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RESEARCH ARTICLE

Impact of Drought on Secondary Metabolites in Medicinal plant *Desmodium gangeticum* (L.) DC

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Abstract

Background/Objective: Various Secondary metabolites in *Desmodium gangeticum* (L.) DC. make this plant medicinally important. Also, this plant is naturally subjected to drought-like conditions; the main abiotic stress relates to low water availability to plants. However, literature is scarce regarding studies on drought stress impact on secondary metabolites content in *Desmodium gangeticum* (L.) DC. **Methods:** Plants were exposed to water regimes to generate drought-like conditions, i.e. 200, ml water was given at 3-day intervals for mild stress, 4-day intervals for moderate stress and 6-day intervals for severe stress. The flavonoids, phenolic and alkaloid content of the leaf were estimated at 15 and 30 days after drought stress (DAS). **Findings:** *Desmodium gangeticum* plant expressed differential responses in leaves number and size, secondary metabolite contents to water deficit based on the duration of stress applied. Leaves grown per plant decreased as the severity of drought stress increased (mild-11, moderate-8, severe-6 leaves) compared to control (14 leaves). Flavonoid content increased significantly at 15 DAS and decreased significantly by 50% at 30 DAS. Meanwhile, 15 and 30 days after drought stress (DAS), a significant decrease was observed in phenol and alkaloid content. **Novelty:** Our study highlights the intricate relationship between drought stress and the biosynthesis of biologically active secondary metabolites, flavonoids, phenolic and alkaloids, which can influence the therapeutic properties of *Desmodium gangeticum*.

Keywords: Drought stress; *Desmodium gangeticum* (L) DC; Secondary metabolites; Flavonoids; Phenols; Alkaloids

1 Introduction

The anthropogenic global climate change causes a progressive increase in atmospheric temperature and carbon dioxide (CO_2) level, leading to global warming, low rainfall

and discontinuous monsoon patterns generating drought or water deficit conditions[1]. Drought stress, the prominent abiotic stress, significantly impacts multiple paradigms of plants' physiological processes like growth, development, productivity and nutritional quality of harvests[2]. Synthesis of secondary metabolites against drought stresses is an adaptive and protective response in medicinal plants[3]. These metabolites, including terpenoids, alkaloids, phenolics, flavonoids, and tannins, play vital roles in plant drought stress responses and serve as anti-stressors by acting as antioxidants and antifungal agents[4]. These secondary metabolites and their breakdown products play a key role in enhancing the value of medicinal plants[5]. A very large number of medicinal plants are used in the Indian continent's traditional medicine systems such as Ayurveda, Siddha and Unani treatments[6,7]. Yet, the impact of drought stress on secondary metabolites is studied in small fraction of medicinal plants[8,9]. *Desmodium gangeticum* (L.) DC. is a medicinally important plant and is subjected to drought-like conditions more often. However, this plant has not yet been subjected to study under drought-like conditions. *Desmodium gangeticum* is commonly known as Salparni, has been used in ancient medicine for its diverse therapeutic properties. It is also significant in traditional medicine systems such as Ayurveda, Siddha and Unani treatments to treat various ailments, including respiratory disorders like asthma and bronchitis, digestive issues, and liver disorders[10]. It is an ingredient of tonics like 'Dashmoolarishta', 'Dashmoolakwaath', and Chawanprash[11]. It is enriched in bioactive secondary metabolites such as alkaloids, phenols and flavonoids, contributing to its medicinal properties[12]. Flavonoids such as quercetin and rutin exhibit anti-inflammatory properties and antioxidants, enhancing cardiovascular health and immune system benefits[12,13]. Flavonoids help in scavenging free radicals and protecting cells from damage[14]. Phenols like gallic acid, ellagic acid and vanillic acids play a crucial role in the plant's therapeutic effects, including its ability to support liver health, reduce inflammation, and potentially protect against chronic diseases[15]. Alkaloids are nitrogen-containing organic compounds known for their diverse pharmacological properties such as antimicrobial, anti-inflammatory, potentially anticancer properties, neuroprotective, and analgesic effects[8]. Having vast commercial medicinal use of Desmodium gangeticum, the study of the impact of drought stress on secondary metabolites will help the efficient use of the *Desmodium gangeticum* (L.) DC grown in natural conditions. Realizing this research gap, by generating drought-like conditions in the laboratory, we aim to study the impact of drought stress on secondary metabolites in *Desmodium gangeticum* (L.) DC.

