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An assessment of andrographolide production in *Andrographispaniculata* grown in different agro-climatic locations

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An interlocational study was carried out to assess the effect of different agro-climatic locations on the secondary metabolite production in the medicinal plant *Andrographis paniculata*. Three accessions of *A. paniculata* were grown in three different locations of India differing in climatic conditions in RBD experimental plots. The andrographolide content in the plant samples of three accessions of *A. paniculata* was estimated from plants collected from these three locations, namely Anand, Kalyani and Solan. The three accessions with no morphological differences were characterised using RAPD and ISSR profiles. As the plants were grown in different agro-climatic conditions, as expected, there was variation in the andrographolide content in the three accessions. Samples collected from Kalyani were found to contain maximum andrographolide content as compared to the other two locations.

Key words: *Andrographispaniculata*, andrographolide, locations, high-performance liquid chromatography (HPLC), random amplified polymorphic DNA (RAPD).

INTRODUCTION

Plant growth and development are complex biological phenomena that depend upon genetic and environmental variables (Waller and Nowacki, 1978). There is indication by many workers that gene × environment interaction plays a crucial role for production of active ingredients in medicinal plants. Both growing location and seasonality play important roles in the production of active metabolites in the plant. Plant growth development in relation to season has distinct impact on accumulation of secondary metabolites (Teiz and Zeiger, 1998). *Andrographispaniculata* is an important medicinal plant. The leaves contain andrographolide, an important medicinal chemical. It is used both in indigenous and modern systems of medicine for treating several diseases. The plant was reported to respond to application of organic and fertilizer nutrients (Rajeswara et al., 2004). In the present experiment, three accessions of *Andrographispaniculata* were grown in three locations to study the influence of agro-climatic conditions of these locations and plant age on the secondary metabolite (andrographolide) production.

MATERIALS AND METHODS

Three accessions of *Andrographispaniculata* were planted at three locations namely Kalyani, West Bengal (tropical type), Anand, Gujarat (arid type) and Solan, Himachal Pradesh (semi-tropics type) differing widely in agro-climatic conditions in an RBD and the

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Table 1. Experimental materials and locations.

S/N	Accessions	Place of collection
1	1	Trichy, Tamil Nadu
2	2	Dangs, Gujarat
3	3	Anand, Gujarat

Experimental locations

S/N	Location of study	State	Temp. °C (max, min)	MSL (m)	Average rainfall (mm)	Co-ordinates
1	Kalyani	West Bengal	4, 45	13.5	1496	22.99°N; 88.45°E
2	Anand	Gujarat	12.5, 45	44	730	22.60°N; 72.93°E
3	Solan	Himachal Pradesh	-10, 22.8	1600	910	30.91°N; 77.09°E

