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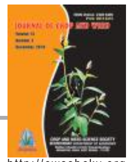

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Nodal plant materials of *Valeriana jatamansi*, a higher altitude-specific medicinal plant typically retained the previous lower altitude exposure and modulated above-ground probes

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ABSTRACT

Valeriana jatamansi, a critically endangered high-altitude (HA) specific medicinal plant was chosen to evaluate the lower altitude (LA) exposure for one, two and three seasons on above ground characters. The result revealed that one season LA exposure did not modulate above-ground probes understudy in comparison to control, the HA-grown plant. But LA exposure for two seasons significantly modulated 'the largest leaf length', 'the largest leaf breadth', 'the largest petiole diameter', 'total number of branches', and 'total number of nodes' in comparison to that of the control. Marginal one season's LA exposure difference between two and one as well as three and two seasons LA exposed plants showed a significant difference on 'the largest leaf breadth', 'the largest petiole diameter', and 'the total number of nodes' in comparison to control. The summary of the finding indicated that the previous LA exposure was retained into vegetative nodes and inherited into the next generation.

Keywords: *Valeriana jatamansi*, higher altitude plant, lower altitude effect, above ground characters.

Valeriana jatamansi (Jones) (family: *Caprifoliaceae*) is a critically endangered and Himalayan region-specific medicinal plant, naturally adapted in high altitude like 1000 to 3000m above sea level (ASL) (Bhattacharjee, 2000).Usually, this medicinal plant was found on primary litter and loamy porous soil enriched with organic matter like humus. Characteristically, *Valeriana* preferred to traditionally grow on hill slopes, moist places, damp woods, ditches and along the streams. The over exploitation of root and rhizome due to its high medicinal value categorized into a critically endangered medicinal plant (Sher *et al*., 2011). The rhizome and/or adventitious root enriched with active components, namely valerenic acid and valepotriates, was traditionally used for curing obesity, skin diseases, epilepsy, insanity,and snake poisoning (Prakash,1999, Mishra, 2004). The rhizome was used in the Ayurvedic system of medicine as a raw tonic, stimulant, antispasmodic, and to treat hysteria, convulsions and epilepsy. The adventitious root was reported to treat insomnia and blood, circulatory, and mental disorders. *Valeriana* was instantly recognised as one of the most famous and important of the Ayurvedic herbs, recorded for better and deep sleep, a calm mind, and a bewildering variety of other mental/emotional benefits. In valuable addition, *Valeriana* was an indigenous brain nervine excellent tonic and a cognitive enhancer. When a higher altitude (HA) specific plant was planted into lower altitude (LA), a lot of consequences may be recorded. These in common are: apparent inability to typically survive, reduced vegetative and gradual growth, inability to flower initiation, inability to seed setting, etc. Interestingly, *Valeriana* can satisfactorily complete the life cycles in the foot hill of the Himalayan region to three generations based on our records. During visual observation, the morphology was deviated from the plant grown from HA region. But there was no scientific report on quantum of the change was evidenced due to LA exposure of the HA adapted plant. As follows, this HA adapted plant will realistically be an excellent material for recording the vegetative changes when exposed to LA for one, two and three seasons sequentially. Moreover, one or two LA exposure differences will give valuable clues about the accumulative LA exposure effect and carried forward past LA exposure-based memory. The reverse exposure of one and two seasons' LA exposed nodal plant material to one season HA exposure will give a ray on retention of the past LA exposure experience accumulated into vegetative nodal plant material.

To properly evaluate the profound LA effect in the early time point, the utmost precious is to generate LA exposed nodal plant materials. The vegetative nodal plant material was typically harvested from HA grown plants and abbreviated as 'ha' (plant material) as no specific information was documented how many countless generations were completed in the natural environment. But HA grown nodal plant material mediated planting material when completed one life cycle in HA it will be abbreviated as '1ha-ha' indicating

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one season HA exposure of the base material, 'ha'. From direct observation, this plant satisfactorily completed its life cycle successfully in the foothills of the Himalayan region, known as Terai zone. The 'ha' nodal plant material mediated planting material when exposed to one, two and three seasons from LA environment, it will be abbreviated as '1la-ha' (one season LA exposure of the HA grown nodal plant material), '2la-ha' (two seasons LA exposure of the HA grown nodal plant material) and '3la-ha' (three seasons LA exposure of the HA grown nodal plant material). This material was considered to perceive the effect from lower altitude (LA) exposure for one, two, and three seasons LA exposure effect in comparison to control, '1ha-ha' (one successful season HA exposure of the 'ha' nodal plant material). The observed difference between '1ha-ha' and '1la-ha' (symbolized as 1ha-ha/1la-ha) was due to environmental which include altitude difference, weather difference, and soil environmental difference, etc. The difference between '1la-ha' and '2la-ha' (one successful LA season exposure difference and symbolized as '1la-ha'/'2la-ha'), and '2la-ha' and '3la-ha'(one season LA exposure difference and symbolized as '2la-ha'/'3la-ha'), '1la-ha' and '3la-ha' (two seasons LA exposure difference and symbolized as '3la-ha'/'1la-ha') was undue to environmental as every material include already one season LA exposure. Thus, an additional LA exposure would play a role if any significant difference was evidenced among the understudy characters. Interestingly, when '1la-ha' and '2la-ha' nodal plant material mediated planting material exposed from one season HA environment, it will be abbreviated as '1ha-1la-ha' (one successful season LA exposure of the 'ha' nodal plant material followed by one season HA exposure), and '1ha-2la-ha' (two seasons LA exposure of the 'ha' nodal plant material followed by one season HA exposure) to check the resuming effect of the characters understudy. The characters may be resumed and was comparable to control, '1ha-ha' or attenuated from resumption, rather it would be a permanent change in epigenetic level. Like so, this material will act as an excellent scientific material for advancing knowledge of epigenetic changes in response to altitude variation in future if sequential LA exposure effect was evidenced as significant, or the one season LA exposure difference between '1la-ha' and '2la-ha' or '2la-ha' and '3la-ha' or two seasons LA exposure difference between '1la-ha' and '3la-ha' was evidenced. The differential effect will address the fine-tuned epigenetic changes and advancing knowledge in the domain of epigenetics in altitude variation. Before that, this present scientific communication presented a base work on some above-ground probes from sequential exposure from LA environment in comparison to HA grown plant.

