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ORIGINAL ARTICLE

Response of secondary metabolites in *Aristolochia bracteolata* Lam. under water stress regimes

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Effect of water stress on leaf water relations and some secondary metabolites in root stem and leaf of *Aristolochia bracteolata* Lam. has been studied. Mature potted plants were imposed to short term water stress. Moisture content, succulence index, mesophyll succulence index showed a very meager decline under water scarcity thereby showing its ability to maintain water status even under water deficiency. Significant rise in proline content in leaf followed by stem and root observed under water stress. Polyphenol and tannin content has increased considerably in all parts but more pronounced effect is seen in root. Maintenance of high succulence even under water stress and concurrent accumulation of secondary metabolites helps in membrane protection and osmoregulation influences for induction of facultative CAM.

Key words: Weed, drought, abiotic, polyphones, Crassulacean Acid Metabolism

Aristolochia bracteolata Lam. is commonly known as worm killer in English belongs to family Aristolochiaceae. This Plant is one of the worst weeds of black cotton soil. This prostrate herb is with ovate leaves and flowers October to May. Whole plant parts are nauseously bitter.

The occurrence of this weed in a variety of habitats and soil types reveals its uniqueness which has prompted us to understand this weed in more elaborative way. Interestingly, earlier Deshmukh and Murumkar, (1995) have reported occurrence of Crassulacean Acid Metabolism in *A. bracteolata* Lam. It exhibit facultative CAM as environmental adoptive response supports its occurrence throughout the year (Kadam and Murumkar, 2012).

Production of the secondary metabolites by the plants is regarded an adaptive capacity in coping with stressful constraints may involve production of complex chemical types and interactions in the structural and functional stabilization through signaling processes and pathways (Edreva, 2008) and produce secondary metabolites during growth this may be signaling towards defense mechanism (Isah, 2019).

Aristolochia bracteolata Lam. survives even under hot dry summer conditions, keeping in this mind it is subjected to short term water stress and analyze the water relations along with response of secondary metabolites to study strategies developed under water scarcity.

MATERIALS AND METHODS

Plant Material and stress treatments

The healthy seeds of *Aristolochia bracteolata* Lam. were grown in earthen pots with a soil, sand, vermi-compost mixture (2:1:1) and watered daily. Well grown plants were subjected treatment of short term water stress. To study the effect of water stress mature plants, four treatments were given. First set as under Control (Well Watered), second group under water deficit for one day (24 hr), third group under water deficit two days (48 hr) and fourth group under water deficit for three days (72hr).

Assay for water relations:

Leaves of treated plants were subjected to moisture content determination by measuring difference in weight after oven drying for 10 days at $80^{0}C$. Succulence was calculated as ratio of fresh and dry matter as well as mesophyll succulence and surface expansion (Kluge and Ting, 1978).

Assay for Secondary metabolites

Treated plants were used for analysis of secondary metabolites. For this, plants were uprooted and roots were washed carefully with water to clear away the adhered soil particles. Intact plants were brought to the laboratory. Healthy and mature plant parts were separated carefully and separated plant parts like leaf, stem and roots were subjected for analysis of secondary metabolites from fresh or oven dried plant material.

Free proline content was determined according to the method of Bates *et al.;* (1973). Plant material was homogenized in sulphosalicylic acid and the filtrate along with glacial acetic acid and ninhydrin reagent was reacted in water bath. The reaction was terminated by transferring the reactant to ice bath. The developed colour was extracted with toluene and the absorbance of toluene chromophore was measured at 520nm spectrophotometrically. The results are expressed as mg/ g^{-1} dry tissue. The standard curve was prepared by employing L-Proline.

Total polyphenols were determined according to Folin and Denis (1915). Plant material homogenized in 80 % acetone and filtrate with 20 % Na_2CO_3 and Folin Denis Reagent was reacted for 20 min. Absorbance of developed color was read at 660 nm spectrophotometrically using reagent as blank and results are expressed as mg g^{-1} fresh tissue.

The tannin content in dried plant parts was estimated by Folin-Denis method (Schanderl, 1970). Plant material was hydrolysed by distilled water in a boiling water bath for 30 minutes and filtrate treated with Folin-Denis reagent, Sodium carbonate Reacted for 30 minutes. Which is further used for spectrophotometric analysis at 700 nm against the blank and results are expressed as mg g^{-1} dry weight.

Statistical Analysis:

The data obtained in all experiments was subjected to statistical analysis. Also the primary data was analyzed using MINITAB software and the values presented in tables and figures are statistically processed to ANOVA at the significance level of 5%.

