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Effect of Salicylic Acid on *Valeriana wallichii* DC syn *Valeriana jatamansi* Jones under Drought Stress

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ABSTRACT

Valeriana jatamansi is a perennial medicinal herb of the western to eastern Himalaya also known as *tagar* or *somaya* growing at an altitude of 1200-2000m asl. Although vulnerable, it is extremely useful for different treatments like headache, eye trouble, epilepsy, hysteria, as a stimulant, carminative and antispasmodic and is also used in various local traditional treatments. As the global temperature is increasing it may cause scarcity of available water which will impact survival and functioning of plants. Water stress in plants takes place due to increment in water depletion or insufficient water absorption or scarcity of available water in soil. Salicylic acid (a plant growth regulator) is synthesized in plant to mitigate the detrimental impact of abiotic stress and enhance metabolic efficiency of plant. Therefore, a pot experiment was conducted to assess the effect of salicylic acid at different concentrations (0 mM, 0.25 mM, 0.50 mM, 0.75 mM and 1 mM) in four different water stress conditions (100% field capacity, 75% FC, 50% FC and 25% FC) at vegetative and flowering stages. During the experiment it was observed that, 0.25 and 0.50 mM concentrations of salicylic acid had positive effect on most of the morphological as well as biochemical traits under study. Under both these concentrations an increase in the number of leaves, shoot length, shoot and root dry matter, protein, proline, phenol and chlorophyll content at vegetative and flowering stages was observed in comparison to control and other treatments with increasing water stress conditions. Therefore, it can be concluded that the lower concentration of salicylic acid had a beneficial effect under drought stress conditions.

***Key words*:** Tagar, *Valeriana wallichii* syn. *V. jatamansi*, Drought stress, Salicylic acid, Medicinal plants

Medicinal plants are widely used from long ago in the various systems of medicine. The active constituents of the plants play a remarkable role in curing diseases. These constituents can be used to improve healthcare as it is directly based on plant extraction and show minimal side effects compared to conventional allopathic medicines. *Valeriana jatamansi* Jones synonymous *V. wallichii* DC. is a very high value medicinal plant commonly known as Indian valeriana or Tagar. This medicinal and aromatic plant belongs to the family Caprifoliaceae (earlier family Valerianaceae) and genus *Valeriana*, encompassing about 250 species worldwide APG 2009. It originated from Latin word "Valere'' meaning to be in good health. It is distributed in many countries of Asia- Nepal, India, southwest China, Afghanistan, Bhutan and Myanmar [1-2]. It is an herbaceous perennial plant of 14-45cm height and grows at an altitude of 1200-2000 asl. It can reproduce sexually (seeds) and asexually (rhizomes) [3]. Different parts of the plant like root, rhizome, seed and flower have medicinal importance. The root has strong Valerianous odour due to presence of volatile oils and other compounds. It contains bioactive compounds like alkaloids, flavonoids, saponins, tannins and essential oil. Valerenic acid and valepotriates / iridoids are the major active constituents [4]. *V. jatamansi* has a long history of uses and is mentioned in various texts such as, Rig-Veda, Charka Samhita and Modern medicine system. The plant has wide applicability as a potent tranquilizer, antispasmodic and hypotensive, stimulant and improves gastrointestinal disorders. It can also work for anxiety, insomnia, epilepsy and hysteria, eye-trouble, skin-diseases, nervous-system and snake-poisoning. The plant shows antimicrobial and antifungal activity [5].

Due to adverse environmental conditions plants are subjected to various abiotic stresses that affect growth, metabolism and yield [6-7]. Among these "drought stress" is most threatening to crop yield [8-10]. It reduces leaf area and dry matter of the plant [11-13]. Somehow, plants can hamper the droughtful condition by increasing root system and leaf thickness (to lessen evapo-transpiration) and altering the physiological processes (by excessive production of ROS which can lead to senescence due to photosynthetic pigment breakdown) [14-16]. Therefore, application of Salicylic acid (SA) can play a major role in moderating the tolerance to several abiotic stresses which in turn have positive effect on