MATERIALS AND METHODS

Plant material

Already mentioned, *Valeria jatamansi* was used for this study. The base material was collected from Sikkim and maintained in the Kalimpong, West Bengal at Regional Research Station. The three-node containing plant material was used for the next generation. *Valeriana jatamansi* Jones was a gynodioceious in nature. The female plants produced pistillate flowers without an androceium and four white petals. Hermaphrodite plants with five petals and three stamens were produced. The vegetative propagation through nodes was followed to maintain the genetic background of the plant.

The uniform quality planting material (QPM) production

A hermaphrodite plants with five petals was selected from Kalimpong, West Bengal. The node mediated emergence was transplanted to both LA and HA plot. As the next generation was considered through vegetative means, the genetic background was maintained. The aerial nodal plant material having three nodes from one and two seasons' LA exposure was harvested. All nodal plant materials were transplanted into artificial soil in the controlled environment conditioned with 20°C, 70% RH, and 14h of 80.18 umol/s/m^2 light for raising the uniform quality planting material production. The 60 days uniform quality planting material was transplanted into HA and LA plots (Fig. 1.A.).

Above ground characters under study

The above ground characters like 'length (cm) of the largest leaf', 'breadth (cm) of the largest leaf', 'petiole diameter of the largest leaf', 'total number of nodes' and 'total number of branches' were considered for understudy. The data was taken from all experimental plants (Fig. 1.B).

Place of experiment

The experiment was carried out in the foothill region, known as the Terai zone in West Bengal, India. The LA farm is located at 28°19'N latitude and 89°23'E longitude and an altitude of 43 m above the mean sea level. The HA farm at Kalimpong is situated at 27°32' N latitude and 88°28'E longitude and an altitude of 1097 m above mean sea level. It is reported that this medicinal plant was naturally grown in high altitude in the hill zone ranging from 1000 to 3000m above mean sea level (ASL). Six ridge valleys were made for each treatment and six plants were maintained in each ridge valley. The number of plants considered for the data record was sixteen for each treatment.

Soil composition

Formulated soil was used for shoot emergence as well as initial quick growth assurance. Perlite, peat moss, and vermiculite (1:1:1; w/w/w) were the components of the artificial soil for better evidence in initial emergence from nodal plant material. It was reported that perlite promoted aeration to ensure an excellent air/water balance which impacted better root growth including better uptake of nutrients in a more effective manner. Peat moss retained moisture for better plant growth which also saved irrigation frequency. Moreover, releases of water and nutrients to the right proportions for optimum plant growth of plants, and reducing the application of manuring were the benefits. Vermiculite improved soil porosity as well as acted as a medium for water and nutrient Exchange. It also helped in the minimization of soil deterioration and ensured vital nutrients were not washed away.

Initial fresh shoot emergence and its environment

The initial environment was very crucial for new shoot initiation which will give uniform planting material. The transparent plastic container with a lid was used for maintaining the humidity. The small plastic pot was filled with artificial soil and kept in the transparent plastic box which was incubated in an environment of 80.18 umol/s/m^2 light intensity, 14-h light condition per day, 70% of relative humidity. At least 14 days were followed for assuring the emerging of new shoot formation.

The preparation for the experimental planting material

In the first year, nodal plant material was harvested from HA grown single plant. Fifty percent nodal plant material mediated plantlets were transplanted into LA plot and another fifty percent nodal plant material mediated plantlets were transplanted into HA plot (Fig. 1.B). In the second year, nodal plant material was harvested from all plants ('1ha-ha' and '1la-ha') grown from LA and HA plot (Fig. 1.B). Fifty percent nodal plant material mediated plantlets from all plant types were transplanted into LA plot and another fifty percent nodal plant material mediated plantlets were transplanted into HA plot. In the third year, nodal plant materials were harvested from both HA and LA plot (Fig. 1.B). All the nodal plant material mediated plantlets ('ha', '1la-ha' and '2la-ha') were transplanted into HA and LA plot. The number of plantlets were considered sixteen for each type of plant material.

