COMPARATIVE PHYSIOMORPHOLOGICAL PERFORMANCE OF HALF-SIB SEEDLINGS OF TEN TEAK CLONES UNDER SUBOPTIMAL AND OPTIMAL ARBUSCULAR MYCORRHIZAL COLONISATION

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VERMA, R. K., KUMAR, P. & ANSARI, S. A. 2001. Comparative physiomorphological performance of half-sib seedlings of ten teak clones under suboptimal and optimal arbuscular mycorrhizal colonisation. The physiomorphological response of half-sib seedlings of 10 teak (*Tectona grandis*) clones to native and introduced arbuscular mycorrhizal (AM) fungi inocula was tested under nursery conditions. Exogenous application of AM fungi benefited most teak clones, except APNPL-5. However, the response of various physiomorphological characteristics to AM fungi inoculum was positive (albeit variable) in all 10 teak clones. The AM fungi inoculum significantly improved root colonisation in all clones, leaf P level in all clones except UP-C2, leaf number in MHSC-A2, stomatal conductance in all clones except APNPL-5, transpiration rate in MHAL-A5, MHAL-P7, MHSC-A2, ORANP-7, photosynthetic rate in all clones, except APNPL-5 and MHSC-A3, water use efficiency in MHAL-P1, MYHD-2, UP-C2, nitrate reductase activity in 6 of 10 clones, plant dry weight in most clones except APNPL-5, MHAL-5 and MHAL-P1 as well as seedling volume in APNPL-5, MHAL-A5, MHSC-A3, MYHV-5, ORANP-7 and UP-C2. The present study revealed (1) the importance of AM symbiosis in growth and water flux in teak and (2) the existence of separate compatibilities between the AM fungi inoculum used and teak clones for colonisation. Inoculation of half-sib seedlings of various teak clones with AM fungi is recommended for production of sturdy seedlings that will ensure high survival and better performance in the field.

Key words: AM fungi colonisation - nitrate reductase activity - photosynthetic rate - teak (*Tectona grandis*) clones - stomatal conductance - transpiration rate - water use efficiency

VERMA, R. K., KUMAR, P. & ANSARI, S. A. 2001. Prestasi fisiomorfologi bandingan anak benih separuh kandung bagi 10 klon pokok jati di bawah pengkolonian mikoriza arbuskel suboptimum dan optimum. Tindak balas fisiomorfologi anak benih separuh kandung bagi 10 klon pokok jati (*Tectona grandis*) terhadap inokulum kulat mikoriza arbuskel (AM) diuji di tapak semaian. Aplikasi eksogen kulat AM memberi faedah

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kepada kebanyakan klon pokok jati, kecuali APNPL-5. Walau bagaimanapun, tindak balas pelbagai ciri fisiomorfologi terhadap inokulum kulat AM adalah positif (meskipun pelbagai) dalam kesemua 10 klon. Inokulum kulat AM meningkatkan dengan bererti pengkolonian akar dalam semua klon, tahap P daun dalam semua klon kecuali UP-C2, bilangan daun dalam MHSCA2, konduktans stoma dalam semua klon kecuali APNPL-5, kadar transpirasi dalam MHAL-A5, MHAL-P7, MHSC-A2, ORANP-7, kadar fotosintesis dalam semua klon kecuali APNPL-5 dan MHSC-A3, kecekapan penggunaan air dalam MHAL-P1, MYHD-2, UP-C2, aktiviti reduktase nitrat dalam enam klon, berat kering tumbuhan dalam kebanyakan klon kecuali APNPL-5, MHAL-5 dan MHAL-P1 dan juga isipadu anak benih dalam APNPL-5, MHAL-A5, MHSC-A3, MYHV-5, ORANP-7 dan UP-C2. Kajian ini menunjukkan (1) kepentingan simbiosis AM dalam pertumbuhan dan fluks air dalam pokok jati dan (2) wujudnya kesesuaian yang berbeza antara inokulum kulat AM yang diguna dan klon pokok jati untuk pengkolonian. Penginokulatan anak benih separuh kandung pelbagai klon pokok jati dengan kulat AM disyorkan untuk penghasilan anak benih yang lebih lasak yang dapat menjamin kebolehhidupan serta prestasi yang lebih baik di lapangan.