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economical yield. This phenolic compound can regulate physiological processes involving respiration, stomatal movement, photomorphogenesis, seed germination and senescence. It is also an important signaling molecule of irregularity in plants when plants respond to biotic and abiotic stresses [17]. SA can protect plants from side effects by regulating antioxidant enzymes against active-oxygen compounds [18-19]. Concentration of 0.05 mM SA increased tolerance to cadmium in barley by increasing its antioxidant activity [20]. Similarly, it is also reported that during salinity stress, 0.1mM SA shows increase in dry and fresh weight of root and aerial organs and leaf area in corn [21]. In addition, it increases polyamine putrescine, spermidine and spermine which contribute to membrane conservation and uniformity under drought stress [22]. Treatment of SA and its derivatives can increase plant tolerance to stress [23]. It has been observed that SA can considerably affect the sensitivity of plants to a number of abiotic stresses [24]. Detailed investigations on medicinal and aromatic plants have been less frequent than on agricultural crops especially under drought stress. The present study was designed to investigate the effect of different treatment of SA (as a plant hormone), on morphological and biochemical parameters and to overcome the impact of drought stress in *V. wallichii* and to increase yield.

MATERIALS AND METHODS

Time, location and climatic conditions of the experiment

The research was conducted in the department of High Altitude Plant Physiology Research Centre (HAPPRC), H.N.B. Garhwal University, Srinagar Garhwal, Uttarakhand, India at an altitude of 560m (amsl) lies between space 30°13'-13'30" North Latitude and 78°45'- 47'4" East Longitude that, receives average rainfall of 1371 mm and an average maximum temperature goes to 37.8°C during summer and minimum to 6°C during winters during the experimental period 2018-2020.

Design characteristics of the experiment and planting procedure

Planting material was collected from the research field station (*Tala, Ukhimath*). A pot experiment was conducted based on a randomized complete block design. Transplanting was carried out in October in the pots (20 cm in diameter at the top edge and 15 cm in height). Fine compost along with garden soil was used as a growth medium in the polybags which were filled with 2.15kg soil. Plants having 3-4 leaves per plant were transplanted in each pot from the nursery. The twenty experimental treatments were made by combination of 100% (no stress), 75% (low stress), 50% (medium stress) and 25% (very high stress) of FC with four concentrations of SA (0.25, 0.50, 0.75 and 1 mM), and all these interactions were compared with their respective controls (0 mM SA). All concentrations were applied by foliar spray on three alternate days before vegetative and flowering stages. Different morphological parameters viz., number of leaves, shoot length (cm), shoot dry matter (gm), root length (cm), root dry matter (gm) and leaf area (cm^2) and biochemical parameters: Changes in chlorophyll (SPAD Value), protein content [25], proline content [26] and phenolic content [27] were observed after 15 days of foliar spray of SA during vegetative and flowering stages.

RESULTS AND DISCUSSION

In the experiment, it was observed that during vegetative and flowering stage, increasing levels of drought stress (75%, 50% and 25% FC) decreases different morphological and yield attributing characters in *V. wallichii* whereas, the application of SA (0.50 mM and 0.25 mM) shows significant increase in number of leaves, leaf area, shoot length, root length, shoot dry matter and root dry matter as compared to overall maximum control value (Table 1).

When various treatments of drought stress (100%, 75%, 50% and 25% FC) were compared to their respective control values (0 mM SA concentration), it showed highest increase in the morphological characters in the interaction of different drought stress with 0.25 mM concentration of SA during vegetative stage and 0.25 mM and 0.50 mM SA concentration during flowering stage (Table 1). Foliar spray of SA resulted in higher plant fresh and dry weight, number of leaves in cucumber and maize [28]. SA increases the leaf area in sugarcane [29-30], which is also consistent with our results in *V wallichii*. The ability of SA to increase plant dry mass, ameliorating the adverse effect of water stress, may have significant implications in improving the plant growth and overcoming the yield barrier arising from conditions of limited water availability. SA had also reported to increase plant growth parameters (number of branches, number of leaves and leaf area) in marigold [31], marjoram and oregano [32].

