



Scan to know paper details and
author's profile

An Empirical Investigation on the Scope of Genetic Improvement of *Lavandula angustifolia* Mill. using Somaclonal Variation in the Context of Indian Agro-Climatic Environment

Sougata Sarkar & Deepak Sharma

ABSTRACT

Traditional cultivation of lavender occurs through vegetative means in mostly all lavender growing counties of the world. As a result genetic variation in the germplasm is negligible, which offers serious limitations in classical breeding programs of lavender. This led us to explore the potential of plant tissue culture methods (developing somaclonal variants followed by the selection of putative variants) for inducing genetic variation which would eventually serve the purpose of genetic improvement in lavender. Induction of heritable genetic variations in agro-economic traits through in-vitro micro propagation techniques is the main aim of this study.

Sterile cultures of lavender were produced. The regular phases of somaclonal plantlet development i.e. callogenesis followed by caulogenesis, rhizogenesis was modified by an intervening cell suspension culture phase represented by: callogenesis-1 (calli-1 derived from organised structures i.e. explants) followed by the intervening cell suspension culture (derived from calli-1), callogenesis-2 (calli-2 derived from cell suspension culture), caulogenesis (from calli-2), and finally rhizogenesis.

Keywords: correlation; genetic improvement; hs-gcms; *lavandula angustifolia*; post-hoc tests; somaclonal variation.

Classification: DDC Code: 576.54 LCC Code: QH401

Language: English



London
Journals Press

LJP Copyright ID: 925674
Print ISSN: 2631-8490
Online ISSN: 2631-8504

London Journal of Research in Science: Natural and Formal

Volume 22 | Issue 7 | Compilation 1.0



© 2022. Sougata Sarkar & Deepak Sharma. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 4.0 Unported License <http://creativecommons.org/licenses/by-nc/4.0/>, permitting all noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

An Empirical Investigation on the Scope of Genetic Improvement of *Lavandula angustifolia* Mill. using Somaclonal Variation in the Context of Indian Agro-Climatic Environment

Sougata Sarkar* & Deepak Sharma

ABSTRACT

Traditional cultivation of lavender occurs through vegetative means in mostly all lavender growing counties of the world. As a result genetic variation in the germplasm is negligible, which offers serious limitations in classical breeding programs of lavender. This led us to explore the potential of plant tissue culture methods (developing somaclonal variants followed by the selection of putative variants) for inducing genetic variation which would eventually serve the purpose of genetic improvement in lavender. Induction of heritable genetic variations in agro-economic traits through in-vitro micro propagation techniques is the main aim of this study.

Sterile cultures of lavender were produced. The regular phases of somaclonal plantlet development i.e. callogenesis followed by caulogenesis, rhizogenesis was modified by an intervening cell suspension culture phase represented by: callogenesis-1 (calli-1 derived from organised structures i.e. explants) followed by the intervening cell suspension culture (derived from calli-1), callogenesis-2 (calli-2 derived from cell suspension culture), caulogenesis (from calli-2), and finally rhizogenesis. The aspect of quantifiability was incorporated by the modified approach. Twenty somaclones exhibited significant differences in twenty one traits reflecting the induction of somaclonal variation within them. Somaclones 5, 6, and 10 among twenty somaclonal variants were genetically improved promising candidates.

Keywords: correlation; genetic improvement; hs-gems; *lavandula angustifolia*; post-hoc tests; somaclonal variation.

Author: Genetic Resource & Agrotech Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu- 180001, INDIA.
email: iimsougata@gmail.com

I. INTRODUCTION

Lavandula officinalis Chaix. synonym *L. angustifolia* Mill. (lavender) is a perennial, branched, bushy shrub belonging to the family Lamiaceae. Lavender is one of the most prestigious cash crop in the world, grown for its expensive essential oil having use in medicine (therapeutic effects as anti-anxiety disorders, sedative, spasmolytic, antiviral, and antibacterial agent), food (as a natural flavouring for beverages, ice cream, sweets, baked goods, and chewing gum), perfumery (cosmetics), in aroma-therapy (as a relaxant) (Da Porto et al. 2009), and a steady agro-industrial business has come up with this aromatic crop during the last few decades (Lis-Balchin 2002). Amongst all other species of *Lavandula*, only three species are of industrial importance e.g. *Lavandula angustifolia*, *Lavandula latifolia* and *Lavandula hybrida* (*Lavandula latifolia* × *Lavandula angustifolia*), which produce lavender oil, spike lavender oil, and lavandin oil respectively (Lis-Balchin 2002; Lubbe and Verpoorte 2011).

Colonial (British) India suffered from abortive attempts for commercial cultivation of lavender. The history of lavender cultivation in independent India dates back to the year 1957, when Sir Col. R.

N. Chopra introduced this plant in the Kashmir valley on a very small scale due to which commercial exploitation could not be possible. Changing political scenario in the province of Jammu and Kashmir also accounted for the failure of his efforts (Singh et al. 2007). It was after the systematic intervention of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow during early 80's, that about 100 hectares of land was brought under lavender cultivation in Kashmir along with development of appropriate agro-technologies. Essential oil obtained from steam distillation of flowering spikes produced at Pulwama farm (presently situated in the Union territory of Kashmir) since then met the international standards and are still being traded to the perfumery industry (Verma et al. 2010; Shawl et al. 2000; Handa et al. 1957). Apart from the successful introduction and sustainable cultivation of lavender in Jammu and Kashmir, CSIR-Institute of Himalayan Bio resource Technology (IHBT), Palampur during year 2000, had also been successful to introduce and cultivate lavender on a semi-commercial scale covering the district of Chamba in Himachal Pradesh (Singh et al. 2007). In the recent past, much of the efforts to popularise lavender cultivation in newer pockets in the Union Territories of Kashmir and Jammu was undertaken by CSIR-Indian Institute of Integrative Medicine (IIIM). In the light of the plant's habit and habitat, cultivation and extension programs, lavender has been restricted to higher altitudes (The Himalayan landscape) in India.

The aerial parts of perennial bushy lavender consists of an erect woody stem, its branches, leaves that are simple, entire, opposite, lanceolate, aromatic, greyish-green in colour with slight hairy appearance. Every branch terminates in a spike packed with small violet aromatic flowers. For luxuriant growth and development of lavender, a well-drained soil system such as sandy, sandy loam or gravelly soil is a prerequisite with its *pH* between 6.5 to 7.5. Lavender, in India is traditionally propagated by softwood cuttings. Propagation through hardwood cuttings are generally avoided due to delayed root initiation.

In India, flowers of lavender bloom mainly in the valley of Kashmir along with some scattered pockets in Jammu having similar agro-climatic conditions. Hence, vegetative methods of propagation is popular, favoured and relied (for raising uniform population of genotypes, quality and quantity of essential oil yield) over seed propagation methods (as genetic uniformity is compromised in the progeny populations) by the growers across this province.