Introduction

Teak (*Tectona grandis*) is a major source of high quality timber in India, Indonesia, Myanmar and Thailand. This high value timber was introduced to the tropical regions of Asia, Africa and Central America at the beginning of the 19th century (Wood 1993). It is a slow growing species and current production fails to meet the demand for its timber. Hence, the genetic improvement of teak was initiated about four decades ago. Superior provenances or individual (plus) trees were selected from natural populations, the growth of their progeny was tested and clonal seed orchards were established to supply improved seed and planting stocks (Wood 1993). Bud grafts of identified plus teak trees from India were prepared and established as clonal seed orchards in various agro-climatic regions throughout India. However, clonal seed orchards, including one at Chandrapur, Maharashtra, face a severe problem of poor fruit set and seed germination (Anonymous 1992), upsetting the benefits of teak improvement programs. Raising healthy seedlings from available improved seeds may improve performance in the field. Since most afforestation sites are poor in nutrients and arbuscular mycorrhizal (AM) fungi, pre-inoculation of seedlings with AM fungi is expected to confer additional advantages.

Verma & Jamaluddin (1995) examined AM fungi colonisation in teak forests and teak plantations of various ages at several sites. Subsequently, AM fungus isolates obtained from teak roots were multiplied to produce AM fungi inoculum for treating teak seedlings at the Tropical Forest Research Institute (TFRI) in Jabalpur. Our study described the effect of AM fungi inoculum and its interaction with teak genotypes on physiomorphological performance of seedlings raised from open-pollinated (half-sib) improved seeds of 10 teak clones maintained in a clonal seed orchard at Chandrapur.

Materials and methods

Preparation of AM fungi inoculum and inoculation of seedlings

Arbuscular mycorrhizal fungi were sieved from soil of the teak rhizosphere collected from Chandrapur, Maharashtra using 45 µm sieve. The spores of AM fungi were recovered by wet sieving and decanting/density gradient centrifugation (Sylvia 1994). Six species were identified from the isolates as *Acaulospora scrobiculata* (TF20), *Glomus intraradices* (TF24), *G. mosseae* (TF26), *G. etunicatum* (TF27), *Gigaspora* sp. (TF21) and *Scutellospora pellucida* (TF28). A mix of all the AM fungi species was multiplied in earthen pots in sterilised medium, namely, sand and soil (1:1 v/v), using teak seedlings as trap plants. The voucher culture (VC 3) of the mixed AM fungi was maintained at the TFRI. Six-month-old inoculum, including the medium, infected root pieces, spores and hyphae, containing an average of 292 infective propagules per ml (Liu & Luo 1994), were used to inoculate one-year-old seedlings during transplantation into 16 × 30 cm polyethylene bags. The root zone of each seedling received 10 ml of inoculum.

Experimental design

The experiment was laid out to test the effect of two treatment factors (AM inocula and teak clones) and their interactions on various physiomorphological parameters in complete randomised design. One hundred seedlings from each clone were divided into two groups, (1) control (M1) receiving no exogenous AM fungi inoculum and (2) treated (M2) receiving exogenous AM fungi inoculum. There were five replicates for each characteristic measured.

Raising teak seedlings

Open-pollinated (half-sib) seeds were collected from 10 teak clones belonging to five states of India: Andhra Pradesh (APNPL-5), Maharashtra (MHAL-A5, MHAL-P1, MHAL-P7, MHSC-A2, MHSC-A3), Karnataka (MYHD-2, MYHV-5), Orissa (ORANP-7) and Uttar Pradesh (UP-C2). These clones have been maintained as bud grafts at Lohara Chandrapur, Maharashtra since 1969 (Anonymous 1992). The collected seeds were weathered mechanically and germinated in nursery beds at Lohara, Chandrapur. Seedlings were uprooted after one year, made into stump (root-shoot) cuttings (seedlings) and brought to the TFRI, Jabalpur. These were subsequently planted individually in 16 × 30 cm polyethylene bags containing 5 kg soil mix (soil and sand 1:1 v/v). The physical and chemical properties of soil mix determined as described by Jackson (1973) were sandy loam soil texture, pH 7.6, EC 0.35 mmho cm^{-1}, organic matter 1.3% and available N, P and K contents of 144.8, 10.3 and 18.8 mg kg^{-1} respectively. Seedlings were then grown for one year before evaluation.