Chlorophyll, protein and phenol content are generally reduced under severe drought stress. Maximum decrease in chlorophyll content in comparison to overall control values were found in 50% and 25% FC (Fig 1A). The decrease in chlorophyll content as affected by water deficit is because of production of reactive oxygen species (ROS), such as O_2 and H_2O_2, which lead to lipid peroxidation and consequently, chlorophyll destruction [33]. Water stress had resulted reduction in chlorophyll and carotenoid content to a large extent in lemon due to instability of protein complexes and destruction of chlorophyll by increased activity of chlorophyllase enzyme under stress [34].

Whereas, maximum increase in chlorophyll was observed in treatment 25% FC combined with 0.50 mM SA and 1 mM SA during both vegetative and flowering stages respectively (Fig 1A). Increased in chlorophyll after SA applications were observed in both control and stressed plants and were explained by the positive action of hormone in plant nutrient uptake as greater contents of Fe, Mg and Ca can stimulate the biosynthesis of Chlorophyll [35-36]. Similar results were also observed in *Coriandrum sativum* plants treated with 0.50 mM SA [37]. The positive effect of SA on photosynthetic pigment is attributed to its stimulatory effects on Rubisco activity and photosynthesis. SA induces synthesis of kinases protein, which is important in regulating cell division, differentiation and morphogenesis [38-39]. It was also reported that application of SA improved chlorophyll content in *Brassica napus* [40].

Maximum decrease in protein content was found in 100% FC (vegetative stage) and 75% FC (vegetative and flowering stage) while at both vegetative and flowering stages, interaction of 50% FC + 1mM SA and 75% FC + 0.25 mM SA concentration resulted in maximum increase in protein content compared with overall control values (Fig 1C). SA increases protein content in basil and marjoram under normal conditions [41]. Whereas, during vegetative stage 0.25 mM and 1 mM SA concentration increased the protein content among all the interactions with respect to their control and

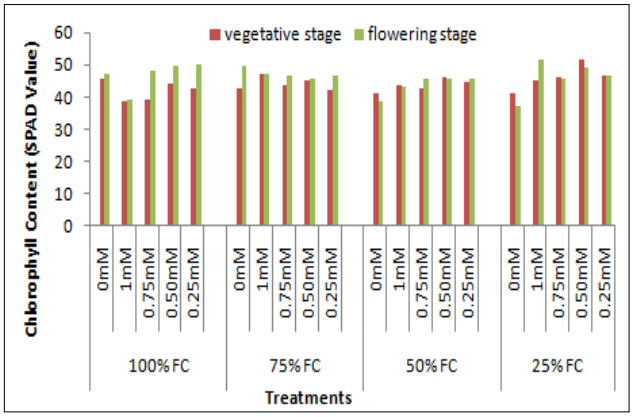
during the flowering stage, maximum increase was found in 0.25 mM SA concentration among all the treatments (Fig 1C). It was found that the content of proteins in plant leaves increases during severe drought [42]. However, SA could affect the defensive proteins including kinases and RubisCO [43]. It is because that there is an induction in genes expression of proteins that cause resistance [44]. It has been reported that protein accumulation in all treatments of *Melissa officinalis*, is responsible for protection of plants during considerable water loss [45].

Table 1 Effect of Salicylic Acid (0, 0.25, 0.50, 0.75 & 1 mM) on mean performance of the *Valariana walichii* DC for morphological traits under drought stress (100%, 75%, 50% & 25% FC) during vegetative and flowering stages

