GROWTH, PHOTOSYNTHETIC COMPETENCE AND OLEO-GUM RESIN PRODUCTION OF GUGGAL (*COMMIPHORA WIGHTII*) ACROSS SOIL MOISTURE AND NITROGEN GRADIENT

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SAMANTA JN, SARAVANAN R, GAJBHIYE NA & MANDAL K. 2012. Growth, photosynthetic competence and oleo-gum resin production of guggal (*Commiphora wightii*) across soil moisture and nitrogen gradient. Guggal (*Commiphora wightii*) is a small tree, widely distributed in the forest of arid western India and Pakistan, which yields oleo-gum resin (guggul). The present study was an attempt to increase the growth rate of guggal with external inputs such as nitrogen and irrigation. Individual guggal rooted cuttings were grown in pots and supplemented with 17 or 34 mg kg^{-1} nitrogen through urea, potassium nitrate or glycine. However, nitrogen did not have significant effect on the plant growth rate, leaf chlorophyll, photosynthesis rate, and the yield and quality of guggal compared with control. Similarly, rooted cuttings were grown at five different soil moisture regimes (30–10%, at 5% intervals labelled as SM30–SM10). Plants at higher soil moisture depletion reacted by stomatal closure to reduce transpiration rate. Total leaf chlorophyll content drastically declined from 1.5 (SM30) to 0.5 mg g^{-1} (SM10). Consequently, plant growth rate was hampered significantly with depleting soil moisture. SM30 supported best growth rate as indicated by plant height and leaves per plant. Guggul production declined by 11–20% at SM15–SM10 compared with SM30 but its quality gradually improved with the increase in moisture deficit. Hence, soil moisture will be important in organised farming of this species.

Keywords: Burseraceae, drought stress, leaf gas exchange, macronutrient

SAMANTA JN, SARAVANAN R, GAJBHIYE NA & MANDAL K. 2012. Pertumbuhan, kecekapan fotosintesis dan penghasilan resin gam oleo guggal (*Commiphora wightii*) merentasi cerun kelembapan tanah serta nitrogen. Guggal (*Commiphora wightii*) merupakan pokok kecil yang tersebar luas di hutan di barat India yang gersang serta Pakistan dan menghasilkan resin gam oleo (guggul). Kajian ini merupakan cubaan untuk meningkatkan kadar pertumbuhan guggal dengan manipulasi luaran iaitu kandungan nitrogen serta pengairan. Keratan berakar guggal ditanam dalam pasu yang ditambah 17 mg kg^{-1} atau 34 mg kg^{-1} nitrogen dalam bentuk urea, kalium nitrat atau glisina. Bagaimanapun, nitrogen tidak memberi kesan signifikan terhadap kadar pertumbuhan pokok, klorofil daun, kadar fotosintesis dan hasil serta kualiti guggal berbanding dengan kawalan. Selain itu, keratan berakar ditanam pada lima kelembapan tanah (30%–10%, pada selang 5% dan dilabel SM30–SM10). Pokok yang ditanam pada kehilangan kelembapan yang lebih tinggi bertindak balas dengan menutup stoma untuk mengurangkan kadar transpirasi. Jumlah klorofil daun menurun dengan mendadak dari 1.5 mg g^{-1} (SM30) ke 0.5 (SM10). Akibatnya, kadar pertumbuhan pokok terbantut dengan signifikan apabila kelembapan tanah berkurangan. SM30 memberi kadar pertumbuhan terbaik dari segi ketinggian pokok serta bilangan daun setiap pokok. Penghasilan guggul menurun sebanyak 11% hingga 20% pada SM15–SM10 berbanding dengan SM30 tetapi kualitinya semakin bertambah baik apabila defisit kelembapan air bertambah. Justeru, kelembapan tanah penting dalam perladangan spesies ini.