Physiomorphological observations

Photosynthetic rate (P_N), stomatal conductance (gs), transpiration rate (E), water use efficiency (WUE) were recorded at the end of one year using a portable photosynthesis system (LI 6200, LI-COR Inc., Lincoln, NB, USA). Nitrate reductase activity in randomly collected leaves was measured according to Jaworski (1972). The percentage of root colonised by AM fungi, leaf number, collar diameter, plant height and dry weight (after drying for 72 h at 70 °C) were determined after the seedlings were uprooted from the polyethylene bags. The seedling volume was computed by using the formula: (root collar diameter)^2 × (plant height) (Ruehle *et al.* 1984). Fine roots weighing 0.5 g from each replication were used for assessment of root colonisation percentage. The roots were stained with trypan blue according to the method of Phillips & Hayman (1970). The percentage of colonised root length was determined by the grid-line intersect method (Giovannetti & Mosse 1980). Leaves were oven dried (72 h at 70 °C), powdered and acid-digested according to Lindner (1944) to liberate its phosphorus content. Phosphorus in the digest was then measured by the method of Fiske & Row (1925).

Statistical analysis

The data obtained for various physiomorphological characterstics were subjected to factorial statistical analysis, employing two way analysis of variance (ANOVA) and "F" test for significance, and LSD at $p \le 0.05$ ($LSD_{0.05}$) for comparison of means of AM fungi inoculum, teak clones and their interactions. A Pearson Product (moment correlation coefficient) between AM fungi colonisation and leaf nitrate reductase activity (NRA) was examined (Gomez & Gomez 1984).

Results

Root colonisation (%)

Inoculation with AM fungi enhanced root colonisation by an average of 126% over controls (Table 1). The roots of APNPL-5 exhibited the highest AM colonisation which was, however, not significantly different than that of MYHV-5, UP-C2, MYHD-2 and ORANP-7. The roots of MHAL-A5 and MHSC-A3 had the lowest colonisation.

Plant growth

Generally, the half-sib seedlings treated with AM fungi inoculum exhibited 55.5% greater plant dry weight than controls. Inoculation significantly increased biomass accumulation of ORANP-7, MHSC-A2, UP-C2 and MYHV-5, whereas AM inoculum had no effect on biomass of APNPL-5, MHAL-A5 and MHAL-P1. Inoculated seedlings had 40% greater volume than the uninoculated control

Table 1 Percentage root length colonised by AM fungi, growth characteristics and leaf P concentration of half-sib seedlings of teak one year after inoculation with AM fungi.

Teak clones	Root colonisation (%)			Plant dry weight (g)			Seedling volume (cm³)			Leaf number			Leaf P concentration (mg g⁻¹ dry weight)		
	M1	M2	Clones	M1	M2	Clones	M1	M2	Clones	M1	M2	Clones	M1	M2	Clones
ORANP-7	28.4	75.4	51.9	25.3	51.6	38.4	7.5	14.7	11.1	10.0	11.4	10.7	2.1	3.4	2.8
MYHV-5	36.0	76.9	56.4	25.9	42.6	34.2	9.9	15.1	12.5	10.4	10.0	10.2	2.2	3.2	2.7
MHSC-A3	21.8	65.2	43.5	25.1	35.0	30.0	5.4	8.9	7.1	8.0	5.6	6.8	2.5	2.9	2.7
MHAL-P1	29.1	72.2	50.7	30.6	36.6	33.6	9.4	10.0	9.7	7.2	7.6	7.4	2.3	2.9	2.6
MHSC-A2	29.4	67.2	49.8	15.1	51.1	33.1	7.2	8.4	7.8	7.4	12.6	10.0	2.3	2.8	2.5
MHAL-A5	22.1	63.2	42.6	28.6	35.3	32.5	7.7	12.8	10.2	9.8	10.4	10.1	2.2	2.9	2.5
APNPL-5	40.6	75.5	58.0	33.2	41.3	37.2	7.7	13.7	10.7	10.2	7.4	8.8	2.2	2.7	2.4
MYHD-2	30.1	74.4	52.2	20.7	31.5	26.1	7.3	6.7	7.0	5.8	5.2	5.5	1.8	2.8	2.3
MHAL-P7	33.0	66.8	49.9	27.0	38.7	32.8	4.2	5.6	4.9	8.6	7.2	7.9	1.9	2.4	2.2
UP-C2	38.4	69.3	53.8	31.2	45.7	38.4	14.0	15.9	14.9	7.0	5.6	6.7	2.0	2.2	2.1
AM inocula	30.9	70.6		26.3	40.9		8.0	11.2		8.4	8.3		2.1	2.8	