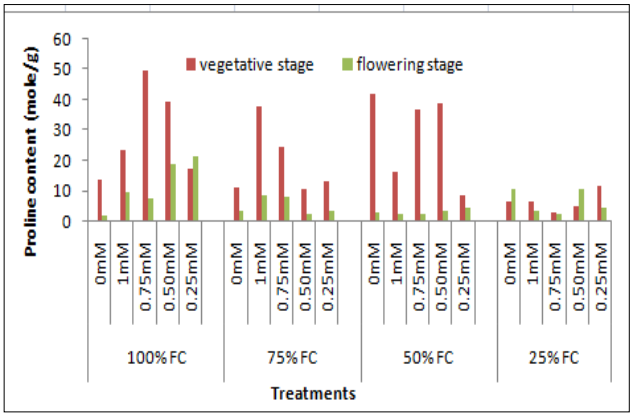
Treatments	No. of leaf (±SE)		Leaf area (cm^2) (±SE)		Shoot length (cm) (±SE)		Root length (cm) (±SE)		Shoot dry matter content (%) (±SE)		Root dry matter content (%) (±SE)	
	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering	Vegetative	Flowering
100%+0mM	10.33 ±1.07	15.00 ± 1.20	14.07± 1.32	25.29 ±1.77	27.79 ±0.25	28.43± 0.44	20.04± 0.37	15.88± 0.48	9.44± 0.06	8.21± 0.02	24.53± 0.09	18.85± 0.02
100%+1mM	9.78± 0.58	11.33 ± 1.34	18.85± 0.99	26.83 ±3.05	21.45 ±0.28	30.75± 0.40	16.09± 0.42	11.15± 0.26	10.63 ± 0.16	9.423 ±0.20	23.43± 0.27	26.59± 0.17
100%+0.75mM	9.88± 1.78	11.22 ± 0.95	16.86± 1.14	27.83 ±3.44	22.35 0.19	31.82± 0.36	23.36± 0.47	15.95± 0.41	9.49± 0.08	10.42 ±0.11	15.53± 0.15	25.57± 0.06
100%+0.50mM	9.11± 0.44	9.77± 0.77	18.22± 1.37	30.31 ±2.74	29.66 ±0.19	93.58± 0.25	16.76± 0.39	20.10± 0.17	5.38± 0.04	11.64 ±0.17	29.33± 0.01	28.26± 0.06
100%+0.25mM	10.11 ±1.25	11.00 ± 0.51	18.67± 1.30	25.78 ±1.17	38.35 ±0.49	64.64± 0.20	15.29± 0.47	15.39± 0.32	11.48 ± 0.14	7.63± 0.05	22.48± 0.21	21.36± 0.09
75%+0mM	7.66± 0.19	11.89 ± 1.45	19.42± 2.25	24.72 ±1.67	22.74 ±0.35	21.83± 0.62	25.79± 0.46	11.70± 0.16	13.52 ± 0.15	10.48 ±0.13	29.76± 0.10	24.47± 0.12
75%+1mM	12.66 ±1.01	12.33 ± 1.83	19.89± 0.94	23.06 ±3.46	21.06 ±0.39	34.27± 0.40	18.23± 0.61	20.13± 0.26	13.37 ± 0.14	9.24± 0.12	27.60± 0.18	23.63± 0.09
75%+0.75mM	14.00 ±0.69	13.88 ± 0.72	16.13± 1.37	22.090± 1.15	25.23 ±0.49	47.99± 0.19	21.10± 0.53	21.25± 0.62	12.44 ± 0.10	8.20± 0.06	29.20± 0.09	19.39± 0.05
75%+0.50mM	12.22 ±2.78	14.110 ± 2.66	17.72± 1.91	20.97 ±1.77	14.99 ±0.57	94.66± 0.39	18.52± 0.55	19.44± 0.55	15.82 ± 0.08	11.63 ±0.17	26.38± 0.26	24.53± 0.16
75%0.25mM	9.55± 1.11	12.33 ± 1.52	16.37± 1.06	28.27 ±4.79	17.04 ±0.55	60.98± 0.37	11.41± 0.36	18.25± 0.36	10.357± 0.09	9.40± 0.13	20.72± 0.10	19.37± 0.20
