spore counts (Gerdeman and Nicolson, 1963), root infection, stem diameter and plant height were recorded at 30 days intervals (0, 30, 60 and 90 DAT), while root volume and dry weight (Giovannetti and Mosse, 1980) were taken 105 and 110 DAT, respectively. Quality parameters of the calyces evaluated were titratable acidity (TA), soluble solids concentration (SSC), ascorbic acid and total anthocyanin concentration that were assayed following the methods of Wan Zaliha (2009). The data obtained were subjected to an analysis of variance (ANOVA) using GLM (General Linear Model) procedures with SAS 9.1 software package (SAS Institute Inc, Cary, NC, USA). Treatments means were further separated by LSD for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

RESULTS AND DISCUSSION

It is well documented that AM fungi colonization is commonly accompanied by an increase in plant growth (Ikiz *et al.*, 2008) which leads to an improvement of yield for various agriculture crops such as tomato, sorghum, and others (Bryla and Koide, 1998). In the present study, however, vegetative and floral parts of soilless grown roselle were not significantly increased with the AM treatments, as shown in Fig. 1 to Fig. 4. These outcomes may be associated with the changes in responsiveness of the roselle plants to AM colonization at various developmental stages of the plant and the existing fungal spore population. As reported by Bryla and Koide (1998), tomato was not responsive to AM inoculation in the early stages of growth and was only responsive in the later stages of development. Similarly, Dasgan and Ekici (2008) postulated that the responsiveness to AM could be related to "genetic control" of the genotype itself. This may be attributed to the photoassimilate compartmentation process, where the plants that are inoculated with the AM formulations may translocate most of their energy and nutrients to root development first, with vegetative development following only later.

In the present study, root development (root volume and dry weight) in the treated plants was significantly improved following AM inoculation – showing a doubling – as compared with the uninoculated control plants (Fig. 5). This observation is in agreement with Sim and Eom (2006) who noted that AM colonization exhibited a significant correlation with the root development compared to the vegetative plant growth. Also to be taken into account are the observations of Aulia *et al*. (2009) who find that AM infection produces different effects depending on the type of crop, the soil type and its fertility, environmental conditions, and the water irrigation system applied. These factors could be the reason for the inconsistent results in this study.

Sharma and Srivastava (1991) report that AM fungi inoculation improves the yield of various fruit crops. In this connection, Fig. 6 shows a positive

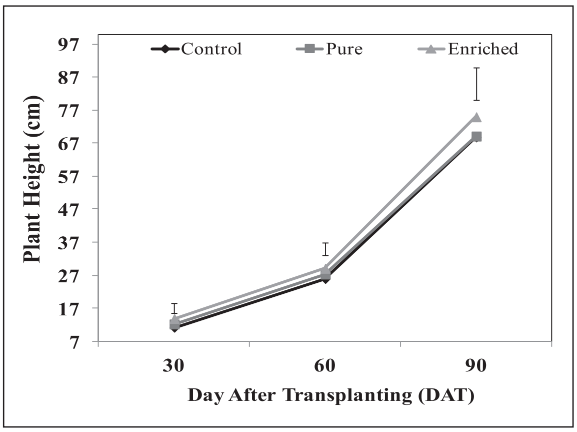

Fig. 1. Effects of different AM on roselle plant height. Vertical bars represent $LSD_{0.05}$.

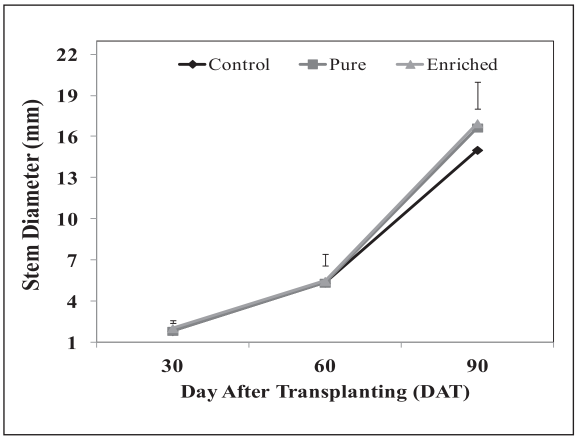

Fig. 2. Effects of different AM on roselle stem diameter. Vertical bars represent $LSD_{0.05}$.

