Photosynthetic Performance and its Relationship with Leaf Water Potential in Desmodium gangeticum L. DC. (Shalaparni) Genotypes under Field Conditions

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Abstract Desmodium gangeticum L. DC. (Family - Fabaceae) is a medicinal legume that grows in dry hilly areas and dried roots are used as general tonic and aphrodisiac. It has a calming, sedative effect and is also used to control inflammation, fever and neurological imbalances. Genetic variability for leaf water potential and photosynthetic activities which attributes for biomass was assessed in 31 accessions collected from various agro climatic zones of India. The accessions were screened for three gas exchange parameters viz., photosynthetic rate ($P_N$), stomatal conductance ($g_s$) and transpiration rate ($E$) during active photosynthetic period and leaf water potential ($\psi_l$) during pre-dawn, noon and dusk. Plant biomass was recorded for the accessions during harvest. There were considerable variations in leaf water potential of genotypes during pre-dawn and noon. The genotypes differed significantly for the gas exchange parameters studied. High positive correlation was observed between $P_N$ and $g_s$ ($R^2 = 0.68$) with $E$ ($R^2 = 0.75$) and significant negative correlation with noon $\psi_l$ ($-0.26$). The genotypes were categorized into 2 main clusters namely, A and B with 4 sub clusters. Cluster A contained 13 genotypes of which 6 had higher photosynthetic performance with superior water relations and cluster B contained 18 genotypes of which 3 accessions (DDG 3, DDG 22 and DDG 33) formed a sub group and exhibited poor photosynthetic performance. The positive correlation of $g_s$ and $E$ with photosynthesis can be helpful for improvement of $D. gangeticum$.

Keywords Desmodium gangeticum; Gas exchange parameters; Water relations; Field performance

Introduction Tick clover (also known as tick-trefoil, Desmodium gangeticum and shalaparni) belonging to the family Fabaceae, is an important herb used in many Indian Ayurvedic medicines. This species is distributed throughout Indian subcontinent and hence considerable genetic diversity exists due to wide adaptation and naturalization in various agro-climatic regions. The herb contains bioactive compounds like indol-3-alkyl-amines, gangetin (a pterocarpan), flavonoids, isoflavonoid glycosides, and aminoglucosyl glycerolipid (Mishra et al., 2005). The dried shoot and roots are the constituents of many ayurvedic formulations such as ‘Dasamoola’, ‘Chyavanaprasam’ and ‘Dhanvantharamtailam’. It is an effective tonic with diuretic and laxative actions used for curing cardiovascular ailments. The herb is utilized in preparation of a nerve tonic (Kirtikar and Basu, 1975) and preparations of the roots are used as antipyrethic, expectorant, and have alternative and diuretic actions. The alkaloids from aerial parts have hypotensive and anticholinesterase activity and act as stimulant of central nervous system (Iwu et al., 1992). It is also forms a major constituent in many patented herbal preparations (Dayand et al., 2010) used for pharmaceutical purposes. D. gangeticum is also utilized for controlling weeds and erosion in sandy areas.

In spite of growing importance as a traditional and alternative medicine, little efforts are undertaken for commercial cultivation of D. gangeticum. Germplasm were collected from various parts of India to conserve, study and exploit the existing variability for crop improvement (NAIP Final Report, 2012 - National Agriculture Innovation Project, 2008-2012). Little information is available about physiological processes and plant water relations contributing to biomass and root yield of these new genotypes. Such information may be helpful for genetic improvement of this species for high biomass.

Photosynthesis is the primary source of dry matter accumulation (~ 90%) and yield in plants (Zelicht, 1982). Photosynthetic processes are regulated by plants in response to various internal and external...
stimuli. Any such stimulus that substantially alters the net rate of photosynthesis has a potential to alter the growth of plants (Pettigrew, 2004). Genetic variability in photosynthesis was well studied in crops such as maize (Heichel and Musgrave, 1969), wheat (Watanabe et al., 1997), cotton (Pettigrew and Turley, 1998), soybean (Wiebold et al., 1981), pea (Mahon and Hobbs, 1981), strawberry (Hancock et al., 1989), chickpea (Anilkumar et al., 1993), bean (Gonzáles et al., 1995) and papaya (Netto et al., 2009). In addition to the photosynthetic rate, stomatal conductance and transpiration rate are important physiological processes which respond rapidly and sustainability to the changes in soil water potential (Munns et al., 2010) and plant water status. Adaptations to overcome the limitations to photosynthesis by genotypes may provide ability to produce higher biomass even under adverse environmental conditions. Thus, it becomes crucial to identify the genetic components that contribute to overcome the limitations of the photosynthetic rates and use them in breeding programs to maximize the yield and productivity. To facilitate the breeding of *D. gangeticum*, we investigated the genetic variability for leaf water potential and photosynthetic performance of thirty one *D. gangeticum* genotypes collected from different parts of India.