Symbols used

The environments like higher altitude and lower altitude were abbreviated as HA and LA. The original material was nodal plant material harvested from HA grown plant and symbolized as '1ha-ha'. One season's HA exposure of the HA grown nodal (1ha-ha) plant material will be 'ha' (Fig. 1.B). The nodal plant material (ha) mediated planting material was grown in lower altitude (LA) for one more season and symbolized as '1la-ha' (Fig. 1.B). The nodal plant material (1la-ha) mediated planting material was grown in lower altitude (LA) for one more season and symbolized as '2la-ha'. The nodal plant material (2la-ha) mediated planting material was grown in lower altitude (LA) for one more season and symbolized as '3la-ha' (Fig. 1.B). The nodal plant material (1la-ha) mediated planting material was grown in higher altitude (HA) for one more season and symbolized as 'ha-1la-ha' (Fig. 1.B). The nodal plant material (2la-ha) mediated planting material was grown in higher altitude (HA) for one more season and symbolized as 'ha-2la-ha' (Fig. 1.B).

Above ground data collection

The full-grown plant was uprooted and recorded the data on 'length (cm) of the largest leaf', 'breadth (cm) of the largest leaf', 'petiole diameter of the largest leaf', 'total number of nodes' and 'total number of branches.

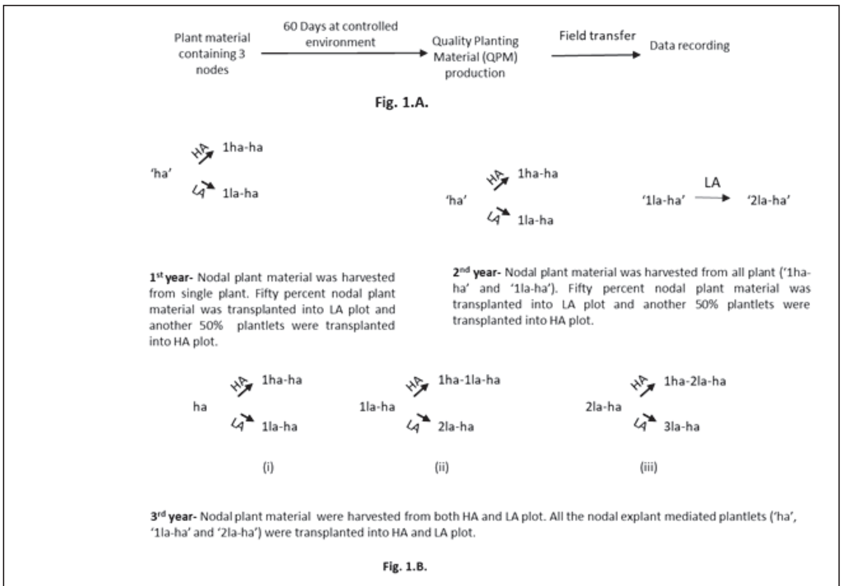
Software used

One-way ANOVA and Tukey's HSD Calculator was used for calculation (https://www.icalcu.com/stat/anova-tukey-hsd-calculator.html) to calculate p values at 0.05% level of significance to see any significant difference was present or not. Analysis of Variance (ANOVA) was calculated online available software (https://www.danielsoper.com/statcalc/calculator.aspx?id=43).

RESULT

Uniform planting material production, LA exposed plants and characters under study

The present study aimed to monitor the 'exposure effect' from the lower altitude (LA) environment of a higher altitude (HA) adapted medicinal plant. The seed-mediated plant propagation was not considered to the next generation for all experiment as cross pollination creates genetic variation. The advantage of vegetative propagation is to keep the genotype purity generation after generation. The nodal plant material was typically harvested from one and two seasons' LA-grown plants and nodal plant material was transplanted into both LA and HA environment (Fig. 1.A). The testable hypothesis in the present study in common was to objectively evaluate on lower altitude effect on above ground characters and it's carried forward the LA exposure through vegetative nodal plant material. Therefore, some above-ground probes like 'the largest leaf length', 'the largest leaf breadth', 'the largest petiole diameter', 'the total number of nodes present', and 'the total number of branches' produced were monitored and focused for data recording. The plant satisfactorily completed its life cycle and produced flowers and viable seeds up to three generations from our experimental record. But from the first LA exposure, the overall morphology has deviated from the HA-grown plant but there was no specific information about that. That's why, a scientific approach was employed to record some above-ground characters mentioned earlier. The uniform planting material growth was a key issue for field transplantation. Therefore, the uniform planting material was produced in the controlled environment (Fig. 1.A). The aerial nodal plant material was typically harvested and planted into artificial soil for the shoot emergence in the controlled environment conditioned with 22°C, 70% RH, and 14h of 6000 LUX light and 10h dark for raising the uniform planting material production. The 60 days uniform planting material was transplanted to both LA as well as HA plots including control.