RESULTS

It can be revealed from the table 1 that all the water relations parameters shows consistent decline in their values due to increasing water deficit. It is interesting to note that even at moderate and severe water deficiency the moisture content has not decreased significantly and it seems that high moisture content has been maintained even in water deficit conditions. It is well evident from the present findings that moisture content is moderately high at normal conditions in this weed. This obviously reflects the succulent nature of this plant. Further, with increasing water stress also, moisture content shows a very meager decline in status, thereby showing its ability to maintain water status even under water deficiency. It is evident from the results that succulence index decreased drastically from 9.18 g. g^{-1} to 4.86 g. g^{-1} with increasing water stress. This may be one of the reasons for its success to survive under arid, dry and hot environmental conditions.

There is very small decline in Mesophyll Succulence Index (MSI) from 0.476 g mg^{-1} chl to 0.308 g mg^{-1} chl. with increasing water stress and almost the mesophyll succulence index has been maintained by this weed even under water deficit conditions. The normal condition has shown high MSI value indicating succulent nature of this weed. The determination of succulence index in the photosynthetic tissues of facultative CAM species helps to establish the relationship between succulence and facultative CAM. Once positive gas exchange had ceased the capacity for photosynthesis was maintained for longer periods of drought and this was directly correlated to the degree of leaf succulence. Thus, only negligible decrease in mesophyll succulence index in *Aristolochia bracteolata* Lam. at water stress can be correlated to facultative CAM nature as well as sustenance of high succulence in this weed.

Under control conditions highest proline content is recorded in leaf followed by stem and root. The water stress showed overall accumulation of proline with increase in water deficit. And significant rise in proline content in leaf followed by stem and root has been observed. Similarly water stress showed overall accumulation of total Polyphenols with increase in water deficit in *A. bracteolata* Lam. Most significant rise in Polyphenol content in root followed by stem and leaf with severe water deficit of 72 hrs (Table 3). More pronounced effect of accumulation of polyphenols in roots is to control membrane and cellular injury, diverting nitrogen from other organic solutes. It has been observed that tannin accumulation is more profound in roots than leaves due to water scarcity (table 4).

Table 1. Effect of Water Stress on of Leaf Water Status in *Aristolochia bracteolata* Lam.

Parameter	Water Stress levels			
	Control	24 hrs	48 hrs	72hrs
Moisture content (%)	89.11 ± 0.04	87.94 ± 0.6**	80.31 ± 0.05*	79.41 ± 0.4
Succulence Index g.g^{-1}	9.18 ± 0.02	8.29 ± 0.05*	5.66 ± 0.06*	4.86 ± 0.06
Mesophyll Succulence index (Sm) g mg^{-1} chl	0.48 ± 0.04	0.35 ± 0.05*	0.33 ± 0.00*	0.31 ± 0.00

± - S. D. of three observations
* - Values are significant at 5% level where P < 0.05.
** - Values are non-significant at 5% level where P < 0.05.

Table 2. Effect of water stress on free Proline content in different parts of *A. bracteolata* Lam.

Plant Part	Free Proline (mg/ g dry tissue)			
	Water Stress levels			
	Control	24 hrs	48 hrs	72hrs
Leaf	1.45 ± 0.01	2.07 ± 0.04*	4.06 ± 0.02*	4.76 ± 0.01*
Stem	0.79 ± 0.01	1.45 ± 0.03*	3.31 ± 0.01*	4.10 ± 0.02*
Root	0.46 ± 0.02	0.91 ± 0.03*	2.40 ± 0.01*	3.31 ± 0.00*

± - S. D. of three observations
* - Values are significant at 5% level where P < 0.05.
** - Values are non-significant at 5% level where P < 0.05.

Table 3. Effect of water stress on total Polyphenol content in different plant parts of *A. bracteolata* Lam.

Plant Part	Total Polyphenol content (mg/ g fresh tissue)			
	Water Stress levels			
	Control	24 hrs	48 hrs	72hrs
Leaf	4.18±0.02	5.57±0.02**	6.96±0.01**	12.35±0.03**
Stem	2.09±0.02	3.65±0.01**	5.22±0.03*	10.27±0.54*
Root	1.22±0.06	2.78±0.02*	4.00± 0.57*	8.18±0.02*

± - S. D. of three observations
* - Values are significant at 5% level where P < 0.05.
** - Values are non-significant at 5% level where P < 0.05.

Table 4. Effect of water stress on Tannins in different plant parts of *A. bracteolata* Lam.

Plant Part	Tannins mg /g dry tissue			
	Water Stress levels			
	Control	24 hrs	48 hrs	72hrs
Leaf	21.34 ±0.02	23.59± 1.16*	25.84±0.21*	31.46±0.02*
Stem	16.29 ±0.02	19.10±0.03*	21.91±0.01*	26.40±0.03*
Root	8.98 ±0.02	11.79±0.02*	14.60±0.17*	21.91±0.11*

± - S. D. of three observations
* - Values are significant at 5% level where P < 0.05.
** - Values are non-significant at 5% level where P < 0.05.

DISCUSSION

Plants adapt themselves to drought conditions by changing their morphology, physiology, anatomy and gene expression and they optimizes metabolism of their organs and cells in order to maximize productivity under the drought conditions.