To this end, in this research article, using an established drought platform and biochemical assays, we have studied the impact of severity and period of drought stress on leaf growth, leaf size and secondary metabolites, mainly flavonoids, phenols and alkaloids in *Desmodium gangeticum*. For generating drought stress, plants were exposed to water regimes i.e. 200 ml water was given daily to control plants, and in stress plants, 200 ml water was given at 3 days intervals for mild stress termed as stress 1, 4 days interval for moderate stress termed as stress 2 and 6 days interval for severe stress termed as stress 3. Drought stress affects the total leaf number and size in the *Desmodium* plant. Flavonoid content increased in severely stressed plants (stress 3) at 15 days and significantly decreased at 30 DAS. Phenolic content showed no significant changes in mildly stressed plants but decreased in severely stressed plants early on. Alkaloid content remained stable under mild stress but declined significantly in severe and prolonged drought conditions. This research better helps optimise the growth conditions for maximal extraction of commercial bioactive compounds from medicinal plants.

2 Methodology

Plants and seeds of *Desmodium gangeticum* were randomly handpicked from D.D.U. Gorakhpur university campus, Gorakhpur during July-August. The plants and seeds were brought to the laboratory for identification and were identified by Prof. Awanish and authenticated with the help of flora Gorakhpurensis written by Dr. T. N. Srivastava.

Seeds of *Desmodium gangeticum* (L.) DC. were surface sterilized with 20% ethanol for 10 minutes and washed with sterilized water. To increase germination, seeds were treated with H_2SO_4 (80%) for 10 minutes and thoroughly washed several times with sterilized water. Seeds were germinated in a growth chamber at $25\pm1^{\circ}$C and 50-60% humidity for one week. Seed germination was 25-30%. Germinated seeds were sown in the sand and grown for 6 weeks with 200 ml water daily and Hoagland nutrient solution at 3 days interval. After 6 weeks, plants were grown in sets of three in control conditions and in drought conditions. 200 ml Hoagland nutrient solution was applied to pots once every 3, 4 and 6 days intervals (different durations of repeated drying and wetting cycles) to generate different extent of drought-like conditions[16]. Soil moisture content was measured using a moisture meter calibrated on a scale from 0 to 10. The moisture meter readings were classified into four distinct categories based on observed plant stress responses under different moisture levels: (1) **Control**: Readings from 8 to 10 reflect optimal soil moisture levels, representing no drought stress. (2) **Stress 1**: Readings from 6 to less than 8 indicate minor moisture deficit with limited plant stress. (3) **Stress 2**: Readings from 3 to less than 6 represent moderate soil moisture deficiency, where plants experience noticeable stress but can still survive. (4) **Stress 3**: Readings below 3 indicate critically low soil moisture levels associated with severe water stress in plants. These thresholds were determined based on the specific soil moisture characteristics observed during preliminary testing. Control plants were watered every day with 200 ml water. Stressed plants were watered with 200 ml

water once every 3, 4 and 6 days intervals to maintain stress 1, stress 2, and stress 3 respectively. At 15 and 30 day samples were collected and dried at 65±1°C in the drying chamber, and biochemical parameters like flavonoid, phenol and alkaloid content were estimated.

The leaf Sample extract was prepared by grinding 10 milligrams of dried leaves in 2 ml of methanol and incubated for 24h for each set of treatments, and used for quantitative estimation of flavonoid, phenol and alkaloid.

Total flavonoid content was estimated in dried leaves of *Desmodium gangeticum* by the aluminum chloride colorimetric method[17]. For each set of treatments in 0.5 ml of methanolic leaf sample extract was added 1.5 ml of methanol, 0.1 ml of $AlCl_3$ and 0.1 ml of Potassium acetate and 2.8 ml of distilled water. Incubated at 25 ± 1°C for 30 minutes. The absorbance of the reaction mix was taken at 410 nm using a spectrophotometer, Thermoscientific varioskan flash. The amount of total flavonoid content was computed with the standard curve prepared from quercetin (Figure 3 **B**).

Total Phenol content was estimated in dried leaves of *Desmodium gangeticum* by Folin-Ciocalteu method[17]. For each set of treatments, 0.5 ml of methanolic leaf sample extract was added to 0.5 ml of Folin-Ciocalteu reagent and 5 ml distilled water. The reaction mix was incubated for 10 minutes. Then, 0.5 ml of sodium carbonate was added and incubated for 45 minutes at 25 ± 1°C. Absorbance of blue colour was taken at 760 nm using spectrophotometer, Thermoscientific varioskan flash. The amount of total phenol content was computed with standard curve prepared from Gallic acid (Figure 4 **B**).