INTRODUCTION

Drought-induced water deficit stress in plants is the result of complex relationship between soil, plant and environment. Plant undergoes several acclimation mechanisms for survival under such hostile environment. Reducing the leaf area is one of the most common responses by plants towards moisture deficit stress (Curran et al. 2010). This is achieved in many ways including producing smaller leaves, altering leaf shape and changing venation (Xu et al. 2009). Reduction in photosynthetic activity due to changes in photochemical reactions at various levels is also common under stress (Baquedano & Castillo 2006). Nitrogen is one of the major plant nutrients and contributes towards growth and yield of plants. Increased nitrogen availability

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helps plants to achieve higher leaf area per unit plant biomass (LAR) (Thompson et al. 1988) and strongly enhances foliar photosynthetic capacities and faster growth (Field & Mooney 1986).

Commiphora is a genus which is widely distributed in Africa and Asia. *Commiphora wightii* (guggal) is a small tree, 2–4 m high. In India, the species is endemic to the forest of arid western states, i.e. Gujarat and Rajasthan. Guggal is mostly found growing on rocky, hilly tracts and sloped terrains of some rivers. Apart from India, it is also found in Sind and Baluchistan in Pakistan. Upon tapping, the tree exudes an oleo-gum resin (guggul). In the Indian system of traditional medicine, Ayurveda, guggul is used in several preparations against obesity and rheumatism. Scientific investigations established its anti-inflammatory, anti-rheumatic and hypocholesterolemic properties. Guggulsterone [4,17(20)–pregnadiene–3,16–dione] is the active component of guggul and it lowers the LDL cholesterol level by antagonising farnesoid X receptor, a nuclear hormone receptor (Urizar et al. 2002). Products containing guggulsterones have worldwide demand and India has produced 51 t guggal from 1963–1964 (Atal et al. 1975). However, the natural plant stand has dwindled and it is now placed in the IUCN Red List of Threatened Species (www.iucnredlist.org). Slow growth rate of the species has been identified as one of the reasons for poor plant stand (Soni 2008).

In its natural stand, guggal plant experiences water deficit stress for a long period as rainy days are skewed for 1–2 months (between June and August) during the monsoon season. Similarly, degraded and shallow soils of the natural guggal stand remain poor in terms of major nutrients. The plant is well adapted to such hostile environment due to its slow growth rate and leaflessness during most part of the year. Obviously, these adaptations provide ecological significance even though guggul production is reduced under such circumstances. Two external inputs, namely, irrigation and nitrogen may induce faster growth of this endangered species. Increasing plant growth and guggul productivity under organised cultivation may help survival of this species by reducing overexploitation in natural plant stand. However, information on the soil moisture and nitrogen requirements for guggal and their effects on growth, physiology and economic yield are scarce. In the present study we have grown guggal plants at different soil moisture regimes and nitrogen levels with the objectives of measuring plant growth and different physiological parameters. Oleo-gum resin production, its quality and survival of plants after tapping were also measured.

MATERIALS AND METHODS

Plant growth conditions for irrigation experiments

An experiment was conducted to study the effect of soil moisture on guggul under a rain shadow area (prepared with transparent polythene sheet on top) from July till November 2009. The experiment was repeated once from August till December 2009 at the research farm of Directorate of Medicinal and Aromatic Plants Research (22° 35' N, 72° 56' E). Guggal hardwood stem cuttings (~30 cm long, ~10 mm diameter), one per pot, were directly planted in 2 L plastic pots each containing 2 kg field soil (Ustalfs suborder) in March 2009. The cuttings were rooted and raised with sufficient water till the start of different soil moisture treatments. The experiment was conducted following randomised complete block design (RCBD) with five treatments and each treatment was replicated four times and each replication consisted of three plants. Soil moisture levels (30, 25, 20, 15 and 10% labelled SM30, SM25, SM20, SM15 and SM10 respectively) were maintained gravimetrically by adding water every day. Morphological data were recorded at monthly intervals beginning from the first day of treatment till three months. At this time each plant was tapped by giving a semi-circular, bark-deep incision at about 5 cm above soil surface and smearing the cut surface with a suspension made from freshly harvested gum. Oozed, solidified oleo-gum resin was collected after a month and weighed to record the yield.