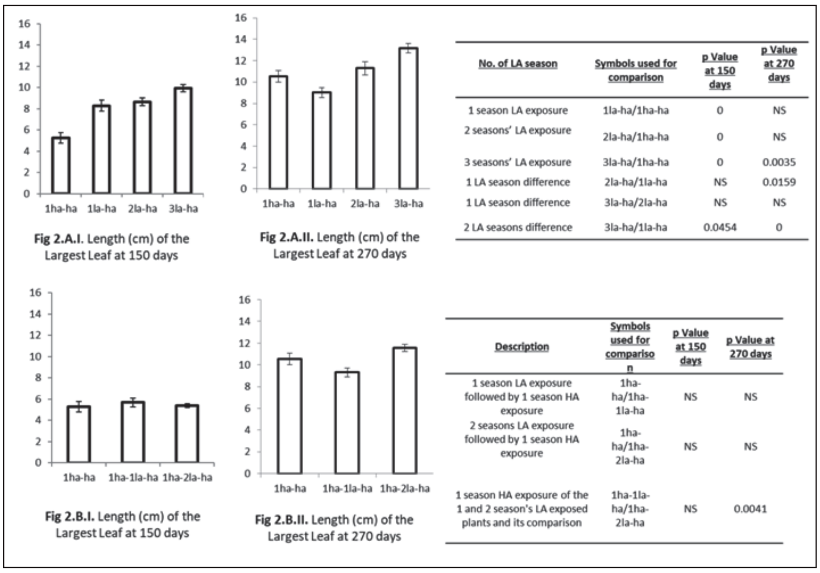
Parameters	Descriptions	Symbols used
Comparison between one season HA exposure from one, two and three seasons' LA exposure.	One season LA exposure difference from one season HA exposure.	1ha-ha/1la-ha
	Two seasons' LA exposure difference from one season HA exposure.	1ha-ha/2la-ha
	Three seasons' LA exposure difference from one season HA exposure.	1ha-ha/3la-ha
One or two seasons' LA exposure difference among LA exposed plants.	One season' LA exposure difference.	1la-ha/2la-ha
	One season' LA exposure difference.	2la-ha/3la-ha
	Two seasons' LA exposure difference.	1la-ha/3la-ha
Reverse exposure (rescue effect)	One season's HA exposure of the one season LA exposed nodal plant.	1ha-ha/1ha-1la-ha
	One season's HA exposure of the two seasons' LA exposed nodal plant.	1ha-ha/1ha-2la-ha
	Comparison of rescue effect between one and two seasons' LA exposed nodal plant.	1ha-1la-ha/1ha-2la-ha

Fig. 1.C.

Fig. 1: The overview of planting material production and LA exposure details under study. Fig. 1. A**.** The process of planting material production from all kinds of LA exposed plant material and subsequent transplantation into the field. Fig. 1. B**.** The aerial nodal plant material of one, two, and three seasons' LA exposure. Fig. 1.C**.** In the present study, the following comparison was consiidered under study: a comparison between one season HA exposure from one, two, and three seasons' LA exposure of the base material, ''ha'; any above ground character difference in one season LA exposure difference or two seasons' LA exposure difference among all LA exposed plants; the reverse exposure to resume the above ground characters in one, and two seasons' LA exposed plants; comparison between one and two seasons' LA exposed plants when exposed to one season HA environment.

Lower altitude effect on length (cm) of the Largest Leaf

The result sufficiently showed that 150 days of LA exposure showed a significant higher leaf length in '1la-ha' as compared to '1ha-ha' (p= 0; Fig. 2.A.I) whereas '3la-ha' showed significantly higher leaf length as compared to '1la-ha'. This indicated precisely that two seasons potential LA exposure was accumulated into a nodal plant material (p= 0.0454; Fig. 2. A.I). At 270 days, one season LA exposure did not include any profound effect on the leaf length (Fig. 2. A. II) whereas leaf length difference was evidenced between '3la-ha' and '1ha-ha' (p=0.0035; Fig. 2. A. II) as well as '2la-ha' and '1la-ha' (p= 0.159; Fig. 2. A.II). This result