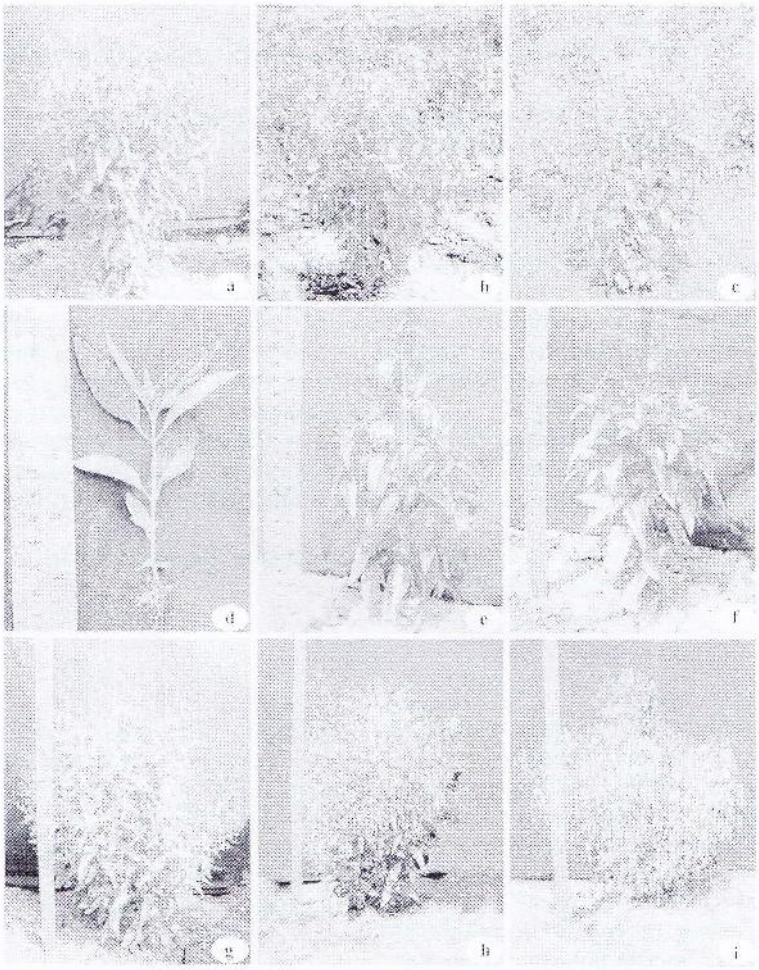

Figure 1. plant habit of (a) Accession 1, (b) accession 2, (c) accession 3 at 80 DAT; different growth phases of *A. paniculata*, (d) seedling at transplanting stage, (e) 40 DAT, (f) 60 DAT, (g) 80 DAT, (h) 100 DAT, (i) 120 DAT.

samples were collected at 20 days interval (Table 1) (Figure 1). Herbage samples were collected at four regular intervals that is, starting from 60 Days After Transplanting (DAT) (DoS -2nd June and DoT - 18th July) till 120 DAT at 20-day intervals from the three

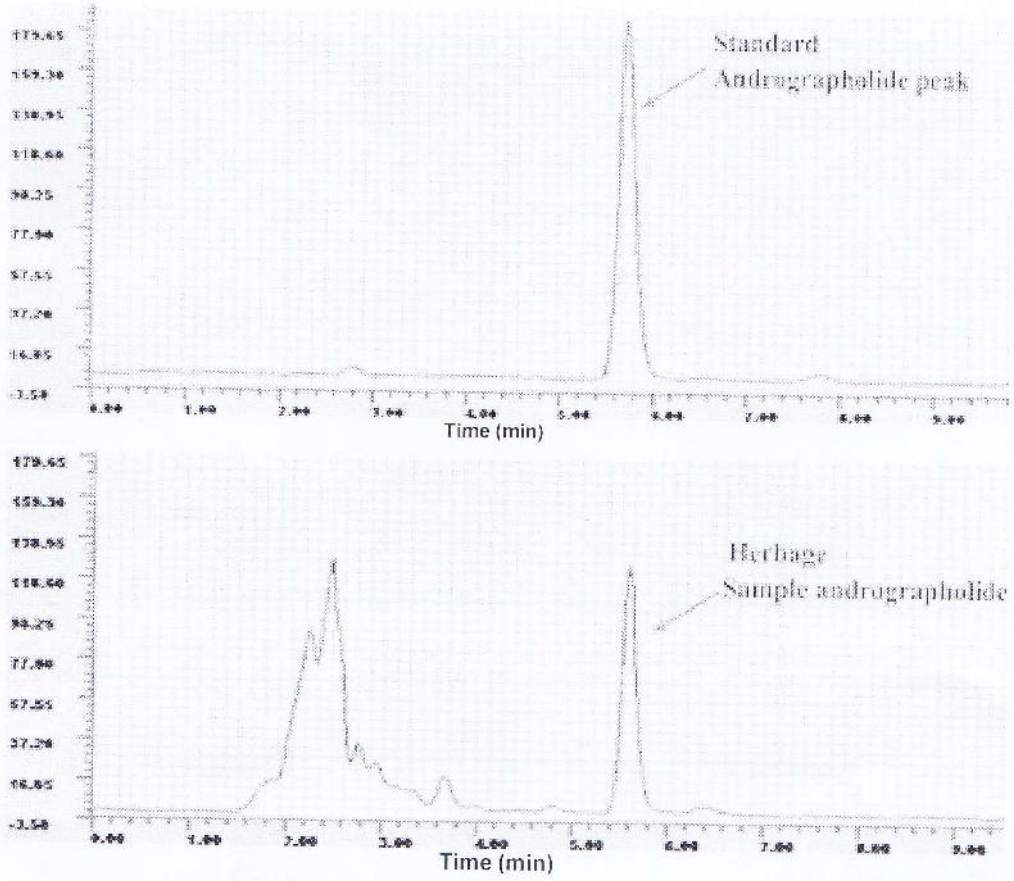

Figure 2. HPLC graph showing the concentration of andrographolide in herbage.