Genetic improvement of lavender through classical plant breeding approach was initiated in India by CIMAP Regional Centre, Kashmir in 1978. Polycross mating design was implemented to achieve maximum diversity among progeny. By the end of 1988, the Centre was credited to develop forty three genotypes of lavender comprising twenty parental lines and twenty three clonal lines. As a result, the variety 'Karlovo' was released as an outcome of introduction and a clone, now known as 'Sher-i-Kashmir' came into existence (Singh et al. 1989). Since then, nothing much significant was done with the aspect of genetic improvement in lavender and the last or may be the only well recognized variety called 'Sher-i-Kashmir' was developed more than three decades ago from Indian perspective. However, genetic improvement programs continued in other scientific communities of the world who improvised and adapted classical plant breeding approaches (Hassiotis et al. 2010), polyploid induction model (Urwin et al. 2007; Urwin, 2009; Urwin et al. 2014), mutation breeding approach (Badawy et al. 2003), transgenics (Muñoz-Bertomeu et al. 2006; Ibrahim et al. 2017; Landmann et al. 2007; Mendoza-Poudereux et al. 2014) and plant tissue culture (PTC) techniques (Tsuru et al. 2009; Keykha et al. 2014; Andrys and Kulpa 2018; Onisei et al. 1994; Onisei et al. 1999) to induce genetic variation in the existing lavender germplasm.

PTC techniques are reported in some *Lavandula* sp. e.g., axillary shoot proliferation was reported for *Lavandula dentata* (Jordan et al. 1998; Sudria et al. 1999, 2001; Echeverrigaray et al. 2005), *Lavandula latifolia* (Sanchez-Gras and Calvo 1996) and *Lavandula vera* (Andrade et al. 1999). Direct regeneration of shoots from different

explants was reported for *L. latifolia* (Calvo and Segura 1989a) and indirect regeneration of shoots (having an intervening callus phase) was reported in *Lavandula angustifolia* (Quazi 1980; Ghiorghita et al. 2009), *Lavandula × intermedia* (Dronne et al. 1999), *L. latifolia* (Calvo and Segura 1988, 1989b; Jordan et al. 1990), *Lavandula officinalis × L. latifolia* (Panizza and Tognoni 1988) and *L. vera* (Tsuro et al. 1999, 2000).

In order to initiate any genetic improvement program, the minimum prerequisite is to have an optimum genetic variation in the existing germplasm of the specified crop. The maximum exploitation of clonal propagation method, for decades, in the context of lavender cultivation in India has led to narrowing its genepool. Considering all the above aspects, it is highly imperative that genetic improvement leading to varietal development in lavender will be a very difficult task through classical breeding approach unless the magnitude of variation in the lavender population rises to an optimal level. Occurrence of variation in nature is very slow hence, induced variation is the only favourable and novel option in this regard. In the light of all these above aspects, application of somaclonal variation technique through plant tissue culture (PTC) approach to induce variability among indirectly regenerated and *in-vitro* raised lavender plantlets were considered as a novel approach in this present study for bridging the gap of genetic improvement endeavours in Indian perspective. A schematic representation of the entire endeavour is reflected in the figure (1).

II. MATERIALS AND METHODS

2.1 Plant material and establishment of sterile *in-vitro* cultures

Healthy apical shoot meristems were excised from lavender plants growing in glass house condition for establishing aseptic cultures. These explants were surface sterilized with Tween-20, followed by 70% ethanol wash for 30s and finally with 0.10% HgCl₂ treatment for 45 s followed by five washings with sterile double distilled water before implanting onto a modified Murashige and Skoog, 1962 basal medium (MS₀) supplemented with

0.20 ppm thiamine-HCl, 1.00 ppm pyridoxine-HCl, 4.00 ppm glycine, 100.00 ppm myo-inositol, 0.70% agar and 3.00% sucrose. The pH of the media was adjusted to 5.80 prior autoclaving at 121.00°C and 117.70 kPa for 15 min. All aseptic cultures were incubated at 25±2°C with a photoperiod of 16 h under fluorescent light (40 to 50 µmol m⁻¹s⁻¹).

2.2 Classification of culture medium

The nodes, internodes, apical shoots, and leaves were used as explants from axenic *in-vitro* plantlets. The 0.5MS₀ (half strength of MS₀) and MS₀ supplemented with (1.00, 3.00, 5.00 ppm) 2,4-Dichlorophenoxyacetic acid (2,4-D) in combination with (0.25, 0.50 ppm) Kinetin (Kin) and 0.5MS₀ and MS₀ supplemented with (0.50, 1.00 ppm) Indole-3-acetic acid (IAA) in combination with (0.50, 1.00 ppm) Indole-3-butyric acid (IBA) were examined for selection of effective callus inducing medium(s) from forty media combinations mentioned above.

A portion of the callus (weighing 1.00 g) was subcultured into 100.00 ml liquid MS₀ (without agar) medium and incubated on a rotary shaker with constant agitation (70 rpm) in the dark until individual cells of the callus could be observed as a uniform suspension in the liquid medium. From this suspension culture, 20 µl was transferred onto the most effective callus inducing medium screened from the above medium combinations. The remaining callus were repeatedly subcultured onto fresh callus propagation medium.

Calli subcultured onto 0.5MS₀, MS₀, and MS₀ supplemented with (1.00, 2.00 ppm) 6-benzylamino purine (BAP), MS₀ supplemented (0.25, 0.50 ppm) Kinetin (Kin) alone and in combination were examined for selection of effective shoot inducing medium(s) from ten media combinations mentioned above.

The healthy adventitious shoots (5-7 cm long) obtained upon indirect regeneration were excised from the culture. These shoots were subcultured onto MS₀, 0.5MS₀, and 0.5MS₀ supplemented with 0.50 ppm Indole-3-butyric acid (IBA) and were examined for selection of effective root inducing

medium(s) from three media combinations mentioned above.

On the other hand, cuttings of apical shoot meristems from a donor lavender plant growing in open field condition were subjected to rooting in (1:2) sand-soil mixture which will subsequently act as checks/controls.

The most healthy and complete *in-vitro* plantlets derived through caulogenesis were selected for acclimatization and were gradually shifted to glasshouse condition for further hardening and evaluation of somaclonal variations.

2.3 Acclimatization and hardening

Twenty-nine acclimatized somaclones (SC) along with two checks were initially transplanted into small pots containing 2:1:1:1 mixture of soil: sand: vermiculite: farmyard manure and hardened in the glasshouse of the experimental farm of CSIR-IIIM, located at Chatha, Jammu, INDIA. These complete plantlets were irrigated with 0.5MS₀ liquid medium without sucrose as and when required during initial days of glasshouse establishment. Later it was entirely replaced by tap water. Twenty regenerated plantlets with two checks finally attained proper vegetative growth and development and were transferred to bigger pots with precautions for minimum root damage. Neither the somaclones nor the checks bloomed post glasshouse transfer.

2.4 Selection of specific traits for estimation of somaclonal variation.

Data of six morphometric traits (Figure 2) like plant height (height of the tallest branch of the potted plant from the pot soil surface measured in centimetres), number of branches (total number of lateral branches emanating from the main/primary stem), fresh weight of plant (total weight of the shoot and root system of the live plant in grams), root length (measure of the total root length in centimetres), stem circumference (circular measurement of the stem just above the pot soil of the potted plant measured in centimetres), branch length (length of a branch from the apical tip to its base on the primary axis measured in centimetres) and fifteen major

essential oil components like - eucalyptol; endo-Borneol; 3-Carene; camphene; α -Pinene; p-Cymene; o-Cymene; cyclobutane,1,2-dicyclopropyl-; bicycle [2.2.1] heptan- 2-ol,1,7,7-trimethyl-, (1S-endo)-; linalool; D-Limonene; (+)-2-Bornanone; α -Phellandrene; β -Phellandrene; linalyl acetate; extracted from sampled leaves using HS-GCMS for twenty somaclones with two checks (i.e. 22 treatments) growing in bigger pots in glasshouse condition were recorded.