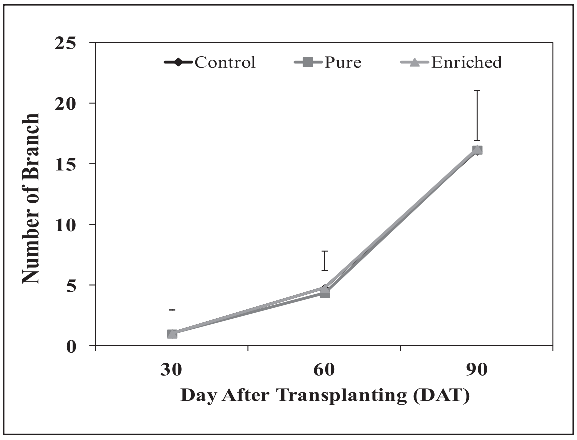

Fig. 3. Effects of different AM on roselle number of branch. Vertical bars represent $LSD_{0.05}$.

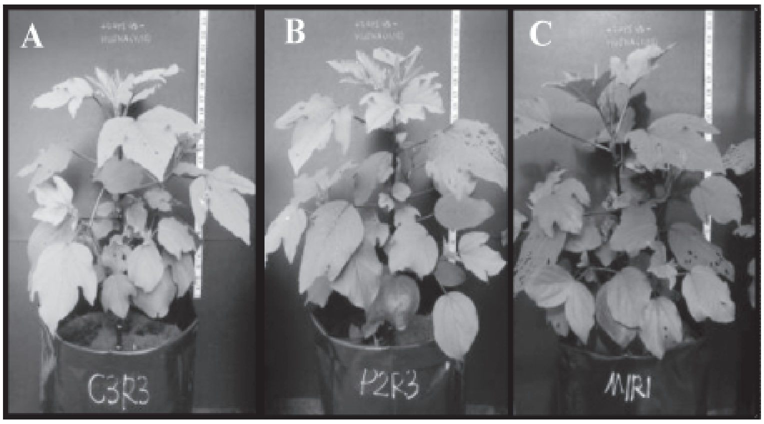

Fig. 4. Roselle growth on 45 DAT. A (Control), B (Pure) and C (Enriched).

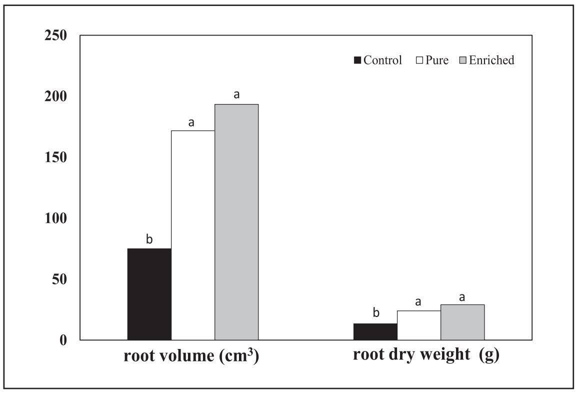

Fig. 5. Effects of different AM on root volume and root dry weight of roselle plant. Means with different letters are significantly different at the 5% level according to LSD test.

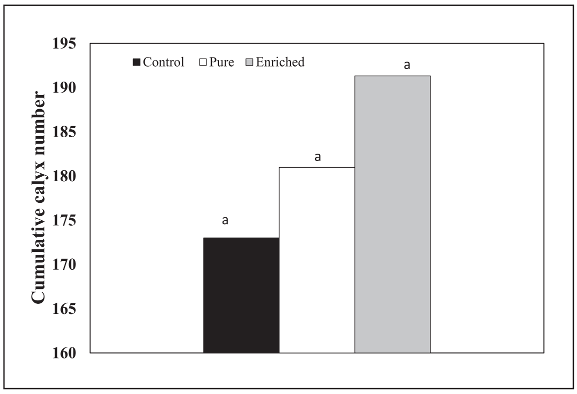

Fig. 6. Effects of different AM on roselle number and fresh weight. Means with different letters are significantly different at the 5% level according to LSD test.

effect of AM inoculation on the yield of roselle calyces, especially when organic plant waste was supplemented, although the yield improvements were not statistically significant. The inconclusive outcomes may be associated with the soilless culture medium used in this research. Besides being a source of phenolic compounds (Abad *et al*., 2002; Ma and Nichols, 2004) the coco peat medium used in the present study has been reported to have high cation exchange capacity (CEC) and exchange capability (EC) values. Dasgan and Ekici (2008) note that high EC in the root medium of the soilless