1 Results

Photosynthetic performances and leaf water potential of thirty one genotypes of *D. gangeticum*, collected from different agro-ecological regions of India and mainatained at DMAFPR, Anand, Gujarat, India were studied. These genotypes, originally naturalized in their natural habitats, were raised in the research farm of ICAR-DMAFPR with proper cultural conditions. The parameters such as net photosynthetic rate (P<sub>n</sub>), stomatal conductance (g<sub>s</sub>) and leaf transpiration rate (T) of the genotypes are presented in Table 1. The 31 genotypes of *D. gangeticum* differed significantly for the gas exchange parameters studied.

1.1 Net photosynthesis rate

The mean photosynthetic (P<sub>n</sub>) rate of 31 genotypes was 18.24 μmol CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup> with the highest P<sub>n</sub> of 33.55 ± 0.9 μmol CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup> recorded for DDG 11 (Figure 1). The genotype DDG 22 performed poorly with lowest P<sub>n</sub> of 8.63 ± 1.9 μmol CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>. Genotypes DDG 28, DDG 8 and DDG 34 had significantly higher P<sub>n</sub> compared to other genotypes studied.

1.2 Stomatal conductance

Similar to P<sub>n</sub>, stomatal conductance also significantly varied among the genotypes studied. The mean g<sub>s</sub> of *D. gangeticum* genotypes was 0.154 mmol H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup> with DDG 8 having the maximum g<sub>s</sub> of 0.198 ± 0.015 mmol H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup> (Figure 2). As a related parameter to P<sub>n</sub>, the g<sub>s</sub> were lowest in DDG 22 with 0.10 ± 0.05 mmol H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>.

1.3 Leaf transpiration rate

Leaf transpiration rate is an important gas exchange parameter which directly relates to the plant water status. The average transpiration rate was found to be 7.73 mmol H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>. Akin to P<sub>n</sub> and g<sub>s</sub>, leaf transpiration rate of DDG 11 and 8 were significantly higher with 9.60 ± 0.44 and 9.39 ± 0.48 mmol H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup> respectively (Figure 3). Whereas, DDG 3 and 22 showed lower transpiration rate compared other genotypes.

### Table 1 Leaf water potential in different accessions of *D. gangeticum*

| Name of accession | (Pre-dawn) MPa | (Noon) MPa | (Dusk) MPa |
|------------------|---------------|------------|------------|
| DDG 1            | -0.62         | -1.34      | -0.69      |
| DDG 2            | -0.54         | -1.95      | -0.58      |
| DDG 3            | -0.18         | -1.51      | -0.67      |
| DDG 4            | -0.36         | -1.72      | -0.51      |
| DDG 5            | -0.82         | -1.78      | -0.72      |
| DDG 6            | -0.88         | -2.33      | -0.62      |
| DDG 7            | -0.50         | -1.88      | -0.44      |
| DDG 8            | -0.30         | -2.15      | -0.45      |
| DDG 9            | -0.38         | -2.03      | -0.67      |
| DDG 10           | -0.31         | -1.39      | -0.34      |
| DDG 11           | -0.23         | -1.82      | -0.51      |
| DDG 12           | -0.35         | -2.13      | -0.49      |
| DDG 13           | -0.38         | -1.71      | -0.35      |
| DDG 14           | -0.25         | -1.71      | -0.35      |
| DDG 15           | -0.30         | -1.93      | -0.42      |
| DDG 17           | -0.44         | -1.59      | -0.46      |
| DDG 19           | -0.35         | -2.21      | -0.36      |
| DDG 20           | -0.43         | -2.17      | -0.31      |
| DDG 21           | -0.25         | -2.03      | -0.44      |
| DDG 22           | -0.39         | -2.01      | -0.53      |
| DDG 23           | -0.30         | -2.05      | -0.45      |
| DDG 25           | -0.52         | -2.05      | -0.35      |
| DDG 26           | -0.40         | -1.70      | -0.36      |
| DDG 27           | -0.38         | -1.93      | -0.43      |
| DDG 28           | -0.33         | -2.20      | -0.48      |
| DDG 29           | -0.18         | -2.09      | -0.43      |
| DDG 30           | -0.32         | -1.99      | -0.42      |
| DDG 31           | -0.37         | -2.01      | -0.52      |
| DDG 32           | -0.35         | -2.07      | -0.51      |
| DDG 33           | -0.27         | -1.36      | -0.45      |
| DDG 34           | -0.41         | -2.05      | -0.43      |
| Mean             | -0.39         | -1.90      | -0.47      |
| Std. Dev.        | 0.15          | 0.26       | 0.10       |
The iWUE varied for the genotypes studied (Figure 4) with DDG 11 showing highest iWUE value (179.1 µmol CO$_2$/mmol H$_2$O m$^{-2}$ min$^{-1}$) followed by DDG 28 (162.3 µmol CO$_2$/mmol H$_2$O m$^{-2}$ min$^{-1}$). These genotypes expressed higher net carbon assimilation per unit water transpired under non-stress conditions.
1.5 Leaf water potential (Ψ<sub>L</sub>)