2.5 Head Space-Gas Chromatography-Mass Spectrometry (HS-GCMS)

The qualitative and quantitative estimation of leaf volatiles for glass house grown treatments were performed on a Shimadzu Nexis GC-2030 hyphenated with GCMS-TQ8040 instrument and samples were introduced through HS-20 headspace sampler. Fresh leaf material of 500 mg sample was kept in 20 ml head space flat base vial fitted with crimp cap and silicon/PTFE 18 mm 35 SHORE septum. The vial was incubated in head space heater for 5 min at 120°C with 50 kps pressure. The loop temperature was 110°C and transfer-line temperature was kept at 120°C. The sample was introduced into the split/splitless injector in the split mode (1:25) at 280°C. The column oven temperature was programmed from 90°C to 120°C at the rate of 3°C/min with final hold of 2 min. High purity helium gas was used as a carrier gas (1 ml/min) and a SH-Rxi-5S MS (30 m \times 0.25 mm; 0.25 μ m film thickness) column was employed for separation. Identification of compounds was based on retention time, elution order, relative retention index using a homologous series of n-alkanes (C₈-C₂₅ hydrocarbons) with those of literature. Further identification was made by matching the recorded mass spectra with those stored in the inbuilt mass spectral library. The percentage determination was based on peak area normalization.

2.7 Experimental design and data analysis

The experiments were conducted using a completely randomized design. The statistical analysis was performed by one-way analysis of variance (ANOVA). Tukey-Kramer's, Scheffe's and Student's T (Bonferoni corrected) post hoc tests,

($p \leq 0.05$) was performed using SPSS statistical software (version 20 for Windows) and MS Excel-2007. Correlation coefficients were calculated amongst all traits and significant positive and negative values were demarcated. The regression equation, $\hat{Y} = c + m_i x_i$, where, ' \hat{Y} ' represents dependent trait, ' x ' represents independent traits, ' m ' represented slope of x , ' i ' represents number of traits and ' c ' represents intercept on \hat{Y} axis was formulated. SET Theory was also involved to demonstrate and simplify the comparison of efficacy among three post hoc tests.

III. RESULTS

3.1 Establishment of *in-vitro* cultures

The apical shoot meristems of *Lavandula angustifolia* Mill. (lavender) growing in glasshouse condition was used as explants for sterile *in-vitro* culture establishment. Almost 75% of the explants completely recovered from the inevitable surface sterilization stress within two weeks of inoculation. These explants exhibited quick growth response in MS_0 . The lateral adventitious shoots emanating from them were subcultured onto shoot proliferation mediums to produce a stock of axenic plantlets which were utilized for all upcoming experiments. MS_0 supplemented with 1.00 ppm BAP and 0.50 ppm Kin exhibited best growth response while 0.5 MS_0 exhibited least growth response to adventitious shoot proliferation in lavender.

3.2 Callogenesis

The nodes, internodes, leaves, and shoot apices of *in-vitro* established lavender plantlets were subcultured onto callus inducing mediums. Within a span of two weeks, although every explant responded to initial callus induction, however the best response was observed in apical shoots followed by leaves, nodes, and internodes. However, in the end of fifth week, internodal explants giving rise to inconspicuous mass of callus, ceased to exist. On the other hand, nodal and leaf explants gave rise to friable callus but maximum proliferation of the same occurred in apical shoot explants (Figure 3). This array of callogenic response of the explants for callus

induction followed by its subsequent growth remained unaltered irrespective of plant growth regulators (PGRs) used in the medium. MS_0 and 0.5 MS_0 each supplemented with a combination of 0.50 ppm IAA and 0.50 ppm IBA, MS_0 and 0.5 MS_0 each supplemented with 0.50 ppm IAA and MS_0 supplemented with 5 ppm 2,4-D and 0.25 ppm Kin proved to impart better callogenic effect in lavender than other combinations (Table 1, Figure 4). Comparatively, apical shoot explants exhibited best callogenic capacity compared with other explants. It also exhibited quickest callogenic response (callus induction followed by its growth) in MS_0 supplemented with 5 ppm 2,4-D and 0.25 ppm Kin giving rise to friable yellowish callus. With all these notable advantages, callus cultures derived from apical shoot explants was unanimously carried forward for establishing cell suspension culture.

3.3 Cell suspension culture

The friable callus obtained from the apical shoot explants (Figure 5c) growing on the semisolid MS_0 supplemented with 5 ppm 2,4-D and 0.25 ppm Kin were used to establish suspension cultures (Figure 5f). 1 g of this callus was transferred into 100 ml liquid MS (without agar and PGRs) medium and incubated on a rotary shaker with constant agitation (70 rpm) in the dark until individual cells of the callus could be observed as a uniform suspension in the liquid medium.

3.4 Suspension culture mediated callogenesis

A volume of 20 μ l from this cell suspension culture (Figure 5f) was transferred onto the already screened, most effective, callus inducing semisolid medium i.e. MS_0 supplemented with 5 ppm 2,4-D and 0.25 ppm Kin (Figure 5g). This was materialised to ensure that a uniform mass of callus be produced (Figure 5h) and utilized for every experiment(s) related to indirect regeneration and to have an accurate estimation of the frequency of regenerants to be produced from callus per media combination(s) as laid down in the subsequent experiments.

3.5 Caulogenesis

The suspended cells present in the liquid MS medium when transferred to MS₀ supplemented with 5 ppm 2,4-D and 0.25 ppm Kin, led to prolific growth of a uniform callus mass weighing 20 g (approx). The callus obtained through direct mode (Figure 5e) as well as through cell suspension mediated mode (Figure 5h) were identical to each other in nature and appearance. However, the later was unambiguously more effective for an accurate numeric estimation of indirect regeneration response.

This callus mass weighing 20 g (approx) was divided into four equal parts weighing 5 g (approx) each, that were subcultured onto shoot inducing mediums with a specific distribution pattern (Distribution 1 - 5) as represented in figure(6).

The morphogenesis of adventitious buds from callus (derived through cell suspension mediated mode) were discernible within three weeks of culture when the callus was subjected to shoot inducing medium(s). MS₀ supplemented with BAP and Kin exhibited better caulogenic response than rest of the combinations of PGRs used alone or with 0.5MS₀. The best combination of medium with prolific organogenic frequency (considering growth and development of adventitious shoots arising from callus) was that of MS₀ supplemented with 1.00 ppm BAP and 0.50 ppm Kin followed by MS₀ supplemented with 1.00 ppm BAP and 0.25 ppm Kin, MS₀ supplemented with 2.00 ppm BAP and 0.25 ppm Kin and MS₀ supplemented with 2.00 ppm BAP and 0.50 ppm Kin (Figure 7a-k). Least caulogenic response was exhibited by 0.5MS₀ medium. A detailed description of the caulogenic response with respect to media combinations are represented in supplementary table (1).

3.6 Rhizogenesis

Indirect regeneration of lavender shoots from cell suspension culture mediated calli came into prominence within six weeks of subculturing. These regenerated shoots grew and developed nodes, leaves and in some cases primary branches. The healthy regenerants were excised

and subcultured onto root inducing mediums. The 0.5MS₀ supplemented with 0.50 ppm IBA exhibited best rhizogenic response in terms of quickest induction of roots as well as development of branches during root growth and development (Figure 8a-l). Hence, indirect regeneration of complete *in-vitro* plantlets of lavender was achieved.