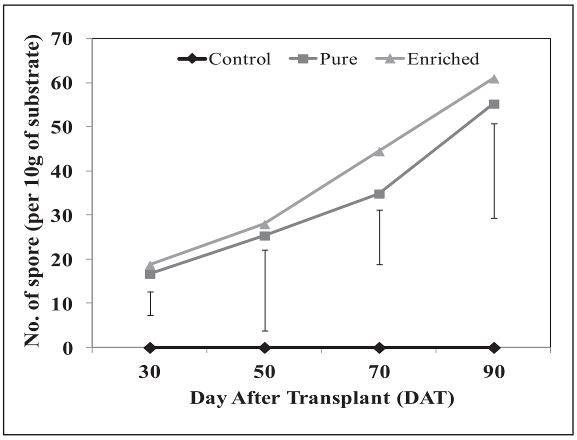

Fig. 7. Effects of different AM on number of spore. Vertical bars represent $LSD_{0.05}$.

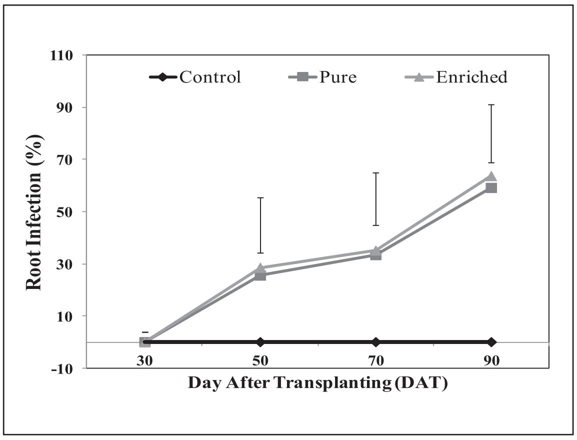

Fig. 8. Effects of different AM on root infection. Vertical bars represent $LSD_{0.05}$.

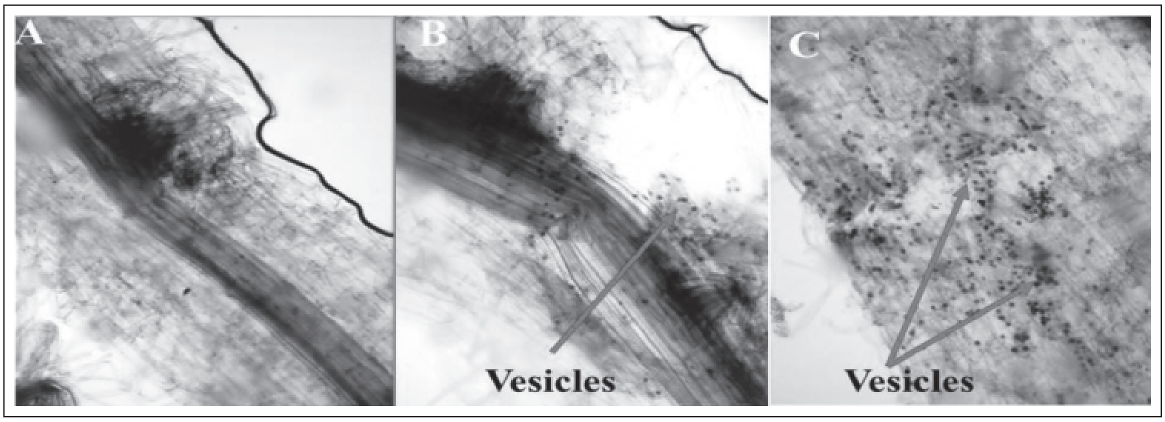

Fig. 9. Vesicle presence indicate as the positive infection in roselle root. A (Control), B (Pure) and C (Enriched).

culture systems could limit fruit set, and hence fruit yield and production. In agreement with those findings, Ma and Nichols (2004) report that high concentrations of phenolic compounds in fresh coco peat are at least partly responsible for poor growth and yield reduction in crop plants.