Plant growth conditions for nitrogen experiments

To study the effect of nutrient doses, an experiment was conducted from June till October 2010 under similar conditions mentioned above, unless mentioned otherwise. Soil used in the experiment was collected from an agricultural plot after growing the nutrient-exhaustive crop, sorghum. Initial available nitrogen in the soil was 96 mg kg^{-1} of soil. Plants were supplied with two concentrations of nitrogen, 17 and 34 mg kg^{-1} soil, in two equal splits, one at the beginning of the experiment and second, after two months of the first application. Nitrogen was applied separately in three different forms, namely, amide (urea), nitrate (potassium nitrate) and organic (glycine). Control pot received no nitrogen supplement. Experiment was conducted following RCBD, each treatment being replicated three times. This experiment was repeated from July till November 2010.

Photosynthesis measurement

Three months after initiation of treatments, photosynthetic parameters were recorded from fully expanded single leaf from every plant anytime between 10.30 a.m. and 12.30 p.m. Readings were taken with an open type infrared gas analyser at ambient CO_2 and relative humidity.

Leaf relative water content

Fully expanded mature leaves (2–3 per plant) were collected from all plants at three months after the start of treatments. Leaf discs (100 mg per replication) were collected from each treatment at the time of photosynthetic measurement between 11.00 a.m. and 12.30 p.m. Relative water content was measured following Barrs and Weatherley (1962).

Chlorophyll content estimation

After gas exchange observations, the same leaves were harvested and used for chlorophyll estimation. Leaf discs (100 mg per replication) were extracted for 1 hour in dimethyl–sulphoxide (DMSO) at 65 °C under darkness. The green solution was decanted, volume was made to 10 ml with DMSO and its absorbance was taken at 649 and 665 nm using a spectrophotometer. Chlorophyll content was estimated using Wellburn's (1994) equations.

Quality analysis of guggul

Guggul sample (1 g) was refluxed thrice in 100 mL of ethyl acetate in a water bath. Pooled extract was evaporated to dryness and redissolved in 20 mL HPLC grade methanol. The extract was then passed through a 0.2 μm filter and used for quantification using authentic guggulsterone-E and -Z standards in HPLC system fixed with an RP-18 column (250 mm × 4.6 mm, 5 μm), quaternary gradient pump, 600 system controller, 2669 PDA detector and Empower software. A mixture of methanol and water (65:35 v/v) was used as mobile phase at a flow rate of 1 mL min^{-1}. Absorbance of the compounds was monitored at 245 nm.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) following RCBD model. Means were compared with least significant difference ($p = 0.05$). Data from two repetitions of the experiment were analysed separately. Since results from both experiments showed similar trend, we reported the mean data of only one experiment in this paper.

RESULTS

Growth responses

Guggal plant growth was strongly but differentially influenced by varying soil moisture levels (Figure 1a). Height was greatest when soil moisture was at field capacity (SM30). Plant height decreased when soil moisture level decreased and was lowest at SM15 and SM10. Plant heights at these two moisture levels were less than ⅓ that of the highest moisture level.

Leaf senescence and abscission increased with increased soil moisture depletion. It was also associated with less production of new leaves. Hence, soil moisture had significant influence on the number of leaves produced per plant (Figure 1b). Leaf number had similar trend as plant height but SM30 and SM25 in the former did not vary significantly ($p = 0.05$). Plants at SM30 had maximum number of leaves at all sampling intervals whereas SM15 and SM10, the least. Three months after treatment, SM25, SM20, SM15 and SM10 had 4.3, 40.2, 90.0 and 91.0% decrease in leaf numbers respectively, compared with SM30.

Applied nitrogen failed to produce any significant difference in plant growth compared with control. One month after the first application, control plants showed 31.1% increase in height while the rest of the plants had values between 31.7 and 37.1% (results not shown). After 2 months