Leaf water potential was studied for 31 genotypes of <i>D. gangeticum</i> (L.) DC at three different time of the day as described above and the results are presented in the Table 1. The Ψ<sub>L</sub> was highest during dawn period and ranged between -0.18 MPa (DDG 29) and -0.88 MPa (DDG 6). Due to active photosynthesis and transpirational loss, Ψ<sub>L</sub> declined during noon and lower compared to Ψ<sub>L</sub> of both, dawn and dusk. During noon it ranged between -1.34 MPa (DDG 1) and -2.33 MPa (DDG 6). The water potential (Ψ) recovered during dusk and ranged between -0.31 and -0.31 MPa. The mean water potential was -0.39 MPa during the dawn period. The water potential of these plants during noon increased to -1.90 MPa and during the dusk period, water potential again fell to -0.47 MPa.

1.6 Correlation of gas exchange and leaf water potential and clustering of genotypes

The Pearson correlation coefficient between the gas exchange and leaf water potential for the 31 genotypes were calculated and presented in Table 2. Net photosynthetic rate (P<sub>n</sub>) had high positive correlation with g<sub>s</sub> (R<sup>2</sup> = 0.68) and T (R<sup>2</sup> = 0.75) with significant negative correlation with noon Ψ<sub>L</sub> (-0.26). Similarly, leaf transpiration rate (T) showed significant negative correlation with noon Ψ<sub>L</sub> (-0.36). Significant and positive correlation between leaf and stem biomass (R<sup>2</sup> = 0.76), leaf and root biomass (R<sup>2</sup> = 0.71), leaf and total plant biomass (R<sup>2</sup> = 0.75), stem and root biomass (R<sup>2</sup> = 0.82), stem and total biomass (R<sup>2</sup> = 0.96) as well as root and total biomass (R<sup>2</sup> = 0.85) in <i>D. gangeticum</i> genotypes studied.

Table 2: Pearson correlation matrix of gas exchange parameters with leaf water potential of <i>D. gangeticum</i> genotypes

|                         | Leaf conductance | Photosynthetic Rate (P<sub>n</sub>) | Transpiration rate | Pre-dawn WP | Dusk WP | Noon WP | Leaf biomass | Stem biomass | Root biomass | Total biomass |
|-------------------------|-----------------|-----------------------------------|-------------------|-------------|--------|---------|-------------|--------------|--------------|---------------|
| Leaf Conductance (g<sub>s</sub>) | 1.00            |                                   |                   |             |        |         |             |              |              |               |
| Photosynthetic Rate (P<sub>n</sub>) | 0.68*          | 1.00                              |                   |             |        |         |             |              |              |               |
| Transpiration rate       | 0.97*          | 0.75**                            | 1.00              |             |        |         |             |              |              |               |
| Pre-dawn WP             | 0.03            | 0.02                              | 0.09              | 1.00        |        |         |             |              |              |               |
| Dusk WP                 | 0.18            | 0.07                              | 0.27*             | 0.44*       | 1.00   |         |             |              |              |               |
| Noon WP                 | -0.28*          | -0.26*                            | -0.36*            | 0.11        | -0.11  | 1.00    |             |              |              |               |
| Leaf biomass            | -0.12           | 0.03                              | -0.16             | 0.09        | -0.28  | 0.14    | 1.00        |              |              |               |
| Stem biomass            | -0.04           | 0.09                              | -0.03             | 0.04        | -0.18  | 0.05    | 0.76**      | 1.00        |              |               |
| Root biomass            | -0.09           | 0.00                              | -0.13             | -0.10       | -0.27  | 0.11    | 0.71**      | 0.82**      | 1.00        |               |
| Total biomass           | -0.08           | 0.06                              | -0.09             | 0.06        | -0.24  | 0.10    | 0.91**      | 0.96**      | 0.85**      | 1.00         |