$LSD_{0.05}$	Root colonisation	Plant dry weight	Seedling volume	Leaf number	Leaf P
AM inocula (A)	2.7	3.1	0.5	ns	0.1
Clones (C)	6.1	7.0	0.2	2.1	1.2
Interaction (A × C)	ns	9.8	1.7	3.0	0.3

Data represent mean of five replicates.
ns - not significant; M1 - receiving no exogenous AM fungi inoculum; M2 - receiving exogenous inoculum.

seedlings. However, seedling volume of MHAL-P1, MHAL-P7, MHSC-A2 and MYHD-2 clones did not respond to AM fungi inoculum. AM inoculum had no overall significant influence on average leaf number. There was significant variation in this parameter among the half-sib seedlings of various teak clones. However, there was a significant interaction, indicating that AM inoculation increased leaf numbers in some clones and decreased them in others (Table 1).

Physiological parameters

Inoculation increased P concentration in leaves by 30.8% over that of the control. All teak clones significantly responded to AM inoculum for leaf P concentration. Three teak clones, namely, ORANP-7, MYHD-2 and MYHV-5 exhibited > 50% gain in leaf P concentration upon inoculation compared to their respective controls (Table 1).

Inoculation increased stomatal conductance (gs) by 31% over the control (Table 2), significantly enhancing gs in all half-sib seedlings, except in APNPL-5, MHSC-A3 and UP-C2. A significant clonal effect was also observed. MHAL-P1 had the highest gs and ORANP-7 had the lowest.

Inoculation also enhanced transpiration rate (E) by 15.4% over the control (Table 2). The clones MHAL-A5, MHAL-P7 and MHSC-A2 had significantly higher E values when inoculated with AM fungal inoculum. E values obtained in MHSC-A3, MYHD-2 and UP-C2 after inoculation with AM were not significantly different from those in suboptimal inoculation (M1). $LSD_{0.05}$ value for comparison of E between M1 and M2 was 1.2.

Net photosynthetic rate (P_N) was increased by inoculation of AM fungi by 27.8% over the control. A significant interaction indicated that some half-sibs responded better to inoculation (Table 2). They are MHAL-P1 (41.3%), MHSC-A2 (40.5%), ORANP-7 (39.4%), MHAL-A5 (37.7%), MYHV-5 (35.4%), MHAL-P7 and MYHD-2 (35.2%) and UP-C2 (20.5%).

Inoculated half-sib seedlings had significantly greater water use efficiency (WUE) than uninoculated seedlings. Half-sib seedlings of UP-C2, MHAL-P1, MYHD-2 showed significant positive response to AM inoculum. The response of other half-sib seedlings towards AM inoculum for this parameter was not significant (Table 2).

Half-sib seedlings treated with AM inoculum exhibited a significant elevation in nitrate reductase activity (NRA) (58.5%) in comparison with the control seedlings. The NRA of half-sib seedlings of ORANP-7 (253.2%), MYHD-2 (173.7%), MHSC-A2 (143.1%), MHAL-P1 (122.6%), MYHV-5 (119.7%) and MHAL-P7 (52.8%) were significantly elevated in the presence of AM inoculum whereas those of the remaining four teak clones did not significantly respond to AM inoculum (Table 2).