Total alkaloid content was estimated in dried leaves of *Desmodium gangeticum* by a previously reported method[18]. Briefly, for each set of treatment, 0.5 ml of methanolic leaf sample extract was filtered using 0.45 micron filter and dried it centrivac at 45± 1°C. Dried samples were dissolved in 500 μl of 2N HCl and washed with 1, 2 and 5 ml of chloroform respectively. After washing, pH was maintained to 7.0 with 10 N NaOH. 500 μl ml of Bromocerol blue and 500 μl of Phosphate buffer (pH- 4.7) was added to the samples and shaken for approx. 30 second. Bromocerol forms complex with alkaloids which was extracted with 5 ml of chloroform. Absorbance of the complex was measured at 470 nm using spectrophotometer, Thermoscientific varioskan flash. The amount of total alkaloid content was computed with the standard curve prepared from Atropine (Figure 5 **B**).

Data was statistically analyzed, and Standard error and multiple t-tests were made as required.

3 Results and Discussion

This research article focus on quantifying the levels of various medicinally significant secondary metabolites, including total flavonoids, phenols, and alkaloids in *Desmodium gangeticum* subjected to varying duration, and pulsation (i.e., repeated drying and wetting cycles) of drought stress[16]. Firstly, The seed of *Desmodium gangeticum* (L.) DC. were collected from regional area of Gorakhpur, India. Then, seeds were taxonomically identified as described earlier and germinated at 25°C and 60% humidity in growth chambers for one week, and planted in pots containing river sand (Figure 1**A** and Figure 1 **B**). Seed germination was 27.5 ± 2.5%. The plant was watered daily as described in the methodology and showed optimal soil moisture content i.e. 75% (Figure 1 **C**)[19].

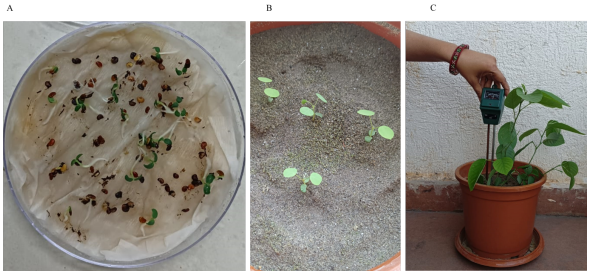

Fig 1. ***Desmodium gangeticum*** **: A: Germinating seeds with Plumule and radicle. B: Image after 20 days grown plant in pots. C: Image of potted plant measuring moisture content with moisture meter**

Leaf number is reduced upon drought stresses given as described earlier in methodology as compared to control. Control plants produced 13 ± 2, stress 1, 11 ± 2, stress 2, 8±1 and stress 3, 6±2 leaves (Figure 2**A**). The leaf number and area of Stress 3 plants were severely affected as compared to leaves number of stress 1 and stress 2 plants (Figure 2**A** and Figure 2 **B**).

The total flavonoid content at 15 DAS significantly increased in stress 1, stress 2, and stress 3 plants compared to the control (p-value 0.0275). At 30th DAS, the total flavonoid content in stress 1, 2, and 3 plants had decreased with age (Figure 3**A**). The initial increase in flavonoid content in stress plants suggests an adaptive response to stress. However, stressed plants compromise flavonoid production upon prolonged drought-like conditions.

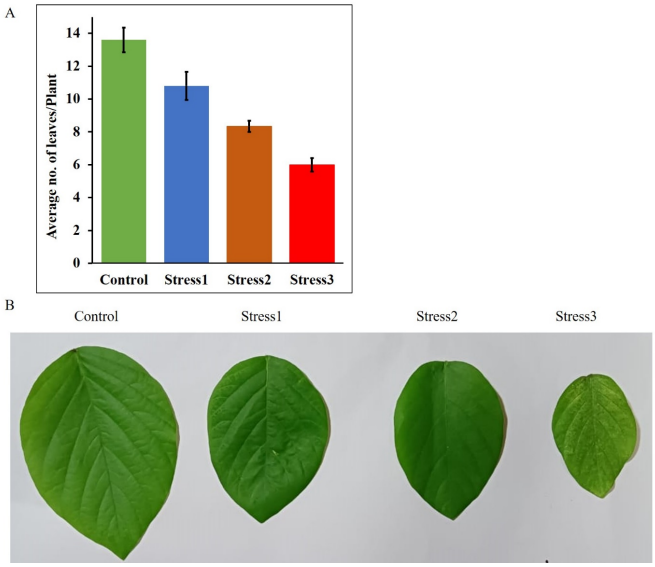

Fig 2. ***Desmodium gangeticum*** **(L.) DC . A: Bar graph showing the average number of leaves in control, stress 1, stress 2 and stress 3 plants. Data are based on means ± SEM (standard error of the mean) of three biological replicates. B: Leaf size in control, stress 1, stress 2 and stress 3 plants at 30 DAS**