locations. The HPLC analysis was done for the samples for estimating the andrographolide content. The Shimadzu HPLC system used for the estimation of andrographolide consists of LC-10AD *VP* pump, Rheodyne sample injector, SPD 10A UV-VIS detector along with Aimil chromatograph data station for data collection and analysis. With the help of this system, selected phytochemicals (standardization, screening and quantification) were carried out at 229 nm wavelength (Chauhan et al., 2000) (Figure 2). The experiment was conducted to investigate whether agro-climate has any influence on the accessions for the andrographolide accumulation. The data obtained from the three locations on the three accessions were analyzed statistically using MSTATC software in the analysis of variance (two factors randomized complete block design with split plot combined over locations) as given by Gomez and Gomez (1976) and the analysis of variance showed differential influences of the locations and accessions on the andrographolide content.

Since the accessions studied in the present investigation were devoid of distinct phenotypic characters, molecular characterization through RAPD and ISSR were carried out for establishing any differences among the accessions at genetic level. For the RAPD analysis, polymerase chain reaction (PCR) was performed based on the protocol of Williams et al. (1990) with minor modifications. Amplification reactions were performed with 2.5 µl of 10X PCR buffer (Bangalore Genei, dNTPs (Fermentas, USA), 5 pmole of the primer, 1U of Taq DNA polymerase (Bangalore Genei, India), 30 ng of genomic DNA. DNA amplification was performed in a thermal cycler (EppendorfAG, Hamberg, Germany) programmed for 43 cycles. ISSR analysis was performed using ISSR primers obtained from BangloreGenei, India. Polymerase chain reaction (PCR) was performed based on the protocol of Zietwiecki et al. (1994) with some modifications.

RESULTS AND DISCUSSION

Differentiation of the three accessions at genetic level

Since the accessions studied in the present investigation were morphologically indistinguishable molecular characterization through RAPD and ISSR were carried out for establishing differences among the accessions at genetic level (Figures 3 and 4). Molecular analysis using Jaccard's coefficient, cophenetic correlation, principal coordinate analysis (PCA) and dendrogram (Figures 5 and 6) of the three accessions also clearly showed the differences and relationship amongst them. Molecular

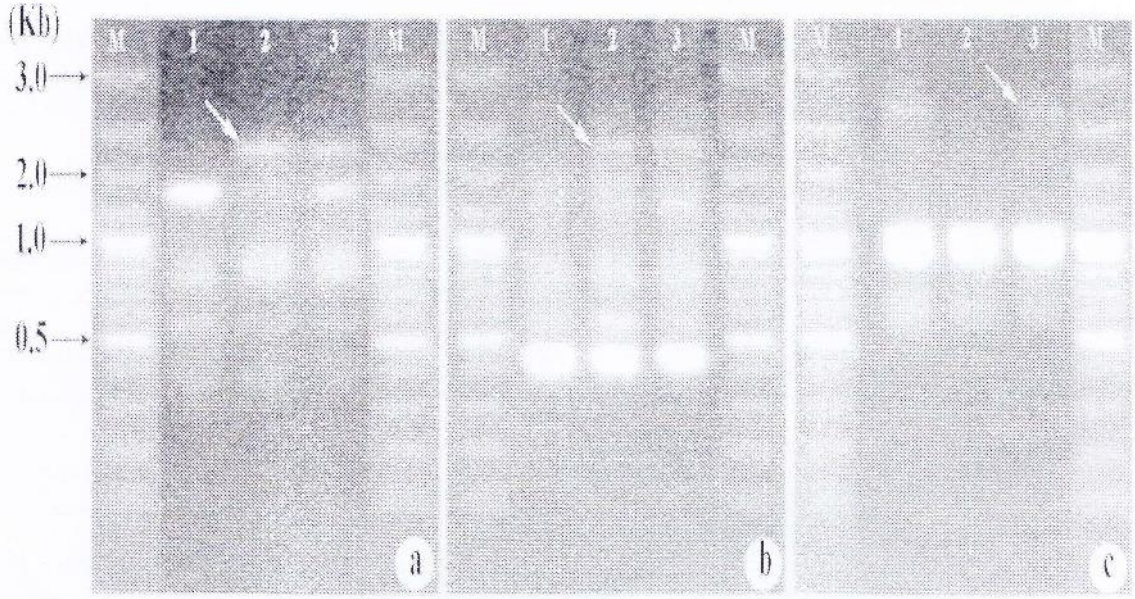

Figure 3. RAPD banding pattern of *A. paniculata* with a) OPA08, B) OPA18 and c) OPN02 [M-100 bp DNA ladder plus, 1- accession 1, 2- accession 2 and 3- accession 3]. *Arrow indicate variation in banding pattern in the three accessions.

