
Influence of arbuscular mycorrhizal (AM) fungi on survival and development of micropropagated *Acorus calamus* L. during acclimatization

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Yadav, K., Singh, N and Aggarwal, A. (2011). Influence of arbuscular mycorrhizal (AM) fungi on survival and development of micropropagated *Acorus calamus* L. during acclimatization. Journal of Agricultural Technology 7(3): 775-781.

The response of AMF inoculation on the survival and development of micropropagated *Acorus calamus* plantlets during acclimatization was studied. The plant height, plant spread, number of leaves per plant, leaf area were significantly higher in AM inoculated plants either alone or in combination as compared to uninoculated plants. This clearly demonstrated that dual inoculation with *Glomus mosseae* and *Acaulospora laevis* not only increased survival rates (93.4%) but also improved plant growth and development.

Key words: *Acorus calamus*, Arbuscular mycorrhiza, *Glomus mosseae*, *Acaulospora laevis*, acclimatization

Introduction

The cultivation of medicinal and herbal plants has assumed greater importance in recent years due to their tremendous potential in modern traditional medicine, cosmetic and fragrance industries. *Acorus calamus* Linn. (family Araceae) is a semiaquatic herb with creeping rhizomes and sword shaped long leaves commonly known as “sweet flag” or “Bach” is an important endangered medicinal plant (The Wealth of India, 2003). The rhizomes are utilized extensively by the Chinese, Indians and American Indians as it possess anti-spasmodic, carminative and anti-anthelmintic properties and also used for treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers and tumors (Anonymous, 2000). Micropropagation facilitates multiplication of high quality and homogeneous plant material in

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mass production. The weaning of the micropropagated plants to greenhouse condition is of the most critical steps in the morphological and physiological adaptation during the preparation of plantlets. In this stage, known as acclimatization phase, plantlets are subject to severe environmental stress, due to poor root and shoot growth and reduced cuticular wax formation, the percentage of mortality is about 30-40%. Vesicular arbuscular mycorrhizal fungi are an integral part of the root system of most of the plants and constitute an important group of microorganisms in the soil microbial community. Inoculation of VAM fungi during an early stage of acclimatization process has become an alternative strategy for better establishment by improving the plant growth. The association of VAM fungi with the medicinal plants had not only enhanced the growth of medicinal plants but also improve the productivity of medicinal compounds (Karthikeyan *et al.*, 2009). The aim was to evaluate the effects of AMF inoculation on the development of *Acorus calamus* in the weaning process and to produce homogenous and high quality medicinal plants on a commercial scale.

Materials and methods

Plant material

Acorus calamus plants (Fig 1) obtained from the Herbal Garden of Department of Botany, Kurukshetra University, Kurukshetra (India) were used in the experiment. Rhizome buds of *Acorus calamus* were excised and superficially disinfected according to the methodology of Debiassi *et al.* (2004). After this process, buds were placed on MS medium (Murashige and Skoog, 1962) supplemented with sucrose (30 g /l), agar (7 g/l) and 2.0 mg/l of -benzylaminopurine, with pH adjusted to 5.8 before autoclaving (Ahmed *et al.*, 2007). Plant cultures were maintained in growth chambers in culture tubes for 45 days, at a temperature of $25 \pm 2^\circ\text{C}$ and a light intensity of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Choice of AM fungus

AM fungi (*G. mosseae* and *A. laevis*) were isolated from the rhizosphere of *A. calamus* by using wet sieving and decanting technique given by (Gerdemann and Nicolson, 1963).

Multiplication of AMF cultures

Both dominant AM fungi were mass multiplied in sterilized sand and soil (3:1) substrate using maize as suitable host in polyhouse conditions. Hoagland

plant nutrient solution containing half of the recommended phosphorus is given once in a week. After 90 days of growth, viable AM spores were isolated by wet sieving and decanting procedure (Gerdemann and Nicolson, 1963).



Fig 1. Micropropagated *Acorus calamus* in plant tissue culture Lab.