Note: * significant at p=0.05, ** significant at p=0.01
Correlation matrix between biomass of different plant parts is depicted in Figure 5. The correlation between leaf and stem biomass, stem and root biomass as well as leaf and root biomass were positive and significant ($P=0.01$) in the *D. gangeticum* genotypes studied. Root biomass is an important parameter as it is a constituent of many medicinal preparations. The selection for higher leaf biomass or stem biomass is an indirect selection tool for higher root biomass and it will enable the breeder to enhance the chances of getting more aerial biomass without compromising the below ground biomass.

![Figure 5 Correlation matrix of leaf, stem and root biomass in different *D. gangeticum* genotypes](image)

The cluster analysis clustered 31 genotypes of *D. gangeticum* into 2 main clusters namely - A and B with 4 sub clusters (Figure 6). Cluster A contained 13 genotypes of which 6 had higher photosynthetic performance with superior water relations. Whereas, cluster B contained 18 genotypes of which 3 genotypes, DDG 3, DDG 22 and DDG 33 formed a sub group and showed poor photosynthetic performance. Cluster A, sub-cluster I contained 5 genotypes DDG 8, DDG 14, DDG 17, DDG 11 and DDG 28 having above average $P_n$, $g_s$ and $T$ activities. In cluster B, DDG 2, DDG 27, DDG 34, DDG 31 and DDG 32 had higher $P_n$ and decreased $\psi_w$ at noon.
Figure 6 Dendrogram of 31 *D. gangeticum* genotypes based on photosynthetic parameters and leaf water potential. Cluster A contained 13 genotypes, whereas Cluster B contained 18 genotypes.

**2 Discussion**

Assessing the genotypic performance in terms of biometrics or photosynthesis can be helpful to estimate the potentials of genotypes to a given environmental conditions. The lower performance of the genotypes like DDG 3, DDG 22 and DDG 33 in terms of photosynthetic rate and associated parameters may explain why these genotypes had lower productivity (data not shown) as compared to the other genotypes but undoubtedly other physiological traits should contribute to this problem. Genotypes DDG 11 and DDG 28 had the maximum $P_n$ values and moderate $g_s$ values which led to these genotypes to consume less water per assimilated CO$_2$ and higher iWUE, whereas other genotypes had lower iWUE. Carbon isotope discrimination ($\Delta$) as a surrogate for water use efficiency to select genotypes in drought-prone environments is successfully employed in wheat breeding. Several selection programs based on $\Delta$ were carried out (Rebetzke et al., 2002 and Juenger et al., 2005) and new wheat cultivars, Drysdale and Rees, with improved water use efficiency have already been released in Australia.

Several genotypes had lower leaf water potential compared to DDG 11 and DDG 28 during noon which attributed for lower photosynthetic rate of these genotypes. Since higher $g_s$ during midday may have positive impact on leaf temperature and *D. gangeticum* canopy temperature were cooler (data not shown) when the $g_s$ and transpiration rate are higher. A difference of 8 °C can be achieved between air and leaf temperature by increased transpiration (Wilkinson, 2004). Amani et al., (1996) showed that wheat cultivars having higher canopy temperature depression (TD) at noon and 4:00 pm produced superior grain yield and there were strong correlation between TD, grain yield and stomatal conductance. Fischer et al., (1998) studied spring wheat cultivars for changes in yield potential in relation to stomatal conductance and other traits. They found that stomatal conductance and maximal rates of photosynthesis were positively correlated with increased yields of advanced cultivars, while leaf temperatures were negatively correlated. Variability in the wheat yield vs. canopy temperature depression (CTD) relationship was also reported by Fischer et al, (1998). However, they found only positive correlation for these traits in all the seasons studied.