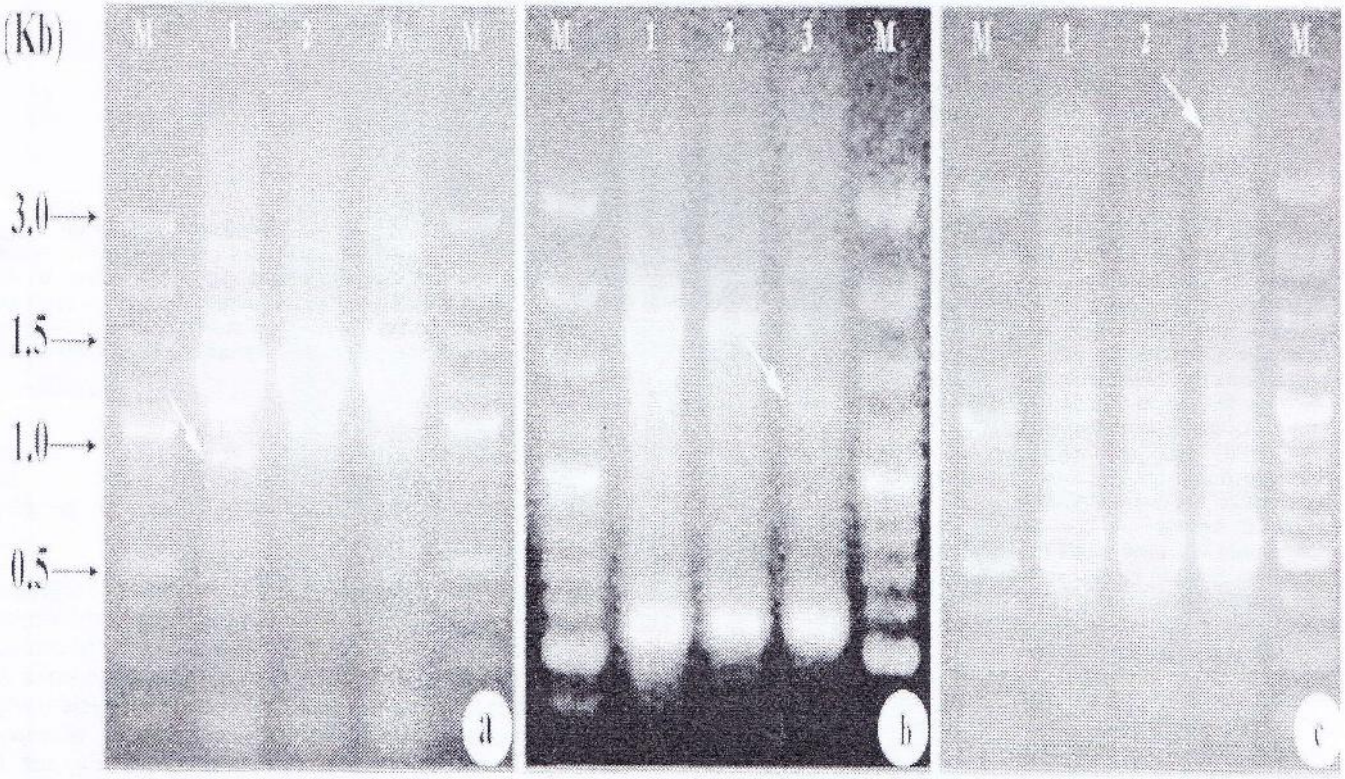

Figure 4. ISSR banding pattern of *A. paniculata* with a) (CT) 9G, B) b) (CT) 8RC and c) (CA) 8AT [M-100 bp DNA ladder plus, 1- accession 1, 2- accession 2 and 3- accession 3]. *Arrow indicates variation in banding pattern in the three accessions.