As expected, the number of fungal spores in the culture medium and percentage success of root infection in roselle planted in soilless culture gradually increased as the experiment proceeded (Fig. 7 and 8). Root infection was scored based on the presence of vesicles, hyphae, or both in the root environment. As shown in Fig. 9, the AM formulations elicited fungal spore production as well as signs of positive root infection of the roselle plant. On day-90, the Enriched and Pure formulation showed a greater number of spores produced (60.9 and 55.2 spores, respectively) as compared to uninoculated plants. In addition, the percentage of root infection in inoculated-Enriched plants was also higher than uninoculated and comparable to Pure. As discussed earlier, Enriched was the combination of AM and plant organic matter which acted as supportive nourishment for the dormant vesicles to grow and survive in the growth medium. Thus, with the greater quantity of nutrients provided in Enriched, the symbiotic interaction of the roselle root with AM was enhanced, as shown in Fig. 7 and 8. In addition, the proportion of arbuscles to vesicles can be influenced by ambient nutrient levels, which act as an indicator of the level of benefit received by each partner of the symbiosis (Johnson *et al*., 2003). Azizah (1999) also states that a higher percentage of organic matter present in the AM-treated BRIS soil would produce a higher number of mycorrhizal fungal spores that could eventually increase root colonization. Similar findings have also been reported by Nur Shuhadah *et al*. (2012). Thus, large numbers of fungal spores indicate better performance of AM symbiosis with the roselle plant through AM interaction with the root. Deshmukh *et al*. (2007) report that the introduction of single colonies of *Glomus mossae* enabled better underground growth performance than that for non-mycorrhizal plants.

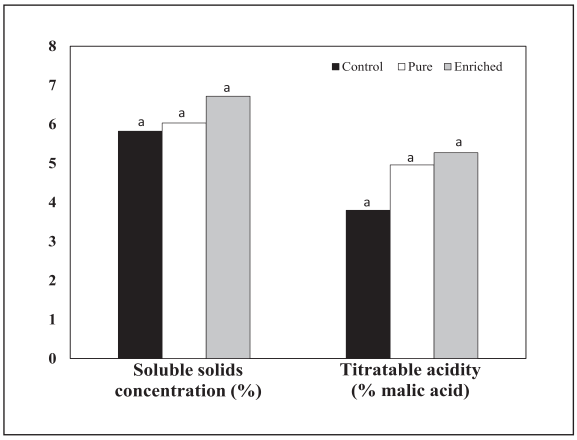

Fig. 10. Effects of different AM on SSC and TA of roselle plant. Means with different letters are significantly different at the 5% level according to LSD test.

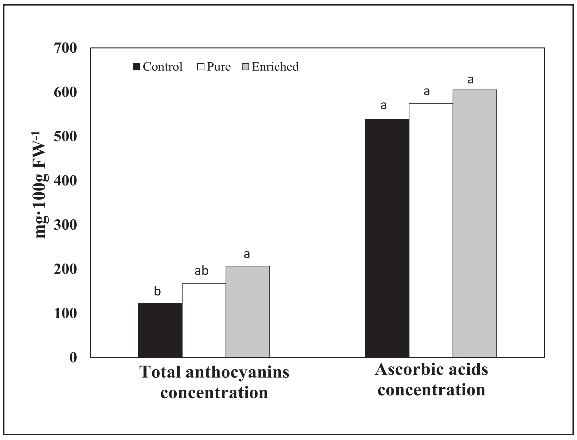

Fig. 11. Effects of different AM on total anthocyanins and ascorbic acid concentrations of roselle plant. Means with different letters are significantly different at the 5% level according to LSD test.

Postharvest parameters such as calyx SSC, TA, and ascorbic acid were not statistically different between treated plants and the controls (Fig. 10 and 11). The exception was in total anthocyanin concentration where significant increments due to the combination of AM and organic plant waste were evident (Fig. 11). This observation is contrary to that of Nur Shuhadah *et al*. (2012) who report that roselle calyx quality is not different between AM-treated and control plants. In the present study, the inoculated plants especially when supplemented with organic plant waste, produced the highest total anthocyanin concentration as compared to uninoculated plants (Fig. 11).

CONCLUSION

AM and organic plant waste significantly increased the number of spore, percentage of root infection, root volume, root dry weight and calyx total anthocyanin concentration in soilless grown roselle. Despite their large numbers, AM spores were not effective in enhancing the above-soil vegetative growth or the yield of roselle calyxes in plants cultivated in the soilless system. In conclusion, AM treatments do not benefit above-soil vegetative growth or yield of soilless-grown roselle. However, AM infection of the roselle roots enhanced their growth substantially, while the calyx anthocyanin content was also increased significantly when AM was further supplemented with organic plant waste.

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