

BRIEF COMMUNICATION

Leaf surface wetness and morphological characteristics of *Valeriana jatamansi* grown under open and shade habitats

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Abstract

The present study was carried out to investigate the degree of leaf wetness and its capacity to retain water droplets in relation to leaf morphological characteristics of *Valeriana jatamansi* J. grown under open and shade habitats. Leaves developed in open habitats had less wettability but higher capacity to retain water droplets and more number of stomata than shade leaves. A significant positive correlation of contact angle (θ) were noticed with trichome length, droplet retention and wax content.

Additional key words: leaf morphology, trichome, wax.

The physiological significance of leaf wetness stems from the fact that CO₂ diffuses about 10 000 times more slowly through a film of water than through air (Nobel 1991). Evidence suggests that photosynthesis in many species may be much reduced because of wet leaf surfaces (Brewer and Smith 1994, 1995). Excess leaf wetness may promote pathogen infection and reduces transpiration of many plant species (Reynolds *et al.* 1989, Evans *et al.* 1992). Leaching of foliar nutrients, pollutant deposition and stomatal occlusion of leaves are also affected by leaf wetness (Cape 1996, Massman *et al.* 1994). Plant species show a broad range of leaf wettability from being covered by a film of water to being completely water repellent (Smith and McClean 1989).

Valeriana jatamansi J. is an important medicinal and aromatic herb found as an understorey of forest area in the temperate Himalayas from 1500 to 3500 m above msl (Pandey and Shukla 1993). The purpose of this study was to evaluate the potential importance of the formation and retention of water droplets as well as of other leaf characteristics of *V. jatamansi* grown under open and shade habitats.

Five months old nursery seedlings of *V. jatamansi* (4 - 5 cm length) were planted during November 1998 at a distance of 40 × 40 cm on ridges in open field (full sunlight) and under shade of nylon net (50 % irradiance) placed about 2 m above ground in the Institute's Biodiversity Experimental Farm at Palampur (1300 m above msl, 32°6' N; 76°33' E). All the observations were taken during October 1999 when plants were about one year old.

From November 1998 to October 1999 daily maximum and minimum temperature were 24.60 °C, 14.35 °C, rainfall 2150 mm, mean relative humidity 54.75 % and wind velocity 4.69 km h⁻¹.

Measurements made on both adaxial and abaxial leaf surfaces included: trichome density, trichome length, water droplet retention, contact angles of water droplets on the leaf surface, stomatal density, guard cell and pore length, and epicuticular wax content. All the measurements were made on ten randomly selected healthy leaves from 5 different plants per habitat with three replications per leaf.

Trichome density was calculated by counting the

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number of trichomes with the help of a haemocytometer (1×1 mm). Trichome length was measured using epidermal peels from both adaxial and abaxial surfaces using ocular micrometer. Stomatal density, guard cell and pore length were estimated from surface impressions (clear enamel nail polish) with a care to avoid veins (Meidner and Mansfield 1968).

Droplet retention (tendency to retain water on leaf surface) was determined by placing a 50 mm^3 droplet of water on a horizontal leaf surface and then measuring the angle of leaf inclination at which the droplet first began to move. Low angular values ($< 20^\circ$) indicate poor water retention.

The degree of water repellency of the leaf surface was determined by measuring the contact angle (θ) of a 2 mm^3 water droplet placed by micropipette on each leaf disc mounted on glass slides using double sided tape. The angle (θ) of a line tangent to the droplet through the point of contact between the droplet and the leaf surface was measured according to Brewer *et al.* (1991). The criteria for judging surface wettability were based on those of Crisp (1963), where $\theta < 110^\circ$ was considered a wettable surface while $\theta > 130^\circ$ was non-wettable.

Epicuticular wax content was estimated according to Barnes *et al.* (1996). Leaf discs of 10-mm diameter were taken for wax estimation. Both surfaces of leaf discs (0.785 cm^2 each) were washed separately with 10 cm^3

chloroform (HPLC grade) dispensed from a burette taking care not to contaminate the waxes extracted from one surface with another. Samples were filtered through pre-rinsed *Whatman No. 1* filter paper and a $0.2 \mu\text{m}$ *Sartorius* filter into pre-weighed evaporating flask. The solvent was reduced under vacuum below 40°C before drying to constant mass at room temperature.

Differences in angular value, contact angle, trichome density and length, stomatal density, guard cell and pore length and epicuticular wax content between the two habitats were statistically analysed employing two factor complete randomized design.

Adaxial and abaxial leaf surface characteristics of *Valeriana* plants grown in open and shade habitats showed significant differences for contact angle (θ), wax content and stomatal density (Table 1). In both the habitats adaxial surface had less wettability and stomatal density and more wax content than the abaxial surface. No significant differences were noticed in any other leaf surface characteristics (Table 1).

When leaves developed under open and shade habitats were compared (Table 2), significant differences were observed in contact angle, retention degree, trichome length, wax content and stomatal density. Leaves developed in open had less wettability, higher water holding capacity, larger trichomes, more wax and stomatal density than the leaves developed in shade

Table 1. Morphological differences in adaxial and abaxial characteristics of *Valeriana jatamansi* leaves grown under open and shade habitats. Different letters in superscript following the values indicate statistically significant differences at $P < 0.05$. Means \pm SE, $n = 30$.