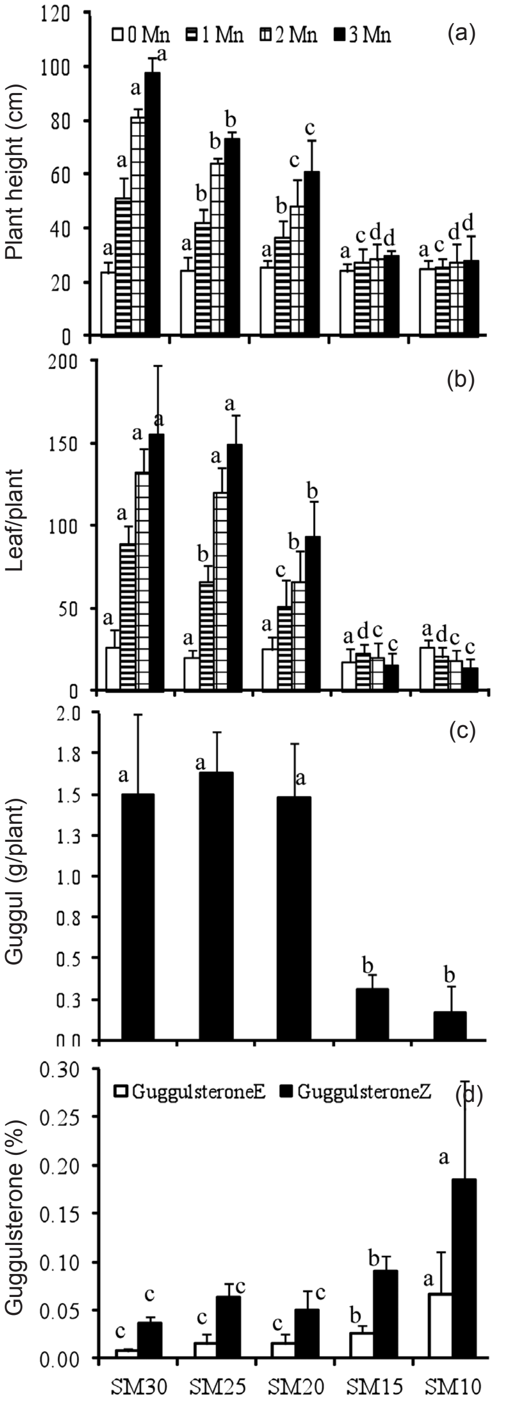

Figure 1 (a) Plant height, (b) leaf number, (c) guggul yield and (d) guggulsterone contents of guggal rooted cuttings under different soil moisture levels (SM30–SM10) at 0–3 months (Mn) after beginning of treatment applications; error bars represent standard deviation, data points of similar time topped by the same letter indicate no significant difference, LSD (p = 0.05) values for plant height = 9.3, 10.2 and 10.2 and leaves = 15.8, 15.0 and 35.3 at 1, 2 and 3 months respectively, LSD for guggul yield, guggulsterone-E and -Z are 0.5, 0.01 and 0.03 respectively

of growth, monthly increments in plant height in treated pots were 54.4–67.5% whereas that of control, 60.1%. At the end of 3 months, height of control plants increased 39.4% compared with beginning of the experiment while treated plants showed 36.3–42.0% increase. Similarly, other growth parameters such as number of branches and number of leaves per plant did not vary significantly between treatments.

Guggul yield and quality

Water deficit stress reduced growth and guggul yield (Figure 1c). Guggul yield was significantly (p = 0.05) affected when soil moisture level was below 20%. Gum yield from plants grown at SM30, SM25 and SM20 was similar. At higher moisture depletion, i.e. SM10 and SM15, plants produced only 11–20% gum compared with SM30. However, none of the plants survived after tapping although survival period was prolonged with higher soil moisture (data not shown).

Guggulsterone contents in guggul samples showed a different trend than that of guggul yield (Figure 1d). Guggulsterone-E and -Z concentrations increased with increasing water deficit stress—highest guggulsterones were accumulated in plants at SM10 followed by SM15. Since guggulstreone is the active constituent of guggul, any increase in its content indicates positive influence of this abiotic stress on the synthesis of guggulsterone and improvement in quality of guggul, as shown by higher active constituents. Nitrogen application had no significant effect on guggul yield and it did not influence the quality of gum in terms of phytochemical constituents.

Plant water status

Plant water status was monitored by measuring relative water content. At the end of the 3-month experiment, plants from SM30–SM20 had similar relative water content which was significantly (p = 0.05) higher compared with plants from SM15–SM10 (Figure 2).