50%+0mM	7.55± 0.80	9.55± 1.05	25.36± 1.36	24.03 ±2.15	22.76 ±0.47	25.090± 0.47	26.08± 0.44	17.147± 0.39	11.57 ± 0.15	29.49 ±0.19	31.34± 0.06	21.76± 0.12
50%+1mM	8.77± 1.12	8.77± 1.39	18.03± 4.25	17.97 ±3.87	19.95 ± 0.31	28.16± 0.66	24.40± 0.39	11.91± 0.07	8.54± 0.17	8.71± 0.25	25.77± 0.18	26.90± 0.01
50%+0.75mM	9.22± 0.96	10.00 ± 0.19	12.11± 1.33	17.54 ±0.72	24.77 ±0.33	40.92± 0.76	22.250± 0.88	17.46± 0.76	11.49 ± 0.27	8.47± 0.21	20.78± 0.18	28.47± 0.15
50%+0.50mM	8.88± 0.29	10.00 ± 0.38	13.11± 3.12	23.77 ±1.26	38.66 ± 0.40	34.51± 0.62	30.28± 0.38	23.71± 0.15	6.36± 0.11	9.47± 0.09	25.34± 0.14	27.57± 0.15
50%+0.25mM	9.55± 1.73	12.44 ± 1.94	18.24± 1.10	26.01 ±3.53	18.04 ±0.42	28.50± 0.34	21.58± 0.58	12.18± 0.23	10.58 ± 0.12	11.78 ±0.08	34.28± 0.05	29.18± 0.04
25%+0mM	7.66± 0.77	10.44 ± 0.96	17.16± 2.60	18.28 ±3.86	16.50 ±0.45	32.84± 0.62	11.47± 0.68	19.05± 0.53	26.74 ± 0.09	10.24 ±0.03	54.49± 0.08	25.423 ±0.21
25%+1mM	6.44± 0.11	9.66± 0.51	11.02± 1.88	20.37 ±3.50	28.66 ±0.57	35.66± 0.75	28.28± 0.30	23.15± 0.26	16.13 ± 0.03	12.74 ±0.05	21.51± 0.19	22.59± 0.19
25%+0.75mM	8.33± 0.69	14.00 ± 1.16	9.44± 1.37	20.16 ±2.15	20.61 ±0.46	33.34± 0.51	14.83 0.57	15.51± 0.63	8.40± 2.70	12.59 ±0.08	26.48± 0.19	28.15± 0.02
25%+0.50mM	8.11± 1.54	12.33 ± 1.34	12.53± 2.12	22.54 ±1.60	30.35± 0.689	28.51± 0.483	41.33± 0.63	17.45± 0.27	13.93 ± 1.23	9.40± 0.17	25.42± 0.11	22.580 ±0.06
25%+0.25mM	6.55± 0.72	11.00 ± 1.16	12.35± 0.72	24.20 ±0.82	28.86 ±0.38	45.72± 0.39	34.58± 0.60	29.29± 0.41	16.27 ± 0.31	15.55 ±0.13	35.40± 0.14	25.45± 0.18
C.D.	3.334	3.234	5.471	5.211	1.281	C1.366	1.518	1.143	1.906	0.409	0.456	0.373
SE(m)	1.160	1.320	1.904	2.746	0.446	0.475	0.528	0.398	0.663	0.142	0.159	0.130
SE(d)	1.641	1.867	2.692	3.883	0.630	0.672	0.747	0.562	0.938	0.201	0.224	0.183
C.V.	21.553	19.787	20.250	20.235	3.152	1.955	4.142	3.868	9.495	2.193	0.999	0.917