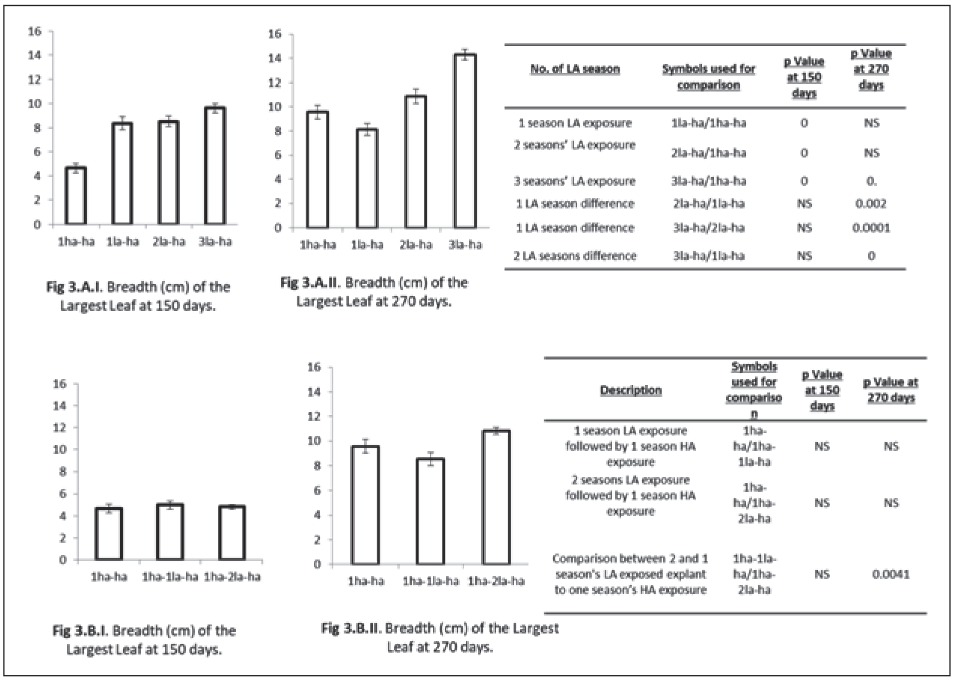


No. of LA season	Symbols used for comparison	p Value at 150 days	p Value at 270 days
1 season LA exposure	1la-ha/1ha-ha	0	NS
2 seasons' LA exposure	2la-ha/1ha-ha	0	NS
3 seasons' LA exposure	3la-ha/1ha-ha	0	0.0035
1 LA season difference	2la-ha/1la-ha	NS	0.0159
1 LA season difference	3la-ha/2la-ha	NS	NS
2 LA seasons difference	3la-ha/1la-ha	0.0454	0

Description	Symbols used for compariso n	p Value at 150 days	p Value at 270 days
1 season LA exposure followed by 1 season HA exposure	1ha-ha/1ha-1la-ha	NS	NS
2 seasons LA exposure followed by 1 season HA exposure	1ha-ha/1ha-2la-ha	NS	NS
1 season HA exposure of the 1 and 2 season's LA exposed plants and its comparison	1ha-1la-ha/1ha-2la-ha	NS	0.0041

Fig. 2: Lower altitude effect on'Length (cm) of the Largest Leaf'. The result showed that one season LA exposure modulated significantly on 'leaf length of the largest leaf' recorded at 150 days (Fig 2.A.I; p=0). At 270 days, the resume in the leaf length was recorded in '1la-ha' and '2la-ha' but not in '3la-ha' (Fig 2.A.II; p=0.0035) when compared to '1ha-ha'. Very interestingly, one season HA exposure resumed 'Length (cm) of the Largest Leaf' which was comparable to '1ha-ha' at 150 days (Fig 2.B. I; p=0.454) and at 270 days (Fig 2.B.II).

signified that previous LA exposure memory was retained into vegetative nodal plant material and transmitted into next generation. The rescue experiment evidenced that one successful HA exposure erased the LA exposure memory from nodal plant material in 150- and 270 days in leaf length of the largest leaf (Fig. 2.B.I and II).



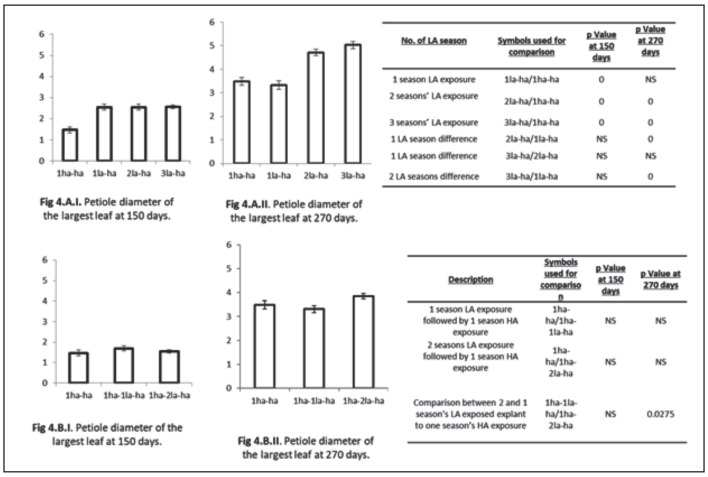
No. of LA season	Symbols used for comparison	p Value at 150 days	p Value at 270 days
1 season LA exposure	1la-ha/1ha-ha	0	NS
2 seasons' LA exposure	2la-ha/1ha-ha	0	NS
3 seasons' LA exposure	3la-ha/1ha-ha	0	0.
1 LA season difference	2la-ha/1la-ha	NS	0.002
1 LA season difference	3la-ha/2la-ha	NS	0.0001
2 LA seasons difference	3la-ha/1la-ha	NS	0

Description	Symbols used for compariso n	p Value at 150 days	p Value at 270 days
1 season LA exposure followed by 1 season HA exposure	1ha-ha/1ha-1la-ha	NS	NS
2 seasons LA exposure followed by 1 season HA exposure	1ha-ha/1ha-2la-ha	NS	NS
Comparison between 2 and 1 season's LA exposed explant to one season's HA exposure	1ha-1la-ha/1ha-2la-ha	NS	0.0041

Fig. 3: Lower altitude effect on'Breadth (cm) of the Largest Leaf'. The result indicated that one season LA exposure enabled significantly higher breadth (cm) of the largest leaf recorded at 150 days (Fig 3.A. I; p=0) but at 270 days, the leaf breadth was resumed and comparable to '1la-ha' and '2la-ha' but not from '3la-ha' (Fig 3.A.II;p=0). Very interestingly, one season HA exposure resumed its original 'breadth (cm) of the largest leaf' which was comparable to '1ha-ha' at 150 days (Fig 3.B. I;p=0.779) and 270 days (Fig 3.B.II).