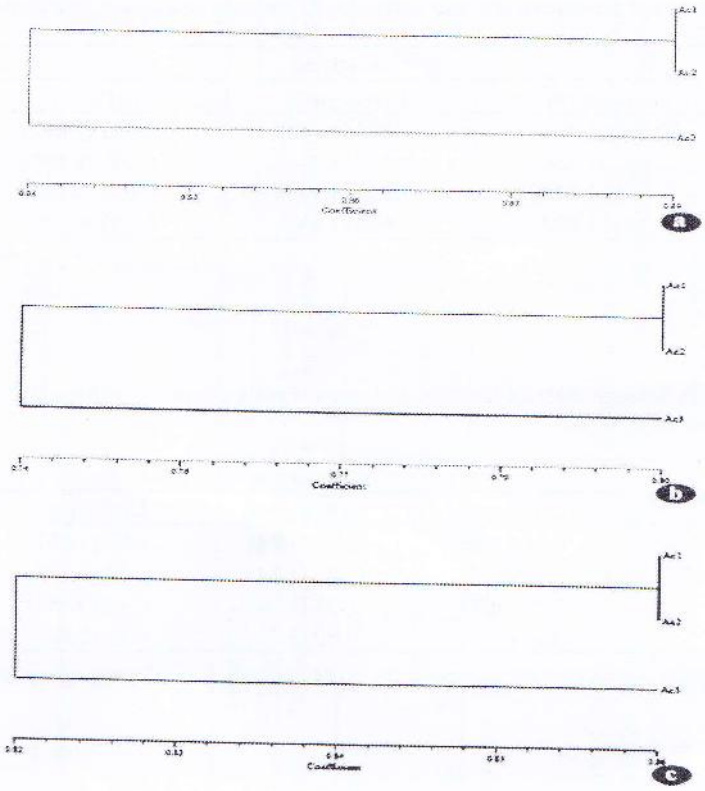

Figure 5. Dendrogram (a) RAPD, (b) ISSR and (c) combined RAPD-ISSR.

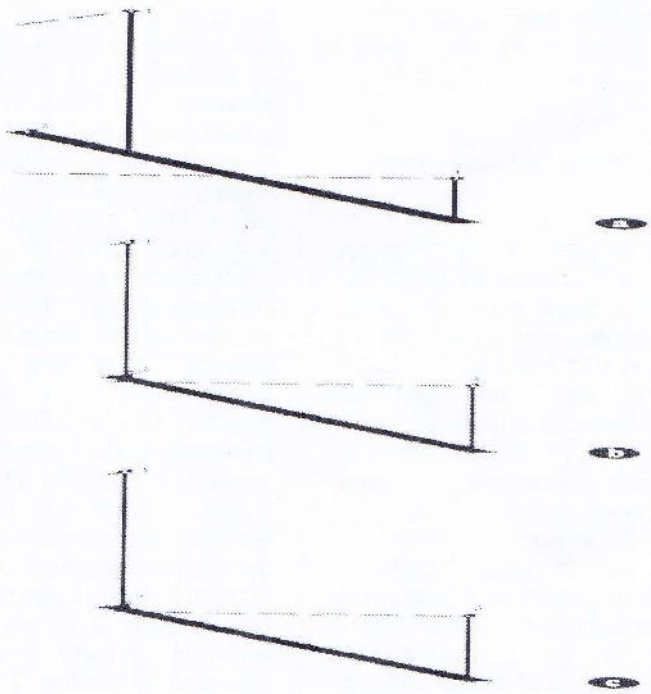

Figure 6. PCA (a), RAPD, (b) ISSR and (c) combined RAPD-ISSR.

Table 2. Effect of locations and accessions and their interaction on herbage andrographolide content (%) at 120 DAT.

Accessions	Locations			Mean
	L_1 (Anand)	L_2 (Kalyani)	L_3 (Solan)	
1	0.909 (1.174)	1.777 (1.483)	0.592 (1.033)	1.09 (1.23)
2	1.492 (1.388)	1.463 (1.384)	0.308 (0.895)	1.09 (1.22)
3	1.261 (1.313)	1.659 (1.446)	0.605 (1.036)	1.17 (1.27)
Mean	1.221 (1.292)	1.633 (1.438)	0.502 (0.988)	

Lsd_{Ac} (p = 0.05): 0.034; CV_{Ac} (%): 5.71; Lsd_{L} (p = 0.05): 0.047; CV (%): 7.66; Lsd_{AcxL} (p = 0.05): 0.058; CV_{AcxL} (%): 5.71. Values in parentheses denote transformed values.