Table 2 Physiological characteristics of half-sib seedlings of teak one year after inoculation with AM fungi.

Teak clones	gs (mol m^{-2} s^{-1})			E (mmol H_2O m^{-2} s^{-1})			P_N (µmol CO_2 m^{-2} s^{-1})			WUE (mol CO_2 $mmol^{-1}H_2O$)			NRA (nmol NO_2^{-1} g^{-1} h^{-1})		
	M1	M2	Clones	M1	M2	Clones	M1	M2	Clones	M1	M2	Clones	M1	M2	Clones
ORANP-7	0.17	0.29	0.23	4.6	6.5	5.5	7.1	9.9	8.5	1.57	1.58	1.57	77	202	139
MYHV-5	0.27	0.35	0.31	7.1	7.9	7.5	6.5	8.8	7.6	0.91	1.12	1.01	61	134	97
MHSC-A3	0.39	0.40	0.39	7.9	7.8	7.8	9.7	9.9	9.8	1.25	1.31	1.28	62	53	57
MHAL-P1	0.39	0.53	0.46	7.1	7.8	7.4	7.5	10.6	9.0	1.07	1.39	1.23	53	118	85
MHSC-A2	0.31	0.42	0.36	7.2	8.5	7.8	7.4	10.4	8.9	1.07	1.24	1.15	51	124	87
MHAL-A5	0.23	0.43	0.33	6.2	9.3	7.7	6.1	8.4	7.2	0.98	0.90	0.94	88	101	94
APNPL-5	0.35	0.33	0.34	6.4	7.4	6.9	8.6	9.1	8.8	1.27	1.24	1.25	175	169	172
MYHD-2	0.16	0.30	0.23	6.1	5.7	5.9	5.1	6.9	6.0	0.83	1.26	1.04	57	156	106
MHAL-P7	0.26	0.40	0.33	6.2	9.1	7.6	6.8	9.2	8.0	1.10	1.05	1.07	50	81	65
UP-C2	0.38	0.38	0.38	5.8	5.3	5.5	7.8	9.4	8.6	1.37	1.80	1.58	151	166	158
AM inocula	0.29	0.38		6.5	7.5		7.3	9.3		1.14	1.29		82	130	

	$LSD_{0.05}$				
	gs	E	P_N	WUE	NRA
AM inocula (A)	0.02	0.4	0.3	0.08	5.0
Clones (C)	0.04	0.8	0.8	0.19	12.0
Interaction (A × C)	0.05	1.2	1.1	0.27	17.0

Data represent mean of five replicates.
ns - not significant; M1 - receiving no exogenous AM fungi inoculum; M2 - receiving exogenous inoculum.

Discussion

A preliminary investigation carried out under sterile conditions showed that teak seedlings planted in polyethylene bags had very poor survival (27% and 4% after six months and one year of growth respectively) in the absence of AM fungus inoculum (Verma 2001). Studies from temperate and tropical regions indicate that species of late seral stages such as teak, tend to show the highest mycorrhizal dependence (Janos 1980a, b, 1987, Allen & Allen 1986, 1990, Allen 1991). Thus, the present investigation was undertaken to test the performance of teak seedlings under suboptimal and optimal AM colonisation.