Lower altitude effect on breadth of the largest leaf

In leaf breadth, the result overwhelmingly showed that 150 days of LA exposure showed a significantly higher leaf breadth in '1la-ha', '2la-ha', '3la-ha' as compared to '1ha-ha' (p= 0; Fig. 3. A.I). In 270 days of LA exposure did not show any effect on the leaf breadth (Fig. 2. A. II) whereas leaf breadth difference was evidenced between '2la-ha' and '1la-ha' (p=0.002; Fig. 3. A. II) as well as '3la-ha' and '2la-ha' (p= 0.0001; Fig. 2.A.II) having marginal one LA season exposure difference. The rescue experiment evidenced that one season of potential HA exposure resumed the leaf breadth in the largest leaf both recorded in both 150- and 270 days (Fig. 3.B.I and II).



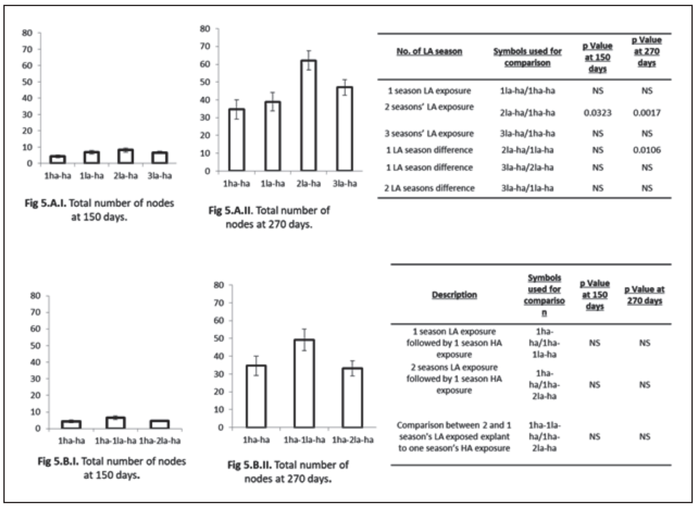
No. of LA season	Symbols used for comparison	p Value at 150 days	p Value at 270 days
1 season LA exposure	1la-ha/1ha-ha	0	NS
2 seasons' LA exposure	2la-ha/1ha-ha	0	0
3 seasons' LA exposure	3la-ha/1ha-ha	0	0
1 LA season difference	2la-ha/1la-ha	NS	0
1 LA season difference	3la-ha/2la-ha	NS	NS
2 LA seasons difference	3la-ha/1la-ha	NS	0

Description	Symbols used for comparison	p Value at 150 days	p Value at 270 days
1 season LA exposure followed by 1 season HA exposure	1ha-ha/1ha-1la-ha	NS	NS
2 seasons LA exposure followed by 1 season HA exposure	1ha-ha/1ha-2la-ha	NS	NS
Comparison between 2 and 1 season's LA exposed explant to one season's HA exposure	1ha-1la-ha/1ha-2la-ha	NS	0.0275

Fig. 4: Lower altitude effect on'petiole diameter of the largest leaf'. The result exhibited that one season LA exposure enabled a significantly higher 'petiole diameter of the largest leaf' recorded at 150 days (Fig 4.A. I;p=0) but at 270 days' exposure, the insignificant petiole diameter of the largest leafwasrecorded in '1la-ha' but not in '2la-ha' and '3la-ha' (Fig 4.A.II; p=0). Very interestingly, one season HA exposure resumed its original 'petiole diameter of the largest leaf' which was comparable to '1ha-ha' at 150 days (Fig 4.B. I;p=0.389) and 270 days (Fig 4.B.II).

Lower altitude effect on petiole diameter

The lower altitude effect on petiole diameter at the middle, the result showed that 150 days of LA exposure showed a significantly higher petiole diameter in '1la-ha', '2la-ha', '3la-ha' as compared to '1ha-ha' (p= 0; Fig. 4.A.I). In 270 days, one successful season LA exposure did not have any effect on the petiole diameter (Fig. 4. A. II) whereas a significant petiole diameter difference was evidenced between '2la-ha' and '1la-ha' (p=0; Fig. 4. A. II) which signified that marginal one LA season difference had a role. The rescue experiment evidenced that one season potential HA exposure



No. of LA season	Symbols used for comparison	p Value at 150 days	p Value at 270 days
1 season LA exposure	1la-ha/1ha-ha	NS	NS
2 seasons' LA exposure	2la-ha/1ha-ha	0.0323	0.0017
3 seasons' LA exposure	3la-ha/1ha-ha	NS	NS
1 LA season difference	2la-ha/1la-ha	NS	0.0106
1 LA season difference	3la-ha/2la-ha	NS	NS
2 LA seasons difference	3la-ha/1la-ha	NS	NS

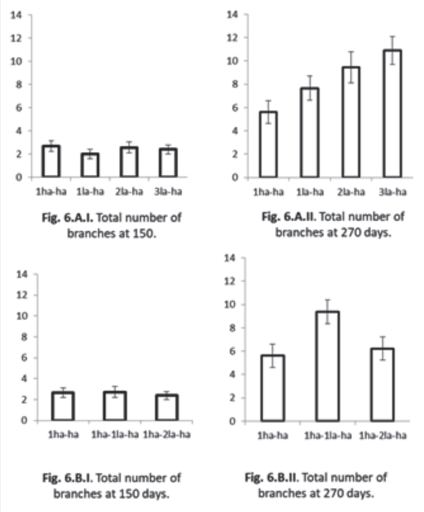
Description	Symbols used for comparison	p Value at 150 days	p Value at 270 days
1 season LA exposure followed by 1 season HA exposure	1ha-ha/1ha-1la-ha	NS	NS
2 seasons LA exposure followed by 1 season HA exposure	1ha-ha/1ha-2la-ha	NS	NS
Comparison between 2 and 1 season's LA exposed explant to one season's HA exposure	1ha-1la-ha/1ha-2la-ha	NS	NS