At the same time, vegetative cuttings of the apical shoot meristems from the same donor lavender plant (used earlier for *in-vitro* establishment) growing in glasshouse condition were subjected to rooting in (1:2)sand-soil mixture. These lavender plants growing in glasshouse condition would subsequently act as checks during the eventual analysis of variation in the somaclones.

3.7 Acclimatization and hardening

The most healthy and complete *in-vitro* plantlets (indirectly regenerated somaclones) were selected to withstand and survive the inevitable stress of acclimatization and hardening processes. As a result, twenty nine acclimatized somaclones (Figure 9) along with two checks were gradually transplanted into small pots containing 2:1:1:1 mixture of soil: sand: vermiculite: farmyard manure and hardened in the glasshouse condition-1 (temperature range $\approx 20.00^{\circ}\text{C}$ - 23.00°C ; humidity ≈ 36.80 - 40.00%). During the initial days of glasshouse transfer, these plantlets were irrigated with liquid 0.5MS₀ without sucrose as and when needed. Later, tap water was used for irrigation. Twenty plantlets along with two checks were able to attain best vegetative growth and development (Figure 10) and were transferred to bigger pots containing 2:1 mixture of sand: soil and further hardened in the glasshouse condition-2 (temperature range $\approx 30.00^{\circ}\text{C}$ - 32.00°C ; humidity ≈ 40.00 - 50.00%) with precautions for minimum root damage. Neither the somaclones nor the checks attained reproductive phase and never bloomed post glasshouse transfer.

3.8 Estimation of variation in somaclones

Quantitative data in the aspects of morphometric and chemometric traits of potted lavender plants (treatments) growing in glasshouse condition were recorded.

IV. MORPHOMETRIC DATA ANALYSIS

Data for six morphometric traits like plant height (T1), number of branches (T2), branch length (T3), fresh weight of plants (T4), root length (T5) and stem circumference (T6) were recorded and subjected to descriptive statistics (Table 2) ANOVA single factor (age of the plant) results indicated significant variation (MSS between groups=1911.26**) in the six morphometric traits (T1-T6) within the twenty two treatments (SC 1-20 and Control 1-2) at 95% level of confidence represented by table(3) and figure(10a_f - g_f). As ANOVA indicated statistical significance, post-hoc tests were used to distinguish significant differences between traits as follows:

4.1 Tukey-Kramer Post Hoc test

There were fifteen paired comparisons made for six traits [$n(n-1)/2$, where n =number of traits] All the paired comparisons were significantly different at $\alpha=0.05$, except three paired combinations viz. number of branches-root length, number of branches-branch length and root length-branch length. But, at $\alpha=0.01$ and 0.001 , plant height-plant fresh weight paired trait became non-significant along with the aforesaid three traits.

4.2 Scheffe Post Hoc test

There were fifteen paired comparisons made for six traits [$n(n-1)/2$, where n =number of traits] All the paired comparisons were significant at $\alpha=0.05$ and Scheffe's critical value=0.457. These paired comparisons remained significant even at $\alpha=0.01$ and Scheffe's critical value=0.633 However, at $\alpha = 0.001$ and Scheffe's critical value=0.880, the only paired trait viz. number of branches-branch length became non-significant.

4.3 Bonferroni corrected Student's T Post Hoc test

There were fifteen paired comparisons made for six traits [$n(n-1)/2$, where n =number of traits] All the paired comparisons were significant at $\alpha = 0.05$, except three paired combinations viz number of branches-root length, number of branches-branch length and root length-branch length.

Therefore, Tukey-Kramer and Bonferroni corrected Student's T proved to be more conservative Post Hoc tests (exhibiting 80% significance each, at $\alpha=0.05$) compared with Scheffe Post Hoc test (exhibiting 100% significance, at $\alpha=0.05$) in relation of this portion of the study.

4.4 Correlation analysis

The correlation study (Figure 11, Module2: area FDEF) represented highest significant positive interaction between plant height-branch length(0.499**), followed by higher significant positive interactions between branch length-number of branches(0.456**), number of branches-plant height(0.427**), number of branches-plant fresh weight(0.412**), followed by high significant positive interactions between plant fresh weight-branch length(0.316**), number of branches-stem circumference (0.310**), plant height-stem circumference (0.305**), followed by significant positive interactions between number of branches-root length (0.223**), root length-branch length (0.128**), stem circumference-branch length (0.101**), stem circumference-plant fresh weight (0.076**), plant fresh weight-root length (0.049**).

The correlation study also represented highest significant negative interaction between root length-stem circumference(-0.529**), followed by plant height-root length(-0.180**) and plant height-plant fresh weight(-0.145**).

4.5 Regression analysis

The regression equation may be represented by all six traits (T1-T6) considered in this study, however the best fit model was represented by the number of branches having the best r^2 value(0.60), highest adjusted r^2 value (0.48) and best p value (0.007) as reflected in the figure (12). Regression equation based on the regression analysis (Figure 12) is $\hat{Y} = -26.76 + 0.76x_1 + 0.40x_2 + 0.37x_3 + 3.95x_4 - 0.02x_5$. This regression model suggests that for every unit change in plant height(x_1), root length(x_2), plant fresh weight(x_3), stem circumference(x_4) and branch length(x_5) the

number of branches(\hat{Y}) is going to be affected by 0.76 times, 0.40 times, 0.37 times, 3.95 times and 0.02 times respectively.

4.6 Chemometric Data Analysis

Morphometric data analysis was followed by essential oil estimation from lavender treatments (twenty somaclones and two checks). As aromatic flowering spikes were unavailable, leaves were sampled from all over the plant from every treatment and were subjected to HS-GC-MS analysis. Analysis of the chromatograms (Figure 13) confirmed that the best fifteen components of the essential oil (denoted by F1 to F15) which are under the present study represented 46.39% - 87.96% of the total essential oil components present in the treatments. This range, itself throws a lot of light to the presence of variation in the essential oil components in the treatments involved.

The somaclone SC5 exhibited the best concentration of three components of essential oil viz. D-Limonene (17.42%), α -Pinene (8.62%) and o-Cymene (5.69%) followed by SC6 exhibited the best concentration of two components of essential oil viz. Camphene (16.35%) and (+)-2-Bornanone (15.00%) and SC10 exhibited the best concentration of two components of essential oil viz. Cyclobutane, 1,2-dicyclopropyl- (8.61%) and α -Phellandrene (6.09%). SC1, SC3, SC7, SC8, SC12, SC18, Control1 and Control2 each exhibited the best concentration of single component of essential oil viz. 3-Carene (22.52%), β -Phellandrene (27.39%), Linalool (1.04%), Linalyl acetate (0.19%), p-Cymene (4.72%), Bicyclo [2.2.1] heptan- 2-ol,1,7,7- trimethyl-, (1S-endo)- (25.56%), Eucalyptol (59.45) and endo-Borneol (9.38%) respectively as reflected in the table (4).

Data for fifteen essential oil components represented by (F1 to F15) were recorded and subjected to descriptive statistics (Table 2). The ANOVA single factor (age of the plant) results indicated significant variation (MSS between groups=949.32**) in the fifteen traits (F1-F15) within the twenty two treatments (SC1-20 and

Control1-2) at 95% level of confidence represented by table (5).