Table 3. Interaction between different locations and stage of plant growth on herbage andrographolide content (%) andrographolide content (%).

Stages of plant age	Locations			Mean
	L_1 (Anand)	L_2 (Kalyani)	L_3 (Solan)	
S1 (60 DAT)	0.62 (1.05)	**2.33 (1.68)**	0.62 (1.06)	1.19 (1.26)
S2 (80 DAT)	1.97 (1.57)	2.20 (1.64)	0.80 (1.13)	1.66 (1.44)
S3 (100 DAT)	1.35 (1.36)	1.31 (1.34)	0.40 (0.95)	1.02 (1.21)
S4 (120 DAT)	0.94 (1.19)	0.69 (1.09)	0.18 (0.82)	0.60 (1.03)

Lsd_{SxL} (p = 0.05): 0.067; CV_{SxL}(%): 7.66; Lsd_{S}(p = 0.05): 0.054; CV_{S} (%): 7.66. *Figures in parentheses denote transformed values.

markers have demonstrated its usefulness to find out genetic similarities and differences between accessions even when a classical morphological description is severely limited. Despite the importance of the crop, very little research has been done to assess the genetic variation of this species using molecular markers (Padmesh et al., 1999).

Effect of locations, stages of plant and accessions on andrographolide content

The samples were collected at different growth stages, where the vegetative stage is found up to 60 to 65 DAT and after that the reproductive stage starts. The study showed that the locations significantly influenced the andrographolide accumulation in the herbage irrespective of accessions or age of the plant. Maximum andrographolide content was recorded in Kalyani (1.63%) with moderate mean temperature (< 28°C) followed by Anand (1.221%) which has dry climate with high mean temperature(> 30°C) and content was lowest at Solan (0.502%) a high altitude (1350 m above msl) which is having temperate climate (20 to 25°C) (Table 2). Chatterjee and Raychaudhuri (1992) expressed that although the biosynthesis of secondary metabolites is genetically controlled, there is a considerable environmental effect on their accumulation. Altitude, photoperiod, developmental stage, NPK application, mineral nutrients and physiological and biochemical factors greatly influence the accumulation of these chemicals. Significant influence of accessions was also noted on andrographolide content irrespective of the locations and sampling times. Andrographolide content was highest in accession 3 (1.17%) which was followed by accession 1 (1.09%) which was at par with that of accession 2 (1.09%) (Table 2). Stages of plant age also influenced the andrographolide content irrespective of the locations and accessions. Significantly highest andrographolide content was recorded at plant age 80 DAT (1.66%), thereafter it was reduced to 1.02% at plant age 100 DAT and 0.60% at plant age 120 DAT (Table 3).

Interaction of locations and accessions significantly influenced the herbage andrographolide content irrespective of stages of plant growth. Maximum andrographolide content was recorded in accession 1 at location 2 (1.777%) which was at par with accession 3 at location 2 (1.659%). However, the minimum andrographolide content was observed in accession 2 at location 3 (0.308%) (Table 2). Location and different stages of plant age had significant influence on the herbage andrographolide content irrespective of the accessions. Maximum content of andrographolide was at location 2 at plant age 60 DAT (2.33%) which was at par with location 2 at plant age 80 DAT (2.20%). The minimum herbage andrographolide content (%) was observed in location 3 sampled at plant age 120 DAT (0.18%) (Table 3). The herbage andrographolide content

Table 4. Interaction between accessions and stages of plant growth on herbage andrographolide content (%).

Stages of plant age	Accessions		
	1	2	3
S1 (60 DAT)	1.18 (1.26)	1.03 (1.20)	1.36 (1.33)
S2 (80 DAT)	1.73 (1.47)	1.59 (1.40)	1.66 (1.46)
S3 (100 DAT)	0.99 (1.21)	0.98 (1.19)	1.10 (1.25)
S4 (120 DAT)	0.47 (0.98)	0.76 (1.10)	0.58 (1.02)

Lsd_{AcxS} (p = 0.05): 0.067; CV (%) $_{AcxS}$: 5.71. *Figures in parentheses denote transformed values.