Fig. 5: Lower altitude effect on'total number of nodes. The result evidenced that '2la-ha' produced a significantly higher 'node number' at 150 days (Fig 5.A. I; p=0.0323) and same trend was evidenced at 270 days (Fig 5.A.II;p=0.0017). Very interestingly, one season HA exposure resumed 'total number of nodes' which was comparable to '1ha-ha' at 150 days and 270 days (Fig 5.B.I and II;p=0.149 and p=0.0727).

resumed the petiole diameter recorded in 150-and270 days (Fig. 4.B. I and II).

Lower altitude effect on number of nodes production

The result showed that 150 days of LA environmental exposure showed a significantly higher number of nodes in ‘2la-ha’ as compared to ‘1ha-ha’ (p= 0.0323; Fig. 5. A.I) when one season LA exposure did not have any effect. In 270 days, the same trend was also evidenced between ‘2la-ha’ and ‘1ha-ha’ (p= 0.0017; Fig. 5.A.II). In addition, a significant difference was evidenced between ‘2la-ha’ and ‘1la-ha’ (Fig.5. A. II) which was indicative of a marginal one LA seasonal effect. The rescue experiment evidenced that one successful HA seasonal exposure resumed the total number of nodes recorded both in 150-and 270 days (Fig. 5.B. I and II).



No. of LA season	Symbols used for comparison	p Value at 150 days	p Value at 270 days
1 season LA exposure	1la-ha/1ha-ha	NS	NS
2 seasons' LA exposure	2la-ha/1ha-ha	NS	NS
3 seasons' LA exposure	3la-ha/1ha-ha	NS	0.0097
1 LA season difference	2la-ha/1la-ha	NS	NS
1 LA season difference	3la-ha/2la-ha	NS	NS
2 LA seasons difference	3la-ha/1la-ha	NS	NS

Description	Symbols used for comparison	p Value at 150 days	p Value at 270 days
1 season LA exposure followed by 1 season HA exposure	1ha-ha/1ha-1la-ha	NS	0.0267
2 seasons LA exposure followed by 1 season HA exposure	1ha-ha/1ha-2la-ha	NS	NS
Comparison between 2 and 1 season's LA exposed explant to one season's HA exposure	1ha-1la-ha/1ha-2la-ha	NS	NS

Fig. 6: Lower altitude effect on‘total number of branch’. The result showed that ‘3la-ha’ showed a significantly higher ‘total number of branch’ at 270 days (Fig 6.A.II; p=0.0097) but not in 150 days (Fig 6.A.I;p=0.012) as compared to ‘1ha-ha’. Very interestingly, one season HA exposure resumed ‘total number of branches’ which was comparable to ‘1ha-ha’ at 150 days and 270 days (Fig 6.B.I and II).

Lower altitude effect on number of branch production

The total number of branches was unvaried among all nodal plant materials at 150 days from LA exposure (Fig. 6. A.I). At 270 days, a significant number of branches was evidenced in ‘3la-ha’ as compared to ‘1ha-ha’ (p= 0.0097; Fig. 6.A.II) when one season LA exposure did not have any effect. Interestingly, third season showed a significant number of branches production. The rescue experiment evidenced that one season HA exposure resumed the character recorded in both 150-and 270 days (Fig. 6.B.I and II) except ‘1ha-1la-ha’ at 270 days.

DISCUSSION

It was a striking fact to know that one season potential LA exposure did not have any effect on any characters recorded in 270 days (Fig. 7) but one season LA exposure effect at 150 days showed a significant variation on ‘length of the largest leaf’, ‘breadth of the largest leaf’, ‘petiole diameter of the largest leaf’ in comparison to control plant, ‘1ha-ha’. This means the LA exposure effect was buffered in the time being within the same LA exposure season in ‘1la-ha’. Interestingly, all plantlets, age of 60 days were produced from a controlled environment. The LA exposure period, 150 days typically showed the LA exposure difference of the above-mentioned characters. Interestingly, two seasons’ LA exposure (‘2la-ha’) showed a significant variation in ‘length of the largest leaf’, ‘breadth of the largest leaf’, ‘petiole diameter of the largest leaf’ and ‘total number of nodes’ recorded at 150 days in comparison to control, ‘1ha-ha’. At 270 days, the ‘2la-ha’ showed a significant variation in ‘petiole diameter’ and ‘number of nodes’ only (Fig. 7). The plant ‘2la-ha’ displayed a significant change in four characters at 150 days of LA exposure, whereas 270 days LA exposure, the plant ‘2la-ha’ showed a significant change in only two characters. This means that 270-150= 120 days of LA exposure, the ‘2la-ha’ plant resumed two characters which were comparable to ‘1ha-ha’. Consequently, there was a tendency of the plant, ‘2la-ha’, to resume the characters towards the control plant, ‘1ha-ha. Three seasons’ LA exposure (‘3la-ha’) also exhibited a significant variation at 150 days in three characters like