4.7 Correlation analysis

The correlation study (Figure 11, Module1: area ABFA) represented highest significant positive interaction between Camphene-(+) with 2-Bornanone(0.680**), followed by higher significant positive interactions between Eucalyptol with endo-Borneol (0.667**), Bicyclo [2.2.1] heptan-2-ol,1,7,7-trimethyl-, (1S-endo)- with Linalool(0.604**), α -Pinene with Bicyclo [2.2.1] heptan-2-ol,1,7,7-trimethyl-, (1S-endo)- (0.581**), β -Phellandrene with Linalyl acetate (0.533**), 3-Carene with α -Phellandrene (0.505**) followed by high significant positive interactions between Camphene with Bicyclo [2.2.1] heptan-2-ol,1,7,7-trimethyl-, (1S-endo)- (0.0.493**), Camphene with Linalool (0.490**), o-Cymene with Limonene(0.482**), followed by significant positive interactions between α -Pinene with D-Limonene (0.459**), 3-Carene with β -Phellandrene (0.402**).

The correlation study also represented highest significant negative interaction between p-Cymene with o-Cymene(-0.864**), followed by 3-Carene with (+)- 2-Bornanone(-0.725**) and endo-Borneol with Bicyclo [2.2.1] heptan-2-ol,1,7,7-trimethyl-,(1S-endo)-(-0.664**).

4.8 Regression analysis

The regression equation may be represented by all fifteen traits (F1-F15) considered in this study, however the best fit model was represented by (+)-2-Bornanone having the best R square value(0.981), highest adjusted R square value(0.944) and *p value*(0.72) as reflected in the table (6). Regression equation based on the regression analysis (Table 6) is $\hat{Y} = 8.556 + 0.29x_1 - 0.02x_2 - 9.13x_3 - 0.05x_4 - 0.26x_5 - 0.42x_6 + 0.32x_7 - 0.19x_8 + 0.17x_9 + 0.37x_{10} + 0.04x_{11} - 0.25x_{12} + 2.39x_{13} + 0.03x_{14}$. This regression model suggests that for every unit change in α -Phellandrene(x_1), β -Phellandrene (x_2), Linalyl acetate (x_3), Eucalyptol (x_4), endo-Borneol (x_5), 3-Carene (x_6), Camphene (x_7), α -Pinene (x_8), p-Cymene (x_9),

o-Cymene (x_{10}), Cyclobutane,1,2-dicyclopropyl- (x_{11}), Bicyclo [2.2.1] heptan- 2-ol,1,7,7- trimethyl-, (1S-endo)- (x_{12}), Linalool (x_{13}) and D-Limonene (x_{14}) the (+)-2-Bornanone (\hat{Y}) is going to be affected by 0.29 times, 0.02 times, 9.13 times, 0.05 times, 0.26 times, 0.42 times, 0.32 times, 0.19 times, 0.17 times, 0.37 times, 0.04 times, 0.25 times, 2.39 times and 0.03 times respectively.

4.9 Total data analysis of morphometric and chemometric traits

Convincing presence of variability in the morphometric traits (T1 to T6) and chemometric traits (F1 to F15) within the twenty two treatments (20 somaclones and 2 checks) were proved. This further opened the opportunity to statistically analyse the existence of variation within these 22 treatments with respect to twenty one cumulative morpho-chemo-metric traits (F1 to F15 and T1 to T6).

Data for twenty one traits were recorded and subjected to descriptive statistics (Table 2). The ANOVA single factor (age of the plant) results indicated significant variation (MSS between groups =1850.63**) in the twenty one traits (F1 to F15 and T1 to T6) for twenty two treatments (SC 1-20 and Control 1-2) at 95% level of confidence represented by table (7). As ANOVA indicated statistical significance, post-hoc tests were used to distinguish significant differences between traits as follows:

4.10 Tukey-Kramer Post Hoc test

There were 210 paired comparisons made for 21 traits [$n(n-1)/2$, where n = number of traits]. Among these, 124 paired comparisons were significantly different while the remaining 86 paired comparisons were not, at $\alpha = 0.05$. So, Tukey-Kramer Post Hoc test was successful in isolating 59.05% significantly different paired comparisons.

4.11 Scheffe Post Hoc test

There were 210 paired comparisons made for 21 traits [$n(n-1)/2$, where n = number of traits]. Among these, 207 paired comparisons were

significantly different while the remaining 3 paired comparisons (endo-Borneol - Cyclobutane, 1,2-dicyclopropyl-; p-Cymene - (+)-2-Bornanone; o-Cymene - Cyclobutane,1,2-dicyclopropyl-) were not, at $\alpha = 0.05$ and Scheffe's critical value=0.079. So, Scheffe Post Hoc test was successful in isolating 98.57% significantly different paired comparisons.

4.11 Bonferroni corrected Student's T Post Hoc test

There were 210 paired comparisons made for 21 traits [$n(n-1)/2$, where n = number of traits]. Among these, 145 paired comparisons were significantly different while the remaining 65 paired comparisons were not at $\alpha = 0.05$. So, Bonferroni corrected Student's T Post Hoc test was successful in isolating 69.05% significantly different paired comparisons.

Therefore, Tukey-Kramer proved to be most conservative Post Hoc test than medium conservative Bonferroni corrected Student's T post hoc test and least conservative Scheffe Post Hoc test at $\alpha = 0.05$ in relation to cumulative assessment of twenty one morpho-chemo-metric traits of twenty two treatments in the present study. This idea is briefly represented with the help of Set Theory in table (8).

4.12 Correlation analysis

The correlation study (Figure 11) may be divided into three modules: Module 1- corresponds to chemometric correlation values demarcated by the large triangular area ABFA, Module 2- corresponds to morphometric correlation values demarcated by the small triangular area FDEF and Module 3- corresponds to morpho-chemo-metric correlation values demarcated by the rectangular area BCDFB. The results of correlation for module 1 and module 2 has already been discussed. However, module 3 is of particular interest as these values depict the combined interaction of morphometric and chemometric traits.

Module 3

The highest significant positive interaction is present between Linalyl acetate with stem circumference (0.503**), followed by high significant positive interactions between α -Phellandrene with plant height (0.380**), D-Limonene with root length (0.364**), o-Cymene with fresh weight of plants (0.353**), 3-Carene with stem circumference (0.325**), α -Phellandrene with branch length (0.308**), p-Cymene with branch length (0.302**), followed by significant positive interactions between o-Cymene with stem circumference (0.293**), α -Pinene with root length (0.283**), β -Phellandrene with number of branches (0.272**).

The correlation study also represented highest significant negative interaction between Bicyclo [2.2.1] heptan-2-ol,1,7,7-trimethyl-, (1S endo) with stem circumference (-0.519**), followed by p-Cymene with stem circumference (-0.444**) and 3-Carene with root length (-0.442**).

Overall correlation

The overall correlation taking module 1, 2 and 3 into account, reflects that the highest positive significant correlation existed between Camphene with (+)-2-Bornanone (0.680**) and the highest negative significant correlation existed between p-Cymene with o-Cymene (-0.864). The overall maxima and the minima both lie in the module 1, represented by chemometric correlation and demarcated by the area ABFA.