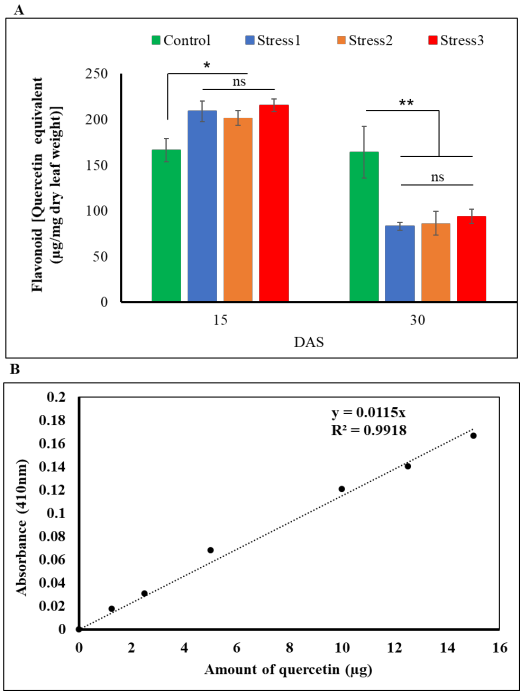

Fig 3. A. ***Desmodium gangeticum*****: Total flavonoid content at different days of plant growth under different drought levels.Data are based on means ± SEM of three biological replicates. B. Standard curve for quercetin and colorimetric quantitation of the total flavonoid. *- p-value < 0.05 and **- p -value < 0.01**

No significant differences in total phenol content were seen in control and stress 1 plants at 15 DAS. However, stress 2 and stress 3 plants exhibited a lower total phenol content compared to control and stress 1 plants at 15 DAS, (p-value 0.0501 and 0.0071 respectively). At 30 DAS, a gradual decline in total phenol content was seen in stress1, stress 2 and stress 3 plants as compared to control (Figure 4**A**). These results suggest that the impact of drought stress on total phenolic content may not be immediate but could become more pronounced or variable over time. The delayed response may be due to either the ability of plants to adapt adverse conditions or the role of environmental factors affecting the synthesis of phenolic compounds.

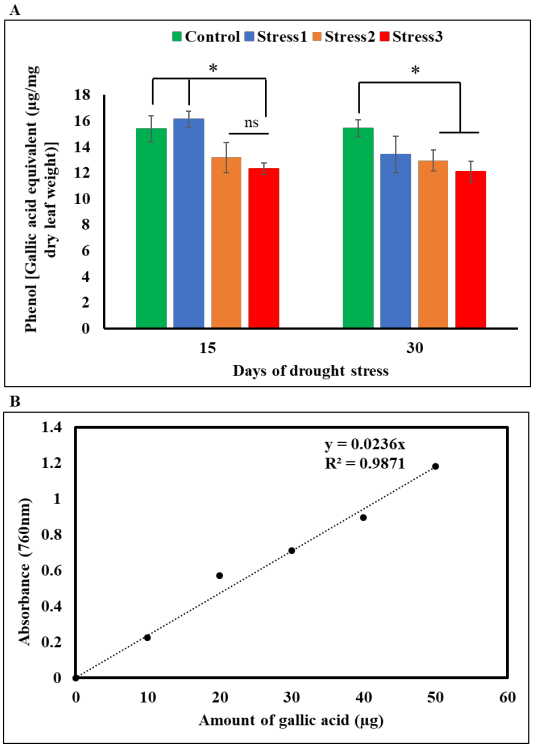

Fig 4. A. *Desmodium gangeticum*: Total phenol content at different days of plant growth under different drought levels. Data are based on means ± SEM of three biological replicates. B. Standard curve for gallic acid and quantitation of the total phenol. *- p -value<0.05

The total alkaloid content at 15 DAS showed a slight decrease in stress 1 and stress 2 plants compared to the control. However, stress 3 plants showed a significant decrease in alkaloid content as compared to control, stress1 and stress 2 plants. At 30th DAS, total Alkaloid content decreased in stress 1, 2 and 3 compared to control (Figure 5**A**). These observations suggest that prolonged drought stress led to a significant reduction in the total alkaloid content in *Desmodium gangeticum* (L.) DC. The decrease in alkaloid production under extended stress conditions may be attributed to the plants diverting their resources away from the alkaloid synthesis to cope with the prolonged stress.