Among all the control values, maximum decrease in phenol content was found in 50% FC during vegetative stage and 100% FC during flowering stage (Fig 1D). In the *Nigella*, drought resulted in decline of phenol compounds [46]. One of the possible reasons for reduction in these compounds under drought stress is related to the antioxidant characteristics of these compounds to scavenging of ROS under drought stress [47]. Whereas maximum increase was observed in treatment with 30% FC + 0.75 mM SA concentration in both vegetative and flowering stages. While 0.75 mM SA concentration during vegetative stage and 0.50 mM SA concentration during flowering stage resulted in maximum increase in all different treatments of drought stress (Fig 1D). Increased synthesis of various phenolic compounds in response to elicitor's application, especially SA, explained by the induction of a state of oxidative stress in plants as exogenous SA, even at low concentrations, interacts with signalling mechanisms of stress [48].

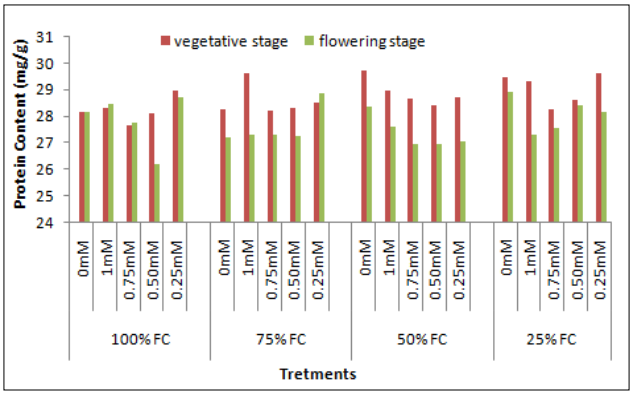
Both during vegetative and flowering stages, maximum proline content was found in 50% FC and 25% FC among all the control values whereas, minimum concentration of proline content found in 25% FC + 0.75 mM SA (vegetative stage) and 75% FC + 0.50 mM SA (flowering stage). While, minimum concentration of proline was found in interaction drought stress with 0.25 mM SA (vegetative stage) and 0.75 mM SA (flowering stage) in comparison with their respective control values (Fig 1B). Proline is a major amino acid in the plant defense system against abiotic stresses [49]. Proline content increases with the increase in the level of drought stress condition. The accumulation of osmolytes allows additional water to be taken up from the environment, thus reducing the immediate effect of water shortage within the plant and they help to stabilize protein tertiary structure and cells [50-51]. Accumulation of proline under stress conditions, supplies energy for survival and growth and thereby enhancing plant tolerance to stress [52]. The level of proline-degrading enzyme proline oxidase activity was inhibited in the roots by drought stress with the increasing gamma-glutamyl kinase activity which is the reason for higher proline accumulation during drought stress in lemongrass and tomato [53-54].



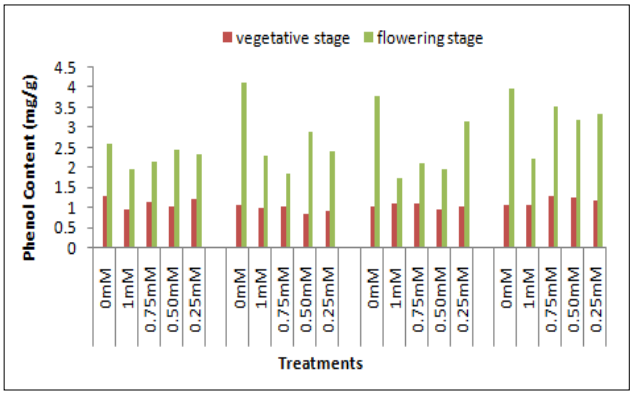
(A) Chlorophyll content



(B) Protein content



(C) Proline content



(D) Phenol content

Fig 1 Effect of different concentrations of Salicylic acid (0, 0.25, 0.50, 0.75 & 1 mM) under drought stress (100% FC, 75%FC, 50% FC and 25% FC) during vegetative and flowering stages

CONCLUSION

Drought is an important abiotic factor influences the growth and physiological characteristics of the plants. Plants responses toward drought stress depend on species and genotype, the length and severity of water deficit, and the age and stage of development. Stress factors are well known to cause a shift in the antioxidant balance in plant cells. This shift is due to an increase in the rate of generation of ROS. The present study highlights the role of SA under drought stress which could be applied as an important signal molecule for modulating plant responses to drought stress and participates in the regulation of physiological processes. The exogenous application of SA at concentrations of 0.25 to 0.50 mM constituted a positive impact on morphological and biochemical characters in *V. wallichii*. However, the low concentration of SA mitigates the increasing level of stress during water scare conditions which can be helpful in escalating the survival rate and yield of plant under drought stress.

Author's Contribution and Competing Interests

Conducted research as a part of doctoral research and there are no competing interests.

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