Chlorophyll content

Leaf chlorophyll a and b contents significantly decreased in stressed plants at the end of the treatment period (Figure 3). Nevertheless, the reduction was greater in chlorophyll a compared with chlorophyll b. The ratio of chlorophyll a to

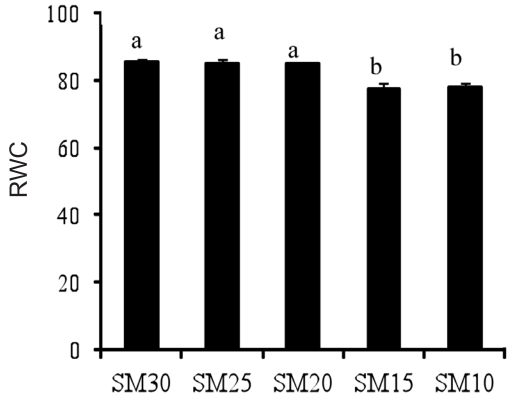

Figure 2 Leaf relative water content (RWC) of guggal rooted cuttings under different soil moisture levels (SM30–SM10) measured 3 months after beginning of treatment applications; error bars represent standard deviation, bars with same letter indicate no significant difference, LSD = 1.8 (p = 0.05)

b also decreased under water deficit conditions from 4.4 to 2.5 in SM30 and SM10 respectively.

In the nitrogen experiment, leaf chlorophyll content did not vary between treatments. Total leaf chlorophyll was within the range of 0.9–1.0 mg g^{-1} between different treatments (results not shown).

Gas exchange parameters

Net photosyshetic rate (P_n) significantly (p = 0.05) declined at lower soil moisture levels starting from SM20. The net photosyshetic rate reduction values in SM20, SM15 and SM10 were 61.7, 84.7 and 96.7% respectively compared with SM30 (Figure 4a). Plants at SM10 were hardly able to maintain any net carbon gain. Reduction in net photosyshetic rate was associated with lower stomatal conductance (g_s). At higher water deficit stress (SM15 and SM10), stomatal conductance was the least and was reduced by 80% compared with well watered plants (Figure 4b). The sharp decline in leaf g_s and transpiration rate (E) under moisture stress implied stomatal response to prevent excess water loss from canopy. These two parameters shared significant correlation (r = 0.94). However, internal CO_2 concentration (C_i) declined by 20 and 30% in SM15 and SM10 plants compared with SM30. Leaf temperature was also higher in the stressed plants (SM15 and SM10) compared with control (data not shown).