All plants show adaptation, both morphological and metabolic, in response to abiotic stress responses. Drought, being the most common abiotic stress to plants, affects most of the physiological and metabolic processes. Given our interest in studying the impact of drought stress on secondary metabolite in *Desmodium gangeticum*, our water irrigation scheme successfully generated the drought-like condition in the laboratory and observed that leaf size is severely decreased in stress plant which indicates the metabolic alteration (Figure 2). Considering the medicinal importance of secondary metabolites, we study the impact of Stress 1, 2, and 3 drought stress on total flavonoid, phenol, and alkaloid contents. Flavonoid content significantly increased upon mild Drought stress at 15 DAS (Figure 3**A**). However, stress 2 showed an insignificant impact on Phenol (Figure 4**A**) and alkaloid content (Figure 5**A**). The prolonged (at 30 DAS) and particularly stress 3 causes a decline in the flavonoid (Figure 3**A**), phenol (Figure 4 **A**), and alkaloid (Figure 5 **A**) contents. Also, flavonoid content increased upon drought stress in multiple plants, including *Desmodium styracifolium*, recently reviewed [20]. However, our and Yang et. al. 2020 studies showed that prolonged duration of drought stress leads to decrease in total flavonoid content [21]. The modulation of phenolic content upon drought stress varies from plant to plant, which has recently been discussed [22]. In our study, stress 2 plants showed insignificant modulation of phenols compared to control, and stress 3 plants led to a decrease in phenols. Unlike our finding

for alkaloids, the alkaloid content increased in *Lycoris aurea*, a medicinal plant, upon drought stress [23]. Thus, the duration and severity of drought stress variably affect various secondary metabolite contents, and it also depends on plant species. This alteration of secondary metabolite production helps plants to protect from drought-like stress.

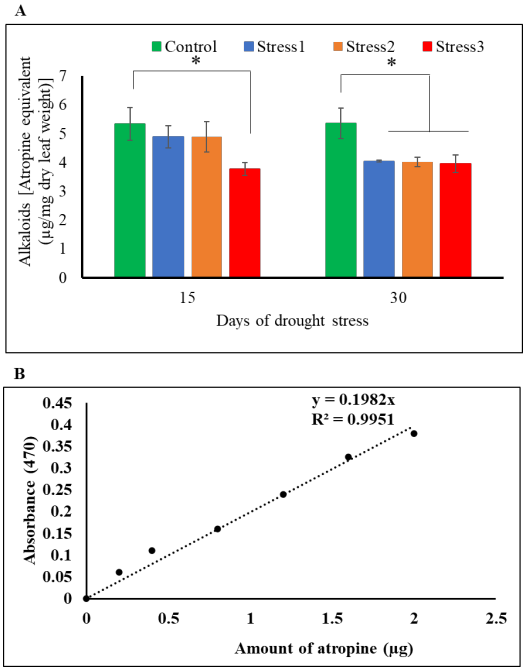

Fig 5. A. *Desmodium gangeticum* : Total alkaloid content at different days of plant growth under different drought levels. Data are based on means ± SEM of three biological replicates. B. Standard curve for Atropine and quantitation of total alkaloid. *- p-value < 0.05

4 Conclusion

This study presents the dynamic and multifaceted responses of *Desmodium gangeticum* (L.) DC. to drought stress, emphasizing that stress duration and plant age influence the production of secondary metabolites. Moving ahead, this article opens several possible uses for optimum levels of drought stress as elicitors, which can stimulate and increase the contents of biologically active secondary metabolites in *Desmodium gangeticum*. For the betterment of human health, this study can be used for further research to obtain the optimum secondary metabolites for pharmacological use. Finally, we suggest that the study of drought stress impact on secondary metabolites using high throughput assays would better resolve various metabolites at the molecular level. Also, it needs to explore genetic mechanisms underlying the observed changes in metabolites.

Author Contributions

Conceptualization, K.B., M.S., A.R. and A.; methodology, K.B., M.S., and A.R Data collection, K.B., A.R., and A. formal analysis, K.B., M.S., A.R. and A.; investigation, K.B, M.S. and A.R; resources, M.S., and A.R; data curation, K.B, M.S. and A.R; writing—original draft preparation, K.B. and M.S.; writing-review and editing, , K.B., M.S., A.R. and A.; visualization, K.B., M.S., A.R. and A.; supervision, M.S. and A.R.; project administration, M.S. and A.R.; funding acquisition, M.S. and A.R.; All authors have read and agreed to the published version of the manuscript.

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