V. DISCUSSION

In the perspective of agro-climatic conditions of Jammu and Kashmir (INDIA), lavender has been predominantly grown as a vegetatively propagable crop since it was introduced in INDIA. The unavoidable clonal propagation practice with lavender germplasm on one hand gave rise to negligible or no genetic variation at all and on the other hand amplified the chance of genetic erosion. The scarcity of genetic variation in the existing germplasm was possibly one of the major bottlenecks for the failure of varietal improvement programs mediated through conventional approaches in regard to lavender genetic

improvement in Indian context. A closer introspection to the problem led to a convincing solution - inducing significant variation *in-vitro* using PTC methods (indirect regeneration) followed by selection of putative candidates. This idea was seldom conceived by former and present experts, but was brought to reality with proper investigations in the present study.

As a regular observation, lavender plants do not attain reproductive phase in the experimental location, hence, seeds as explants were unavailable to initiate the process of *in-vitro* establishment. Alternatively, apical shoot meristems were used to establish a stock of sterile cultures in MS₀ medium (Al Khateeb et al. 2017).

There has been a number of studies in the aspect of micropropagation of *Lavandula sp* in the recent past. In the present study, sterile *in-vitro* cultures of lavender have been quickly established by utilizing those findings for the purpose of inducing genetic variation in the resulting complete plantlets (somaclonal variants). The PGRs - BAP and Kin are proclaimed as multiple shoot producers (Yew et al. 2010). Hence, different combinations of them were used in MS₀ and 0.5MS₀ medium for multiple shoot proliferation in lavender. The best medium for the rapid proliferation of microshoots was MS₀ supplemented with 1 ppm BAP and 0.50 ppm Kin. Unlike the present results for *L. angustifolia*, Al-Bakhit et al. (2007) estimated a much lower concentration of BAP to be most effective for the proliferation of *L. latifolia* microshoots. In another experiment, Zuzarte et al. (2010) confirmed that adding BAP induced higher number of microshoots in *L. pendunculata*. Synergistic effect of higher concentration of BAP and TDZ was most effective for propagation of microshoots in *L. vera* was advocated by Andrade et al. (1999).

The apical shoot explants growing in MS₀ supplemented with 5 ppm 2,4-D and 0.25 ppm Kin exhibited quickest response to callus induction followed by vigorous proliferation of friable yellowish calli. Unlike the present findings, Keykha et al. (2014) used leaf explants to generate callus and observed 2 ppm 2,4-D and 2 ppm BAP

in dark conditions to be the most suitable condition for the callus induction and its growth. Falk et al. (2013) advocated that MS medium supplemented with TDZ to be the best combination for callus induction in *L. angustifolia*.

A cell suspension culture of lavender was established using a known mass of the yellowish friable callus derived from apical shoot meristems. The callus remained in liquid MS₀ (without agar and PGRs) being continuously agitated by circular motion until a uniform suspension of cells was formed. Lappin et al. (1987) also derived the same results like the present study while establishing cell suspensions of *L. angustifolia* from callus induced on agar-solidified MS medium supplemented with 2,4-D and Kin. Watanabe et al. (1982) also established suspension cultures of green *L. vera* cells to screen high vitamin producing cells.

Callus cultures were again generated using a known volume of the suspension culture. The calli so obtained from suspension cultured mediated mode was similar in all respects to the calli derived from apical shoot explants mode. However, the only difference was that, the former was quantifiable but the later wasn't.

The quantifiable mass of calli was distributed in five patterns onto each shoot inducing mediums for indirect regeneration of shootlets. The purpose of this distribution was to ensure maximum exposure of callus cells to the medium. MS₀ supplemented with 1.00 ppm BAP with 0.50 ppm Kin proved to be the best combination for caulogenesis. Synergistic effect of BAP and Kin was evident in the present study unlike Adesoye et al. (2012) in *Sphenostylis stenocarpa*, Al Khateeb et al. (2013) in *Moringa peregrina*, Ahmad and Anis (2014) in *Vitex trifolia*. Formation of complete plantlets occurred after rhizogenesis in 0.5MS₀ supplemented with 0.50 ppm IBA. The most healthy and complete plantlets (somaclones) were put under the inevitable stress of acclimatization and hardening. The resultant somaclones were analysed for morphometric and chemometric variations using statistical tools and techniques.

Six measurable morphological features (morphometric traits) were selected. Statistical analysis of morphometric data was focused mainly on detection of variation(s) induced within the somaclones as a result of indirect regeneration approach. One way ANOVA exhibited significant differences among the morphometric traits with 99.9% confidence. Three follow up post-hoc test (viz. Tukey-Kramer, Scheffe and Bonferroni corrected Student's T) were also performed to detect pair-wise significant differences at 95% confidence level. The purpose of three post-hoc tests was fulfilled when the most effective post-hoc test could be identified among them. Tukey-Kramer and Bonferroni corrected Student's T both proved to be equally most effective post-hoc tests. Tukey-Kramer multiple comparison test was used to distinguish significantly different treatments following the ANOVA test in the studies on *Brassica napus* by Akasaka-Kennedy et al. (2005), on *Grateloupia dichotoma* by Yokoya and Handro (1996) and on *Scilla natalensis* by McCartan, and Van Staden (1998). Tukey-Kramer and Dunnet tests were performed on *Cryptanthus sinuosus* by Arrabal et al. (2002) and on *Caladium bicolor* by Ahmed et al. (2002).

In the absence of flowering spikes, fifteen detectable constituents of essential oil (chemometric traits) from leaves were sampled from each treatment. Similar studies of using leaves in place of or alongwith flowing spikes have been reported by Hassanpouraghdam et al. (2011) in *L. officinalis*, Aburjai et al. (2005) in *L. coronopofolia* and Cristina et al. (1995) in *L. pinnata*. Statistical analysis of chemometric data was focused mainly on detection of variation(s) in the essential oil profile induced within the somaclones as a result of indirect regeneration approach. One way ANOVA exhibited significant differences among the chemometric traits with 99.9% confidence. Correlation and multiple regression analysis have also contributed in explaining the effects of one trait on another. A combined statistical analysis of variance (one way ANOVA) encompassing sumtotal traits exhibited significant difference with 99.9% confidence. Three follow up post-hoc test (viz. Tukey-Kramer,

Scheffe and Bonferroni corrected Student's T) were also performed to detect pair-wise significant differences at 95% confidence level. The purpose of three post-hoc tests was fulfilled when the most effective/suitable post-hoc test could be identified among them. Tukey-Kramer proved to be most conservative post-hoc test when compared with Bonferroni corrected Student's T post-hoc test and Scheffe post-hoc test in relation to assessment of 21 morpho-chemo-metric traits of 22 treatments in the present study. Application of Bonferroni's correction on Student's T test improved the conservativeness of mere Student's T test result from 76.67% to 69.05%. Even then Tukey-Kramer's post-hoc values (59.05%) were far more encouraging than the rest. Tukey-Kramer multiple comparison test was used to distinguish significantly different treatments following the ANOVA test in the studies on *Portulaca grandiflora* by Khandare et al. (2011), on *Lavandula dentata* by Echeverrigaray et al. (2005), on *Allium sativum* by Robledo-Paz et al. (2000) and on *Thapsia garganica* by Makunga et al. (2003).