Munns *et al*. (2000) in their study on water relations and leaf expansion observed that, leaf water status could limit leaf expansion rates during periods of high transpiration. Kluge and Ting (1978) proposed that the succulence index for whole thick leaves and their mesophyll was typical of CAM plants. Deshmukh and Murumkar (1995) evaluated that maintenance of high succulency even under stress is due to CAM pathway in A. bracteolata Lam. The close relationship between succulence and leaf thickness has also been reported by Bussoti *et al.,* (2002) as thicker leaves of *Quercus ilex* showed a greater volume of water per surface area. Under drought conditions, high succulence index, to be used as kind of protection (Bacelar *et al*., 2004). Kluge and Ting (1978) who proposed mesophyll succulence index has to be proved useful in assessing operation of CAM. Ripely *et al.,* (2013) postulated that increasing leaf succulence might be associated with decreasing mesophyll conductance and increasing dependence on Crassulacean acid metabolism (CAM).

Verma and Shukla, (2015) proposed that many of the times, the content and composition of secondary metabolites are affected by the developmental stages of plant. Several reports are available indicating accumulation of secondary metabolites under water stress as in *Crataegus laevigata* and *C. monogyna* (Kirakosyan *et al*., 2004), Sadale (2007) in *Sesbania grandiflora* L., Ashraf & Aafia (2005) in roots of *Phaseoles vulgaris* and *S. acceleato*, Phutela *et al.*

(2000) in leaves and roots of *Brassica juncea,* Jain *et al.* (2006) in pigeon pea roots.

Proline contribute to osmotic adjustment, detoxification of ROS and protection of membrane injury and plays a key role in stabilizing cellular proteins of osmoticum of higher proline content in plants in water stress (Khalil *et al.,* 2010).

Water stress increases concentration of plant phenolics in drought-resistant and drought sensitive spring triticales may be participating in the mechanisms of adaptation of the photosynthetic apparatus to water deficit in leaf tissues (Hura *et al.*, 2009). Shah *et al.*, (2011) found that polyphenols were increased in all cotton genotype under drought condition. Polyphenols act as main compatible solutes in order to maintain osmotic balance, to protect cellular macromolecules, to detoxify the cells and to scavenge free radicals under water stressed conditions in tolerant cotton genotype (Parida *et al.*, 2007). Increase in polyphenols under water stress is reported in Safflower seedling (Yaginuma *et al.,* 2003), *Hypericum brasilience* by Abreu and Mazzafera (2005), M9 apple and MA quince rootstocks (Bolat *et al.*,2014).

Increased tannin content in leaves of *Ceratonia siliqua* due to levels of stress reported by Kouki and Marieta (2002), leaves of *Ocimum gratissimum* L and *Gongronema latifolium* Benth (Osuagwu *et al.,*2010). On the contrary results of the quantitative analysis in *A. wilkesiana* leaves showed that plants under drought tannins production was decreased (Odjegba and Ayodeji, 2013). Identification and manipulation of drought-resistant pathways can be studied in detail by the use of new technological approaches like transcriptomics and proteomics (Ashrafia *et al*., 2018). Prospectively, the use of environmental stresses may provide a potential and profitable way to increase the accumulation of bioactive compounds along with improve quality and reduce over-harvesting pressures of medicinal plants (Wang *et al.,* 2018).

Alterations in Facultative Crassulacean Acid Metabolism mode of photosynthesis is plasticity towards severe drought condition could be beneficial to *Aristolochia bracteolata* Lam. cope up in extreme condition and its success in arid part.

CONCLUSION

The weed *Aristolochia bracteolta* Lam. thrives well in dry semi-arid region of Baramati, and dominates weed flora in summer season. With increasing water stress, in *Aristolochia bracteolata* Lam. leaf water relations showed a very meager decline for its success to survive under arid, dry and hot environmental conditions. But, at the same time water stress has caused a significant decline in surface expansion values in leaves and these values are in accordance with those reported for CAM plants. This feature points clearly towards CAM nature of this weed under drought. Proline accumulation under short term water stress probably contributes to drought tolerance process in this plant under unfavorable conditions. Increased Polyphenol content considerably in root tissue under water stress justifies presence of perennial roots rich in Aristolochic acid this finding is more significant. Positive correlation of enhancement in tannin content with respect to drought in plant *Aristolochia bracteolata* Lam. is indicative of protective oxidative role of tannins under drought.

Secondary metabolites are of great medicinal importance but their synthesis is dependent upon environmental conditions. Thus present studies will be useful to explore medicinal potential of this weed and mass cultivation for commercial production of secondary metabolites. As many of the medicinal properties of weed *Aristolochia bracteolata* Lam. are known in tribal medicine. Preliminary studies in our results will definitely help to undertake research towards fortifying their medicinal value genetically as well as to enable them to tolerate high levels of drought and salinity which otherwise would affect the synthesis of phytochemicals like aristolochic acid and many others in this plant.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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