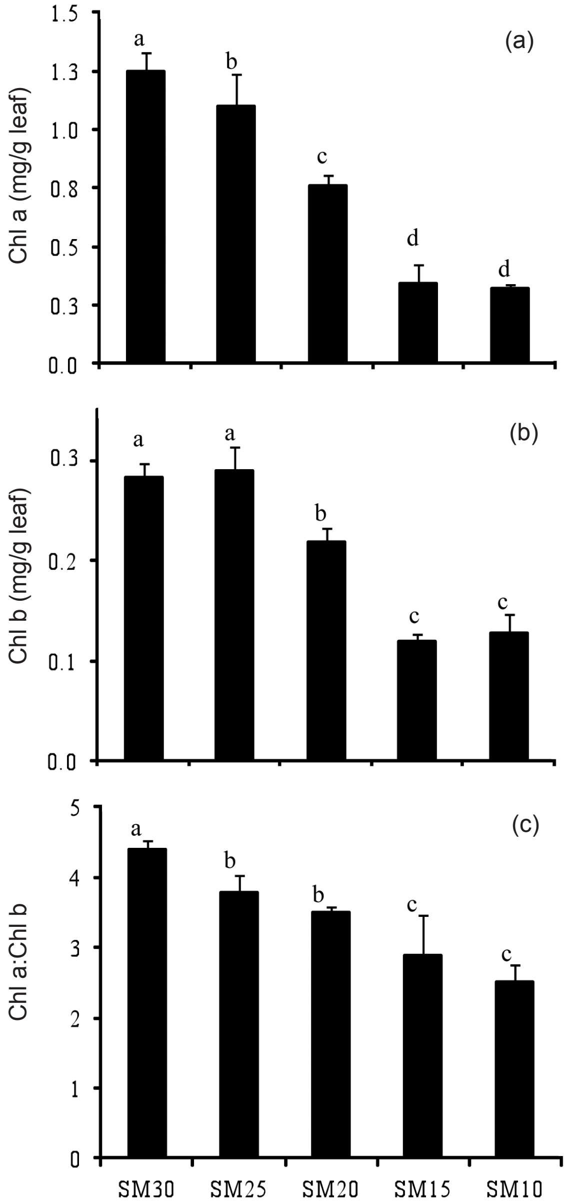

Figure 3 (a) Chlorophyll a (chl a), (b) chl b and (c) chl a:chl b measured at fully expanded leaves of guggal rooted cuttings under different soil moisture levels (SM30–SM10) 3 months after beginning of treatment applications; error bars represent standard deviation, bars with same letter indicate no significant difference, LSD (p = 0.05) values for chl a, chl b, chl a:chl b are 0.1, 0.01 and 0.4 respectively

Nitrogen sources and their concentrations did not have significant influence on net photosynthesis of the guggal leaves.

DISCUSSION

The main aim of the present study was to find a practical method to increase growth rate

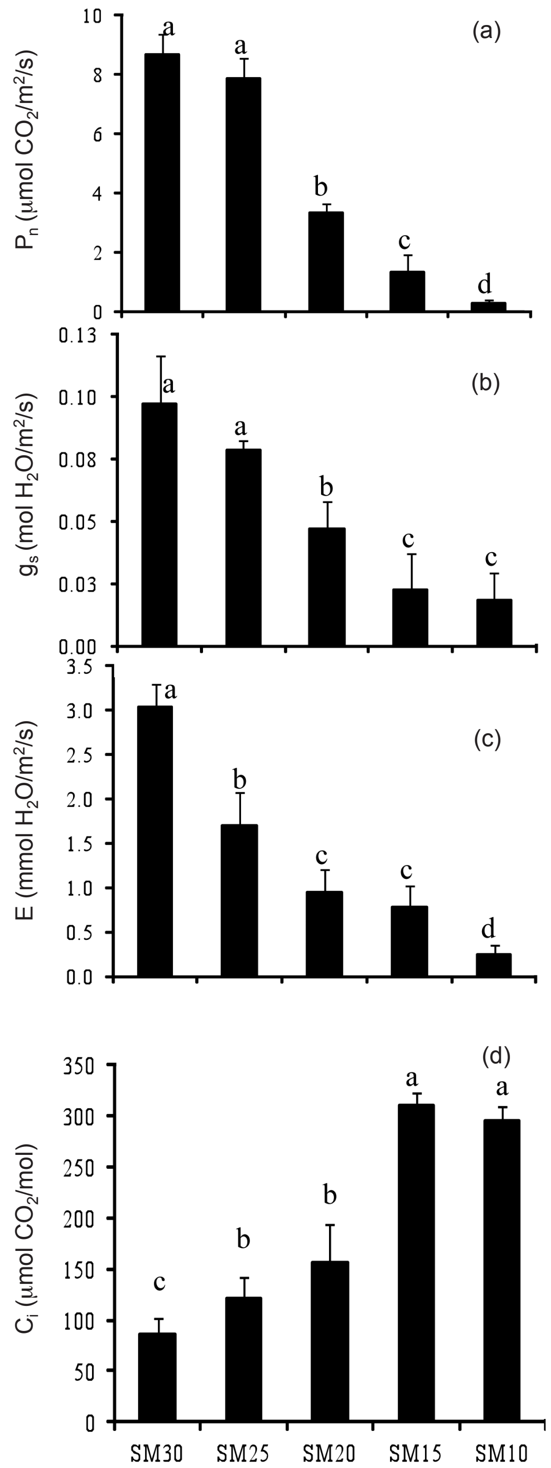

Figure 4 (a) Net photosynthetic rate (P_n), (b) stomatal conductance (g_s), (c) transpiration rate (E) and (d) internal CO_2 concentration (C_i) of guggal rooted cuttings under different soil moisture levels (SM30–SM10) measured 3 months after beginning of treatment applications; error bars represent standard deviation, bars with same letter indicate no significant difference, LSD ($p = 0.05$) values for P_n, g_s, E and C_i are 0.9, 0.02, 0.3, 0.6 and 36.7 respectively