Parameters	Symbols used for comparison	Length (cm) of the Largest Leaf		'Breadth (cm) of the Largest Leaf		Petiole diameter of the largest leaf		Total number of nodes		Total number of branches	
		150 D	270 D	150 D	270 D	150 D	270 D	150 D	270 D	150 D	270 D
1, 2 and 3 seasons' LA exposure effect in comparison to '1ha-ha'.	1ha-ha/1la-ha	0	NS	0	NS	0	NS	NS	NS	NS	NS
	1ha-ha/2la-ha	0	NS	0	NS	0	0	0.0323	0.0017	NS	NS
	1ha-ha/3la-ha	0	0.0035	0	0.	0	0	NS	NS	NS	0.0097
Comparison of one or two seasons' LA exposure difference	1la-ha/2la-ha	NS	0.0159	NS	0.002	NS	0	NS	0.0106	NS	NS
	2la-ha/3la-ha	NS	NS	NS	0.0001	NS	NS	NS	NS	NS	NS
	1la-ha/3la-ha	0.0454	0	NS	0	NS	0	NS	NS	NS	NS
Reverse exposure of the previously LA exposed nodal explant to HA and comparison to '1ha-ha'	1ha-ha/1ha-1la-ha	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.0267
	1ha-ha/1ha-2la-ha	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rescue effect between 2 and 1 season LA exposed plant when both exposed to one season's HA environment.	1ha-1la-ha/1ha-2la-ha	NS	0.0041	NS	0.0041	NS	0.0275	NS	NS	NS	NS

Fig. 7: The summary of the result showed that marginal one season LA exposure difference in ('1la-ha' & '2la-ha') and ('2la-ha' & '3la-ha'') was evidenced in leaf breadth, petiole diameter and number of nodes. The one season HA exposure of the nodes having previous one and two seasons' LA exposure (('1la-ha' and '2la-ha') resumed all characters in just one season HA exposure.

leaf length, leaf breadth, and petiole diameter in comparison to '1ha-ha' (Fig. 7). The same result was also evidenced at 270 days recorded in '3la-ha'. Additionally, the total number of branches was recorded a significant in '3la-ha' in comparison to '1ha-ha'

The marginal one season LA exposure difference either in ('2la-ha' and '1la-1la') or ('3la-1la' and '2la-2la') was not recorded in 150 days but a significant different was evidenced at 270 days in leaf length, leaf breadth, petiole diameter and number of nodes (Fig. 7). Interestingly, all characters were resumed in one season LA exposure in all nodal plant materials (Fig. 7) except total number of branches in '1ha-1la-ha' at 270 days. This is a unique finding that one season LA exposed nodal plant material attenuated the possible resumption of the total number of branches in comparison to '1ha-ha' (Fig. 7). The idea was solidified from two other observations from a significant difference observation from '3la-ha/1la-ha' and '1ha-1la-ha/1ha-2la-ha'. Two seasons additional LA exposure recorded in '3la-ha/1la-ha' evidenced that leaf length, leaf breadth, and petiole diameter were not resumed. The identical characters like leaf length, leaf breadth, and petiole diameter were not resumed between '1ha-1la-ha/1ha-2la-ha'. Therefore, accumulative LA effect was evidenced as well as one season HA exposure erased the past LA exposure experience from vegetative nodal plant material as well. Hence, it will be a good model system for investigating the epigenetic basis of character modulation in reference to altitude variation.

The consistent basis of epigenetics lies on the molecular factors and processes around the DNA packaging assembly that potentially regulate gene activity independent of DNA sequence, and interestingly, it was mitotically stable (Skinner, 2011). The molecular reasons behind epigenetics typically included 'DNA methylation' (Holliday and Pugh, 1975], 'histone modifications' (Turner., 1998), 'non-coding RNAs' (Mattick, 2009; Jodar *et al*., 2013), 'chromatin structure' (Yaniv, 2014), and 'RNA methylation' (Schaefer *et al*., 2010). Epigenetic processes represent the molecular mechanisms for exposure due to changed environments with subsequent gene expression cascades. For example, the epigenetic process represents the driving mechanism to differentiate a stem cell to a different cell type (Skinner, 2011; Boland *et al*., 2014; Avgustinova *et al*., 2016). Therefore, epigenetic phenomena remain an integral part of normal biology. The concept of epigenetic transgenerational inheritance remains germline-mediated inheritance of epigenetic information between generations in the absence of continued direct environmental influences that lead to the phenotypic variation (Skinner, 2011; Skinner, 2008). In our present study, it was evidenced that vegetative plant parts showed previous LA exposure retention. The nodal plant material allowing three nodes with vascular system with effective mass of tissue were responsible for character modulation. Further research on epigenetic level of the nodal plant material tissue definitely gives a clue as to the basis of previous LA exposure retention. The novel molecule accumulation in the vascular system in the nodal plant material in addition gives a ray of hope the specific role of vascular system in previous LA exposure retentionand inheritance.

DECLARATION OF CONFLICTS OF INTERESTS / COMPETING INTERESTS.

The communicating author (HAM) declared that there is no conflict of interest.

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