Inoculation

Inoculation can be given at the *in vitro* stage of rooting, planting out for acclimatization and transplanting to the field after acclimatization. *In vitro* raised plants were taken out from the cultural tubes and washed in sterilized distilled water. Further the roots of those plants were washed with the help of fine brush to remove the agar particles. These rooted plants were then transplanted in cups containing sterilized sand and soil (3:1). Each cup was added 10% inoculum of AM fungi and fine roots of maize colonizing with mycelia/arbuscules/vesicles alone and in combination. The control was maintained without AM inoculation.

Growing condition

The inoculated plants were grown in green house for acclimatization with required humidity and light. However, an extended day length of 16 hours with cool white fluorescent lamp favours the mycorrhizal development. Fifteen replicated were taken with the following combinations treatments: T1- Uninoculated (control), T2- Inoculated with *A. laevis*, T3- Inoculated with *G. mosseae* and T4- Inoculated with *G. mosseae* and *A. laevis*. Plant growth parameters were recorded as plant height, plant spread, number of leaves and leaf area after 15 days interval commencing from the day after inoculation (Fig 2). Three such observations were made during entire growth period of plant. But the data after 45 days was taken (Fig 3).

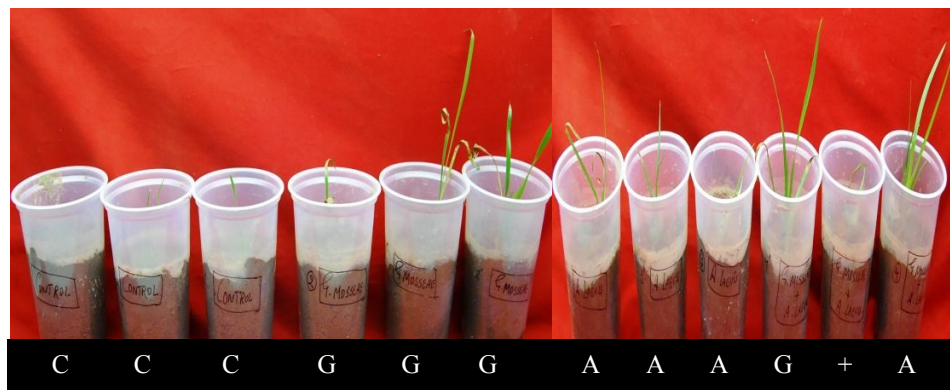


Fig. 2. Plants after 15 days of inoculation.

Table 1. Influence of AM fungi on the various growth parameters of micropropagated *Acorus calamus* after 45 days of inoculation.

Treatments	Plant height (cm)	Plant spread (cm ²)	Leaves (no./plant)	Leaf area (cm ² /plant)
T1(Control)	18.0±0.5	160.8±3.8	4.3± 0.5	13.1±0.3
T2	22.2±0.3	193.7±2.4	4.6± 0.5	17.0±0.5
T3	34.0±0.2	405.2±3.8	5.6± 0.5	28.1±0.2
T4	42.1±0.1	520.3±4.5	6.6± 0.5	36.0±0.3

Data indicate mean ± standard deviation.

Fifteen replicates were used per treatment.

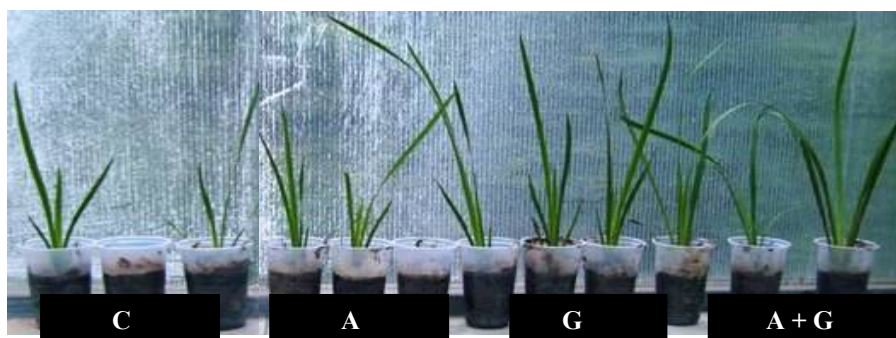


Fig 3. Plants after 45 days of inoculation. C = control, A = *Acaulospora laevis*, G = *Glomus mosseae*.