of guggal. Such method would be useful in accelerating growth and shortening tapping age of plants, thereby attracting farmers to adopt organised cultivation. However, between two external inputs tested for this purpose, nitrogen application failed to produce any significant growth or yield benefit for guggal. Wild plants mostly remain non-responsive to nitrogen and long-term breeding is done to introduce beneficial gene(s) to develop high yielding cultivars. Guggal being a primarily forest species and grows naturally on degraded soil, lacks high nitrogen-utilisation trait. Therefore, it could be concluded that ecological advantage for faster canopy growth under increased nutrient availability was apparently lacking in dry arid regions where guggul normally grew.

In contrary, response of guggal to limited water supply in terms of reduction in plant height, leaf initiation and development was evident. Water deficit stress has differential influence on above- and underground growth of plants and profound negative effect on shoot growth (Xu et al. 2009, Álvarez et al. 2011). In this study, only aboveground observation was conducted because guggal gum ducts were located in the bark tissues. Our results showed that there was significant reduction in plant height and leaf number due to reduced leaf initiation and leaf abscission under severe water stress. Sequential leaf senescence, beginning with the oldest leaf, contributes in supplying nutrients to plants and minimises total leaf area to reduce transpiration loss (Bernstein et al. 1993).

Leaf relative water content did not show such steep decline; it was maintained at 77% in SM10 even with prolonged stress. Plants growing in arid regions are able to reduce water loss by producing epicuticular wax coating on leaves (Gonzalez & Ayerbe 2010). We have observed that under natural stand, guggal produced different leaf morphotypes, thinner and thicker, depending upon high (monsoon) and low (post-monsoon) moisture availability (data not shown).

Although net photosyshetic rate decreased as g_s decreased, the former was not limited by C_i, as indicated by elevated C_i in stressed plants (Figure 4d). Under severe stress, both stomatal and non-stomatal limitations of photosynthesis have been reported and photosynthesis can be inhibited even when the stomatal influence is eliminated by removing epidermis from leaf discs (Tang et al. 2002). Hence at the highest stress intensity, the causes of photosynthesis reduction were impairments in biochemical (altered gas exchange parameters) and photochemical reactions. Non-stomatal factors, such as loss of chlorophyll, turgor and damages to photosynthetic machinery were major reasons for lower net photosyshetic rate under our experimental conditions. It has been reported that photosynthesis (under light- and CO_2-saturated conditions) and quantum yield in the drought resistant *Nerium oleander* (Bjorkman & Powles 1984) and other species (Vitale et al. 2012) reduced in response to water deficit stress under natural conditions.

The potential for oleo-gum resin production is dependent on physiological conditions of the tree. Oleoresinosis in pine and fir stems is restricted by substrate and/or energy availability (Lorio 1988)—a limitation expected under prolonged drought. Water stress diminishes the ability of guggal tree to produce oleo-gum resin. Our results contradicted an earlier report that guggal oleo-gum resin production was enhanced under stress conditions and ceased at high moisture levels (Atal et al. 1975). However, we observed a reverse trend for quality of the product. As secondary metabolites, increase in the concentration of guggulsterones is expected under stress (Zobayed et al. 2007). The present study indicated that higher guggal growth rate and oleo-gum resin yield could be achieved by maintaining soil moisture levels between field capacity and 20%. This can be particularly useful in organised cultivation of the species for commercial guggul farming. However, to ensure better quality of the product, application of water deficit stress through deficit irrigation would be necessary. Future studies should aim at trade-off between these factors to optimise timing, frequency, degree and duration of abundant- and deficit-moisture periods in growing this plant so as to produce guggul with high medicinal properties.

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