VI. CONCLUSIONS

Quantifiable callus cultures raised through cell suspension culture mediated route was instrumental in the indirect regeneration of lavender. Complete somaclonal plantlets were raised, selected and subjected to glasshouse establishment where variation in twenty one morphometric and chemometric traits were analysed for twenty somaclones and two checks. Significant amount of variation existed in all the somaclones raised through *in-vitro* approach which was precisely proved by more than one statistical tests. SC5 (Figure 14) proved to be the most putative somaclonal variant followed by SC6 and SC10.

6.1 Upcoming experiments and hypothesis

The putative somaclonal variants (SC5, SC6 and SC10) are now under vegetative luxuriance along with the rest of the somaclonal variants. These variants will be clonally propagated in the glasshouse to build a population of plants and will

be transferred to that habitat which may eventually lead to the reproductive expression. Selection of early maturing lines, with higher inflorescence count may be obtained from field study which will be carried out soon.

ACKNOWLEDGEMENT

The authors are thankful to Late Dr. Suresh Chandra, CSIR-IIIM, Jammu for his continuous encouragement. Thanks are also due to Dr. Rekha Sapru Dhar, Principal Technical Officer, Plant Tissue Culture Department and Mr. Amit Kumar, Technical Assistant, Instrumentation Division, CSIR-IIIM, Jammu for providing enthusiasm and support during the research. The authors also thank the Director, CSIR-IIIM, Jammu, India, for providing the necessary facilities during the course of this investigation.

Conflict statement

No conflict of interest is declared by the authors

REFERENCES

1. Aburjai T, Hudiab M, Cavrini V (2005) Chemical composition of the essential oil from different aerial parts of lavender (*Lavandula coronopifolia* Poiert)(Lamiaceae) grown in Jordan. Journal of Essential Oil Research 17: 49-51.
2. Adesoye A, Emese A, Olayode O (2012) *In-vitro* regeneration of African Yam Bean *Sphenosty lisstenocarpa* (Hochstex.A. Rich.) Harms by direct organogenesis. Kasetsart. J Nat Science 46: 592-602.
3. Ahmad R, Anis M (2014) Rapid in vitro propagation system through shoot tip cultures of *Vitex trifolia* L.-an important multipurpose plant of the Pacific traditional Medicine. Physiol Mol Biol Plants 20: 385-392.
4. Ahmed EU, Hayashi T, Zhu Y, Hosokawa M, Yazawa S (2002) Lower incidence of variants in *Caladium bicolor* Ait. plants propagated by culture of explants from younger tissue. Scientia horticultrae 96: 187-194.
5. Akasaka-Kennedy Y, Yoshida H, Takahata Y (2005) Efficient plant regeneration from leaves of rapeseed (*Brassica napus* L.): the

- influence of AgNO₃ and genotype. Plant cell reports 24: 649-654.
6. Al Khateeb W, Bahar E, Lahham J, Schroeder D, Hussein E (2013) Regeneration and assessment of genetic fidelity of the endangered tree *Moringa peregrina* (Forssk.) Fiori using inter simple sequence repeat (ISSR). *Physiol Mol Biol Plants* 19: 157-164.
 7. Al Khateeb W, Kanaan R, El-Elimat T, Alu'datt M, Lahham J, El-Oqlah A (2017) In vitro propagation, genetic stability, and secondary metabolite analysis of wild lavender (*Lavandula coronopifolia* Poir.). *Horticulture, Environment, and Biotechnology* 58: 393-405.
 8. Al-Bakhit A, Sawwan J, Al-Mahmoud M. (2007) In-vitro propagation of two *Lavandula* Species: *Lavandula angustifolia* and *Lavandulala latifolia* L. *Medica. Jordan J Agric Sci* 3:16-25.
 9. Andrade L, Echeverrigaray S, Fracaro F, Pauletti G, Rota L (1999) The effect of growth regulators on shoot propagation and rooting of common Lavender (*Lavandula vera* DC). *Plant Cell Tissue Organ Cult* 56:79-83.
 10. Andrade LB, Echeverrigaray S, Fracaro F, Pauletti GF, Rota L (1999) The effect of growth regulators on shoot propagation and rooting of common lavender (*Lavandula vera* DC). *Plant Cell Tissue Org. Cult* 56:79-83.
 11. Andrys D, Kulpa D (2018) In-vitro propagation affects the composition of narrow-leaved lavender essential oils. *Acta Chromatographica* 30: 225-230.
 12. Arrabal R, Amancio F, Carneiro LA, Neves LJ, Mansur E (2002) Micropropagation of endangered endemic Brazilian bromeliad *Cryptanthus sinuosus* (LB Smith) for in-vitro preservation. *Biodiversity & Conservation* 11: 1081-1089.
 13. Badawy E, Sakr S, El-Sharnouby M, Szoke E, Mathe I, Blunden G, Kery A (2003) Production and composition of Lavender plants through tissue culture as affected with gamma irradiation treatments. *Acta-Horticulture* 597: 325-328.
 14. Calvo MC, Segura J (1988) In vitro morphogenesis from explants of *Lavandula latifolia* and *Lavandula stoechas* seedlings. *Sci. Hort* 36: 131-137.
 15. Calvo MC, Segura J (1989a) In vitro propagation of lavender. *Hortscience* 24: 375-376.
 16. Calvo MC, Segura J (1989b) Plant regeneration from cultured leaves of *Lavandula latifolia* Medicus: influence of growth regulators and illumination conditions. *Plant Cell Tissue Org. Cult* 19: 33-42.
 17. Cristina Figueiredo A, Barroso JG, Pedro LG, Sevinete-Pinto I, Antunes T, Fontinha SS, Anja Looman A, Scheffer JJ (1995) Composition of the essential oil of *Lavandula pinnata* L. fil. var. *pinnata* grown on Madeira. *Flavour and fragrance journal* 10: 93-96.
 18. Da Porto C, Decorti D, Kikic I (2009) Flavour compounds of *Lavandula angustifolia* L. to use in food manufacturing: comparison of three different extraction methods. *Food Chem* 112: 1072-1078.
 19. Dronne S, Jullien F, Caissard JC, Faure O (1999) A simple and efficient method for in vitro shoot regeneration from leaves of lavender (*Lavandula x intermedia* Emeric ex Loiseleur). *Plant Cell Rep* 18: 429-433.
 20. Echeverrigaray S, Basso R, Andrade LB (2005) Micropropagation of *Lavandula dentata* from axillary buds of field-grown adult plants. *Biologia Plantarum* 49: 439-442.
 21. Echeverrigaray S, Basso R, Andrade LB (2005) Micropropagation of *Lavandula dentata* from axillary buds of field-grown adult plants. *Biol. Plantarum* 49: 439-442.
 22. Falk L, Biswas K, Boeckelmann A, Lane A, Mahmoud S (2013) An efficient method for the micropropagation of Lavenders: regeneration of a unique mutant. *J Essent Oil Res* 21: 37-41.
 23. Ghiorghita G, Maftai DE, Nicuta D (2009) Some aspects concerning the in vitro reaction of *Lavandula angustifolia* L. *Propag. Ornam. Plants* 9: 47-49.
 24. Handa KL, Chopra IC, Abrol BK (1957) Introduction of some of the important exotic aromatic plants in Jammu and Kashmir. *Indian Perfumer* 1: 44
 25. Hassanpouraghdam MB, Hassani A, Vojodi L, Asl BH, Rostami A (2011) Essential oil