The AM symbiosis promotes acquisition of mineral nutrients especially P by host plants (Cooper & Tinker 1978, Lambert *et al.* 1979). The hyphae of the mycorrhizae effectively enlarge root-soil interface, allowing efficient exploitation of soil around the root (Li *et al.* 1991, Jakobsen *et al.* 1992). In the present study, teak seedlings treated with AM fungi inoculum exhibited an improvement in colonisation and P concentration in their leaves and in overall anabolic activities, e.g., P_N and NRA. These positive effects were manifested as production of more biomass in treated teak seedlings despite no significant improvement in leaf production. Eissenstat *et al.* (1993) noted similar improvement in carbon assimilation in AM fungus-colonised seedlings of *Citrus aurantium.* An increase in the activity of this enzyme in the shoots of mycorrhizal soybeans by Carling *et al.* (1978) and both shoots and roots of mycorrhizal *Trifolium subterraneum* by Oliver *et al.* (1983) have earlier been reported. Buwalda *et al.* (1983) reported that the facilitated uptake of anions, including nitrate, was due to AM symbiosis. Enhanced uptake of nitrate is expected to favour the *de novo* synthesis of nitrate reductase as the ion acts as an inducer and stabiliser of the enzyme (Hewitt & Afridi 1959). This conformed with the existence of a positive correlation ($r = 0.738$, $p \leq 0.05$) between AM fungi colonisation and leaf NRA under AM inoculum treatment in the present study.

The high gs, E and WUE in AM fungi treated seedlings observed here (Table 2) compared favourably with the findings on other species by several workers (e.g., Allen 1982, Allen & Boosalis 1983, Huang *et al.* 1985, Koide 1985). Increased gaseous exchange observed in mycorrhizal onion and sunflower over controls was attributed to more P acquisition by these plants (Nelsen & Safir 1982, Koide 1985 respectively). The increased surface area for water absorption provided by external hyphae together with lower resistance to root radial water flux generated by internal hyphae are responsible for high gs in relation to E and gaseous exchange in AM fungus-colonised plant (Safir & Nelsen 1985). However, the role of AM symbiosis in plant-water relations is still obscure and deserves a thorough investigation especially since it involves two categories of plants, namely, suboptimal and optimal colonised plants which do not have the same size.

Percentage root length colonised by AM fungi was not always correlated to P content of leaves. Half-sib seedlings of ORANP-7 and UP-C2 with moderate AM fungi infection maintained the highest and lowest P levels in their leaves respectively, whereas those of APNPL-5 with the highest AM fungi colonisation contained a moderate leaf P level. This indicated that colonisation and hyphal growth of AM fungi are two separate events, each requiring compatibility between AM fungi inocula and teak genotype. The extraction of nutrients and water from the soil depended more on hyphal growth than on the percentage of root colonisation during AM symbiosis. This argument is in contrast with the studies conducted on myco-mutant plants of *Pisum, Vicia* and *Medicago* (Duc *et al.* 1989, Bradbury *et al.* 1991, Gianinazzi-Pearson *et al.* 1991). This raises the possibility of separate tuning of gene(s) between AM fungi and the host for infection and development of AM fungus hyphae. In addition, an active role of mycorrhizal hyphae in nutrient and water transport from the soil has also been documented (Kothari *et al.* 1991).

Considerable genetical variations were noted in physiomorphological performance of teak clones. For instance, half-sib seedlings of ORANP-7, UP-C2, APNPL-5 and MYHV-5 had significantly superior biomass and seedling volume which may be due to better carbon and nitrogen assimilation resulting from beneficial effect (e.g., high leaf P level) of AM association in these clones. The significance of AM fungal association was further established in the case of MYHD-2 which not only had a poor AM fungal association and the lowest leaf P level but also displayed a dismal physiomorphological performance, including P_N, NRA, biomass production and seedling volume. The facilitated AM fungi colonisation followed by luxuriant growth of fungal hyphae in compatible teak clones led to greater availability of water and nutrients to allow for the fuller expression of the genetic potential of these clones. There are several reports supporting this view (e.g., Smith *et al.* 1992).

Exogenous application of AM benefited most teak clones except APNPL-5. Further, the response of various physiomorphological characteristics to AM fungi inoculum was positive (albeit variable) in all 10 teak clones. Emergence of this trend was expected as AM inoculum indirectly ameliorated growth and physiological characteristics via ensuring augmented supply of water and nutrients, especially P, from the soil.

To conclude, the present study revealed (1) the importance of AM symbiosis in growth and water flux in teak and (2) the existence of separate compatibilities between AM fungus isolates and teak clones for root colonisation. Inoculation of half-sib seedlings of most teak clones with AM fungi is recommended for the production of sturdy seedlings to ensure better survival and performance in the field.

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