Table 5. Interaction of accessions, locations and sampling dates on herbage andrographolide content (%).

Stages of plant age	Locations								
	L_1 (Anand)			L_2 (Kalyani)			L_3 (Solan)		
	Accessions 1	Accessions 2	Accessions 3	Accessions 1	Accessions 2	Accessions 3	Accessions 1	Accessions 2	Accessions 3
S1 (60 DAT)	0.51 (1.00)	0.58 (1.04)	0.76 (1.12)	2.33 (1.68)	2.07 (1.60)	2.59 (1.76)	0.71 (1.10)	0.44 (0.97)	0.73 (1.11)
S2 (80 DAT)	1.59 (1.44)	2.49 (1.73)	1.84 (1.53)	2.58 (1.75)	1.91 (1.55)	2.11 (1.61)	1.01 (1.23)	0.36 (0.92)	1.04 (1.24)
S3 (100 DAT)	0.94 (1.20)	1.54 (1.43)	1.59 (1.44)	1.58 (1.44)	1.19 (1.30)	1.17 (1.29)	0.46 (0.98)	0.21 (0.84)	0.54 (1.02)
S4 (120 DAT)	0.61 (1.05)	1.36 (1.36)	0.85 (1.16)	0.62 (1.06)	0.69 (1.09)	0.77 (1.13)	0.19 (0.83)	0.22 (0.85)	0.11 (0.78)

$Lsd_{Acx Lx S}$ (p = 0.05): 0.116; CV (%)$_{Acx Lx S}$: 5.71. *Figures in parentheses denote transformed values.

was significantly influenced by the interaction of stages of plant growth and the accessions. Maximum herbage andrographolide content was found in accession 1 at plant age 80 DAT (1.73%) which was at par with the content of andrographolide in accession 3 at plant age 80 DAT (1.66%). The minimum herbage andrographolide content (%) was observed in accession 1 at plant age 120 DAT (0.47%) and was at par with the andrographolide content of accession 3 at plant age 120 DAT (0.58%) (Table 4). The locations, accessions and stages of plant growth had significant interaction effect in influencing the andrographolide content of herbage. Maximum herbage andrographolide content was recorded at location 2, in accession 3 at plant age 60 DAT (2.59%) which was at par with accession 1 at plant age 80 DAT at the same location (2.58%), location 1 in accession 2 at plant age 80 DAT (2.49%) and location 2 in accession 1 at plant age 60 DAT (2.33%). Minimum herbage andrographolide content was noted at location 3, in accession 3 at plant age 120 DAT (0.11%) (Table 5) (Figure 7).

Accumulation pattern of andrographolide content in three accessions in three distinctly diverse locations clearly showed that location 2 (Kalyani) was superior to the other two locations that is, Anand and Solan. Location 3 (Solan) has temperate climate (<25°C), low relative humidity (~60%) and high altitude (1600.0 m) and is not suitable for this crop since this species is a native of subtropical region and well adapted to areas of high rainfall (1000 to 2500 mm) and high humidity (> 70%) with moderate temperature (24 to 37°C). Andrographolide content was 3 times higher in Kalyani as compared to Solan. Similarly, it did not perform better in Anand because Anand is a dry area (mean RH ~65%) having moderate rainfall