The plants in this experiment were irrigated optimally and hence did not face water shortage and thus $g_s$ did not decline to a great extent. Genotypes with higher $P_n$ could be the result of higher leaf hydraulic conductance (Schulze, 1994), compensating the higher water losses with the prevention of transient drought stress at midday. Schaffer and Andersen, (1994) reasoned that an efficient stomatal control is adaptation feature to maintain water potential under water deficits, even the transient ones like midday stress. Correlative selection was detected for early
flowering combined with high stomatal conductance in *Impatiens capensis* under water limiting conditions (Heschel and Riginos, 2005). Thus, early flowering and stress avoidance may be key mechanisms behind adaptation to early-season drought in such situations. The positive correlation of $g_s$ and $E$ with photosynthesis may provide support that selection for such traits can be useful and can be incorporated for breeding *D. gangeticum*.

The allocation of biomass to different plant organs depends on species, ontogeny and on the environment experienced by the plant. It was proposed that plants respond to a decrease in above-ground resources with increased allocation to shoots (leaves), whereas they respond to a decrease in below-ground resources with increased allocation to roots (Poorter and Nagel 2000). Allometric growth pattern changes with plant size due to changes in resource allocation pattern. Larger plants will have to invest a larger fraction of their biomass in support structure and have a larger leaf area due to ontogenetic plasticity (Wright and McConnaughay, 2002). The technique of the allometric analysis is utilized for identifying suitable genotypes for specific environments. Thus, strong emphasis in breeding should be on producing genotypes suitable for specific environments. Correlation among the different growth parameters can be utilized to predict the genotypic performance under specific environmental conditions. Under no resource constraints situations, the biomass allocation is least affected and hence we can utilize the above ground biomass as selection tool to achieve higher root biomass in plants. Hence, *D. gangeticum* genotypes with higher above ground biomass production potential are likely to yield higher root biomass.

3 Materials and Methods

The experiments were conducted at the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat, India. The institute lies in latitude of 22.5° North and longitude 73.0° East and receives an average rainfall of 800 mm, maximum and minimum temperature ranges between 12.7 and 42 °C. Thirty one germplasm accessions which were collected from different parts of India were used in this study. They were evaluated during the Kharif season, 2009-2010 in RCBD with three replications. Each entry was sown in single row of 6 m with inter and intra row spacing of 45 x 45 cm respectively. Observations were recorded for 5 individual plants from each replication. The leaf water potential ($\psi_a$) was measured in 3 upper fully expanded leaves using water potential console (Soil Moisture Corporation, Santa Barbara, U.S.A) (Scholander et al.,1965). Leaf water potential measurements were recorded thrice at different time of the day. First measurement was done at pre-dawn (5:00 to 6:30 hours), second during the noon (12:00 to 14:00) and third measurement was noted at dusk (evening) between 18:00 to 19:30 hours. Mature leaf was selected and sharp cut was made at the end of petiole. The leaf was placed inside the chamber with petiole exposed and chamber was pressurized using nitrogen gas slowly increasing pressure with the help of control valve. Pressure was noted in psi when water droplets were visible at the sharp cut of the petiole.

Leaf gas exchange parameters were recorded for the fully matured, active leaf of *D. gangeticum* genotypes grown under field conditions of research farm of ICAR-DMAPR. All the measurements were recorded on clear days between 11.00 and 14.00 hours using portable, open circuit, infra-red gas analysis system (Li-6400, Li-Cor Ins, Lincoln NE, USA). The youngest fully expanded leaves from the exposed outer layer of the canopy were used for gas exchange measurement. Simultaneous measurements of $CO_2$ and $H_2O$ vapour flux, air ($T_{air}$) and leaf temperature facilitated the calculation of leaf carbon assimilation (A), stomatal conductance ($g_s$), transpiration (E), intercellular $CO_2$ partial pressure ($C_i$) and leaf to air vapour pressure difference from the upper leaf (vpd). Leaf chamber of 6 cm$^2$ was used. Care was taken to see that constant sun light was supplied and measurements were recorded after the leaf gas exchange got stabilized inside the chamber. The data obtained were subjected to statistical analysis using R-studio (USA) statistical package.

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