Leaf surface characteristics	Open adaxial	abaxial	Shade adaxial	abaxial
Contact angle [θ]	97.20 ± 1.58^a	81.20 ± 1.72^{bc}	84.00 ± 1.76^b	75.00 ± 0.67^d
Retention degrees	19.30 ± 2.89	16.10 ± 4.20	14.20 ± 2.87	13.50 ± 1.76
Trichome density [mm^{-2}]	5.80 ± 0.24	6.00 ± 0.43	5.60 ± 0.35	5.20 ± 0.36
Trichome length [μm]	416.00 ± 11.94	343.00 ± 17.44	294.40 ± 2.89	293.20 ± 2.31
Wax content [mg dm^{-2}]	1.10 ± 0.03^a	0.68 ± 0.02^b	0.56 ± 0.04^{bc}	0.38 ± 0.03^d
Stomatal density [mm^{-2}]	67.40 ± 4.74^b	163.20 ± 7.57^a	49.60 ± 3.66^{bc}	59.00 ± 6.87^{bc}
Guard cell length [μm]	32.50 ± 0.79	29.00 ± 1.00	32.00 ± 0.94	31.00 ± 0.61
Stomatal pore length [μm]	15.50 ± 0.50	15.50 ± 0.50	17.00 ± 0.94	16.00 ± 0.61

Table 2. Leaf characteristics of open and shade habitats of *V. jatamansi* leaves. Different letters in superscript, following the values in columns, indicate statistically significant differences at $P < 0.05$. Means \pm SE, $n = 60$.

Habitats	Contact angle [θ]	Retention degrees	Trichome density [mm^{-2}]	Trichome length [μm]	Wax content [mg dm^{-2}]	Stomatal density [mm^{-2}]	Guard cell length [μm]	Stomatal pore length [μm]
Sun	89.20^a ± 2.90	17.70^a ± 0.61	5.90 ± 0.23	379.50^a ± 18.31	0.89^a ± 0.12	115.30^a ± 16.72	30.75 ± 0.84	15.50 ± 0.33
Shade	79.50^b ± 1.64	13.85^b ± 0.60	5.40 ± 0.43	293.80^b ± 13.06	0.47^b ± 0.06	54.30^b ± 3.58	31.50 ± 0.55	16.50 ± 0.18

habitat (Table 2).

Contact angle (θ) was found to be positively correlated with wax content (Spearman $r_s = 0.90$; $P < 0.01$), trichome length ($r_s = 0.65$; $P < 0.01$) and droplet retention ($r_s = 0.68$; $P < 0.01$). No significant relationships of θ were found with other leaf surface characteristics.

Many common native and agricultural plant species possess a unique leaf surface characteristics that minimise the amount of surface area in contact with water. In the present study, adaxial and abaxial morphological characteristics of *V. jatamansi* leaves varied significantly within the two habitats of open and shade (Table 1). In both the habitats, the adaxial surface had less wettability (higher θ) and stomatal density but higher wax content than abaxial surface. Higher values of θ and retention degrees (Table 2) alongwith higher photosynthetic rates (unpublished data) of *V. jatamansi* leaves grown under open habitat may be an adaptive feature of such leaves which may lead to enhanced water use efficiency by reducing transpiration as well as increasing photosynthesis (Smith and McClean 1989).

A lower stomatal density and trichome length in shade as compared to open habitat (Table 2) may be due to larger area of leaf in such light limiting environment to maximise photon absorption. Like in other species, the presence of trichomes had a particular strong influence on the formation, repulsion and location of surface water. Besides, they also limit interference of surface moisture with photosynthetic gas exchange by repelling moisture away from epidermis and consequently stomatal pores

(Brewer and Smith 1997). In addition to their well known influence on leaf temperature and light reflection (Brewer *et al.* 1991), trichomes may also represent morphological adaptations to leaf wetting.

No significant differences were noticed in guard cell length and stomatal pore length of the leaves developed under open and shade habitats and this pattern is consistently reported in the literature (Abrams and Kubiske 1990). Higher epicuticular wax content was obtained in the open habitat leaves than shade ones (Tables 2). In shade and sun leaves of ivy (*Hedera helix* L.) the latter had the higher wax content than the former (Hauke and Schreiber 1998). The aerial surfaces of all higher plants develop a layer of epicuticular wax on the outermost surface of cuticle, which alongwith other structures like trichomes form the first line of defence against external influences like air pollutants, high irradiation and attack by pest and pathogens (Percy *et al.* 1994).

The findings of this study and many others (Brewer *et al.* 1991, Ishibashi and Terashima 1995) indicate broad pattern of leaf surface wetness and morphological characters in a given habitat and in individual leaf surfaces. Although surface wetness may have additional important consequences, for example in pathogen infection, the effect of gas exchange has important implications for plant growth. In addition to this, the results reported here could contribute to increasing efficacy of spray chemicals and to our better understanding of the physiological effects of atmospherically deposited pollutants.

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