Results and discussion

In vitro propagated *Acorus calamus* plantlets were acclimatized in the greenhouse to natural conditions. The percentage of mortality was 30-40 % during this phase.

The investigation was carried out in order to evaluate role of AM fungus on the survival and growth rate of *A. calamus*. Inoculation of *A. calamus* with *G. mosseae* significantly increased plant height (34.0 ± 0.2) as compared to control uninoculated plants (18.0 ± 0.5). Similar results were obtained in medicinal plants *Palmarosa* by Gupta *et al.* (1991). Inoculation with *A. laevis* was also increased plant height (22.2 ± 0.3) as compared to uninoculated plant. Researchers have been demonstrated that vesicular-arbuscular mycorrhizal fungi, not only increased phosphorus uptake, but also other diffusion-limited element like Zn, Cu etc (Ellis *et al.*, 1985). The movement of nutrients to plant roots and the rate of absorption of nutrients by roots, especially nitrogen, phosphorus and potassium, is known to be limited by the rate of diffusion of each nutrient through the soil and not by the ability of the root to absorb the nutrient from low concentration in the soil solutions (Nye *et al.*, 1977). Inoculation with AM fungi could increase in nutrient uptake by merely shortening the distance that the nutrients had to diffuse from the soil to the roots. The spread of plant was higher when inoculated in combination (520.3 ± 4.5), followed by *G. mosseae* (405.2 ± 3.8) and *A. laevis* (193.7 ± 2.4) alone. Number of leaves per plant was also significantly more in inoculated treatments as compared to uninoculated treatment, highest being in the treatment *G. mosseae* + *A. laevis* combination (6.6 ± 0.5). An increase in nutrient uptake resulted in relief of nutrients stress and an increase in photosynthetic rate, which obviously rise to an increase in plant growth. Similar trend was observed in the leaf area per plant. In general, mycorrhizal plants are able to translocate more carbon to the roots than non-mycorrhizal plants (Colozzi-Filho and Bolota, 1994). So, mycorrhizal fungi associated with plants were higher survival percentage when planted in the field (Table 2). AM fungi enhance the activity of beneficial soil organisms, like nitrogen fixers and phosphate solubilizers VAM fungi (Lindermann, 1992). AM fungi are also involved in increasing the uptake of water (Huang *et al.*, 1985) and their colonization is also known to suppress the activity of pathogenic organisms and protect the roots from phytopathogenic fungi, bacteria and parasitic nematodes and other soil pathogens invading the roots and thereby increase root growth and nutrients acquisition of the host plants (Bagyaraj, 1984). According to Douds *et al.* (1998) stated that the physiological response of a plant is the interactions between environment, plant and fungus genotype and increases in

growth rate are not always related to colonization. For a rhizome- producing endangered medicinal plant such as *Acorus calamus*, inoculation of AMF to the roots plays a beneficial role on their post transplanting performance. So, ensuring their high survival rate and well growth development resulted in their successful introduction to the field conditions.

Table 2. Influence of AM fungi on the survival rate of micropropagated *Acorus calamus* infield after 45 days of inoculation.

Treatments	Mortality rate (%)
T1 (Control)	39.9
T2	33.3
T3	13.3
T4	6.6

Data indicate mean \pm standard deviation. Fifteen replicates were used per treatment

Acknowledgements

The authors are grateful to University Grants Commission, New Delhi and Kurukshetra University, Kurukshetra for providing financial assistance and laboratory facilities to carry out the work.

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(Received 5 September 2010; accepted 28 March 2011)