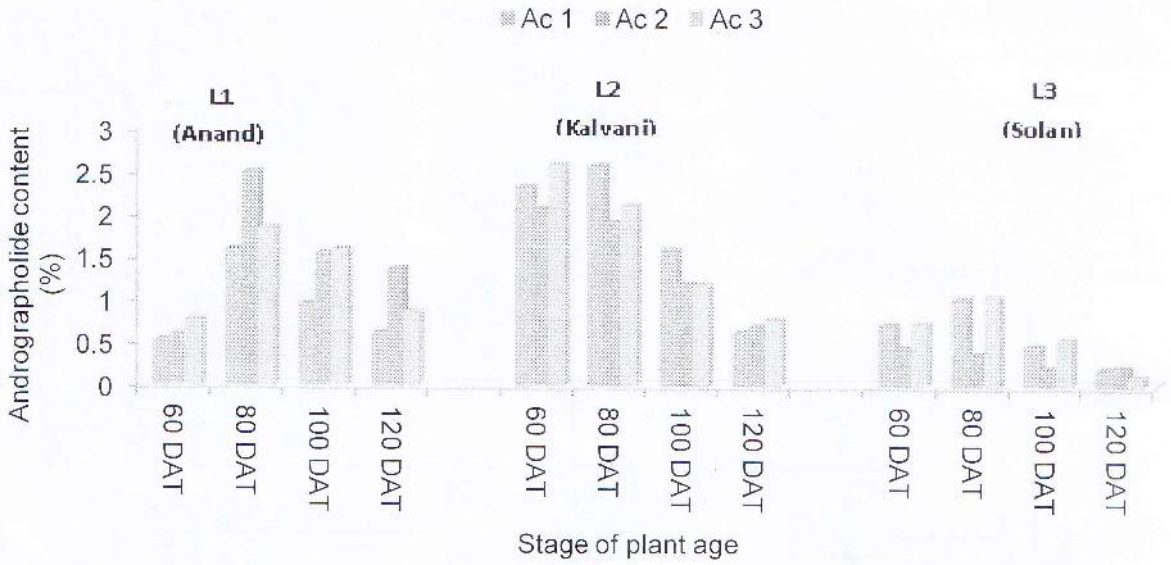

Figure 7. Accumulation pattern of herbage andrographolide content in three accessions across locations.

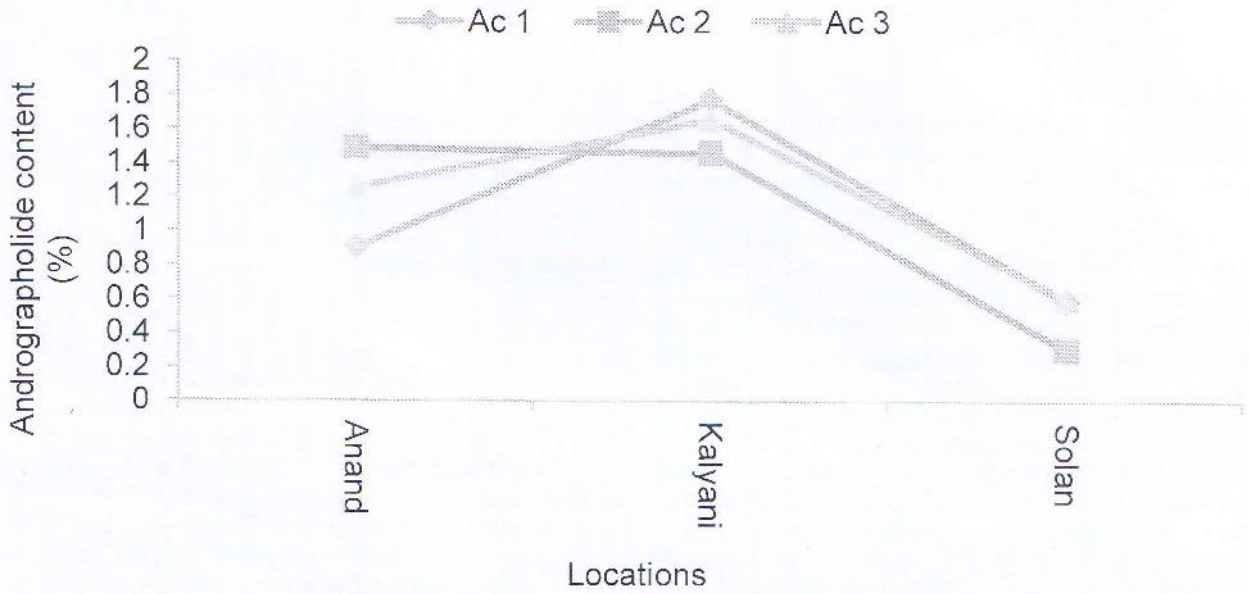

Figure 8. Influence of locations on andrographolide accumulation in three accessions of *A. Paniculata*.

(700 to 800 mm) with high day temperature (> 38.5°C). Performances of all the accessions were superior in Kalyani, although accession 1 was the best because of its adaptability to the weather conditions of Kalyani (average temperature < 28°C, annual rainfall - 2030 mm, mean humidity -84%) which is similar to the place from where the accessions was collected that is, Trichy in Tamil Nadu (Table 1) (Figure 8).

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