- constituents of *Lavandula officinalis* Chaix. from Northwest Iran. *Chemija* 22: 167-171.
26. Hassiotis CN, Tarantilis PA, Daferera D, Polissiou MG (2010) Etherio, a new variety of *Lavandula angustifolia* with improved essential oil production and composition from natural selected genotypes growing in Greece. *Industrial Crops and Products* 32: 77-82.
 27. Ibrahim HM, Salama AM, Abou El-Leel OF (2017) Analysis of Genetic Diversity of *Lavandula* Species using Taxonomic, Essential Oil and Molecular Genetic Markers. *Sciences* 7: 141-154.
 28. Jordan A, Calvo MC, Segura J (1998) Micropropagation of adult *Lavandula dentata* plants. *J. Hortic. Sci. Biotech* 73: 93-96.
 29. Jordan AM, Calvo MC, Segura J (1990) Morphogenesis in callus and single-cell cultures of *Lavandula latifolia* Medicus. *J. Hortic. Sci. Biotech* 65: 49-53.
 30. Keykha F, Khadem A, Bagheri A, Sharifi A, Ameri M (2014) Optimization of lavender (*Lavandula angustifolia*) callus culture. *Plant Tissue Culture and Biotechnology* 24: 279-285.
 31. Keykha F, Khadem A, Bagheri A, Sharifi A, Ameri M (2014) Optimization of lavender (*Lavandula angustifolia*) callus culture. *Plant Tissue Culture and Biotechnology* 24: 279-285.
 32. Khandare RV, Kabra AN, Kurade MB, Govindwar SP (2011) Phytoremediation potential of *Portulaca grandiflora* Hook.(Moss-Rose) in degrading a sulfonated diazo reactive dye Navy Blue HE2R (Reactive Blue 172). *Bioresource technology* 102: 6774-6777.
 33. Landmann C, Fink B, Festner M, Dregus M, Engel KH, Schwab W (2007) Cloning and functional characterization of three terpene synthases from lavender (*Lavandula angustifolia*). *Archives of Biochemistry and Biophysics* 465: 417-429.
 34. Lappin GJ, Stride JD, Tampion J (1987) Biotransformation of monoterpenoids by suspension cultures of *Lavandula angustifolia*. *Phytochemistry* 26: 995-997.
 35. Lis-Balchin M (2002) *The Genus Lavandula*. Taylor & Francis, London and New York.
 36. Lubbe A, Verpoorte R (2011) Cultivation of medicinal and aromatic plants for specialty industrial materials. *Industrial Crops and Products* 34: 785-801.
 37. Makunga NP, Jäger AK, Van Staden J (2003) Micropropagation of *Thapsia garganica*—a medicinal plant. *Plant Cell Reports* 21: 967-973.
 38. McCartan SA, Van Staden J (1998) Micropropagation of the medicinal plant, *Scilla natalensis* Planch. *Plant growth regulation* 25: 177-180.
 39. Mendoza-Poudereux I, Muñoz-Bertomeu J, Navarro A, Arrillaga I, Segura J (2014) Enhanced levels of S-linalool by metabolic engineering of the terpenoid pathway in spike lavender leaves. *Metabolic engineering* 23: 136-144.
 40. Muñoz-Bertomeu J, Arrillaga I, Ros R, Segura J (2006) Up-regulation of 1-deoxy-D-xylulose-5-phosphate synthase enhances production of essential oils in transgenic spike lavender. *Plant physiology* 142: 890-900.
 41. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plantarum* 15: 473-497.
 42. Onisei T, Toth ET, Amariei D (1994) Somatic embryogenesis in Lavender tissue culture: I. Isolation and characteristics of an embryogenic callus line. *Journal of Herbs, Spices & Medicinal Plants* 2: 17-29.
 43. Onisei T, Tóth ET, Amariei D (1999) Somatic embryogenesis in Lavender tissue culture: II. Influence of medium composition and gamma irradiation on embryo development. *Journal of herbs, spices & medicinal plants* 6: 89-99.
 44. Panizza M, Tognoni F (1988) Clonal propagation, callus formation and plant regeneration of Lavandin. *Sci. Hort* 37: 157-163.
 45. Quazi MH (1980) In vitro multiplication of *Lavandula spp.* *Ann. Bot* 45: 361-362.
 46. Robledo-Paz A, Villalobos-Arámbula VM, Jofre-Garfias AE (2000) Efficient plant regeneration of garlic (*Allium sativum* L.) by root-tip culture. *In Vitro Cellular & Developmental Biology-Plant* 36: 416-419.

47. Sánchez-Gras MC, Calvo MC (1996) Micropropagation of *Lavandula latifolia* through nodal bud culture of mature plants. *Plant Cell Tissue Org. Cult* 45: 259–261.
48. Shahl AS, Kumar S (2000) Potential of lavender oil industry in Kashmir. *J Med Aromatic Plant Sci* 22: 319-321.
49. Singh AK, Singh J, Sharma S (1989) Multivariate analysis in relation to genetic improvement in lavender, *Lavandula officinalis* Chaix. *Plant breeding* 102: 302-305
50. Singh S, Singh V, Babu GK, Kaul VK, Ahuja PS (2007) ECONOMICS OF LAVENDER (*LAVANDULA OFFICINALIS* L.). *Journal of Non-Timber Forest Products* 14: 97-100
51. Sudriá C, Palazón J, Cusidó R, Bonfill M, Pinol MT, Morales C (2001) Effect of benzyladenine and indolebutyric acid on ultrastructure, glands formation, and essential oil accumulation in *Lavandula dentata* plantlets. *Biol. Plantarum* 44: 1–6.
52. Sudriá C, Pinol MT, Palazón J, Cusidó RM, Vila R, Morales C, Bonfill M, Cagniguala S (1999) Influence of plant growth regulators on the growth and essential oil content of cultured *Lavandula dentata* plantlets. *Plant Cell Tissue Org. Cult* 58: 177–184.
53. Tsuru M, Ikeda H, Kato H (2009) Efficient genetic transformation in lavandin using *Agrobacterium rhizogenes* as vector. *Journal of the Japanese Society for Horticultural Science* 78:236-241.
54. Tsuru M, Koda M, Inoue M (1999) Comparative effect of different types of cytokinin for shoot formation and plant regeneration from leaf-derived callus of lavender (*Lavandula vera* DC). *Sci. Hort* 81: 331–336.
55. Tsuru M, Koda M, Inoue M (2000) Efficient plant regeneration from multiple shoots formed in the leaf-derived callus of *Lavandula vera*, using the “open culture system”. *Sci. Hort* 86: 81–88.
56. Urwin N (2009) Improvement of Lavender Varieties by Manipulation of Chromosome Number. Rural Industries Research and Development Corporation.
57. Urwin NA (2014) Generation and characterisation of colchicine-induced polyploid *Lavandula* × *intermedia*. *Euphytica* 197: 331-339.
58. Urwin NA, Horsnell J, Moon T (2007) Generation and characterisation of colchicine-induced autotetraploid *Lavandula angustifolia*. *Euphytica* 156: 257-266.
59. Verma RS, Laiq, UR, Chanotiya CS, Verma RK, Chauhan A, Yadav A, Singh A, Yadav AK (2010) Essential oil composition of *Lavandula angustifolia* Mill. cultivated in the mid hills of Uttarakhand, India. *Journal of the Serbian Chemical Society* 75: 343-348.
60. Watanabe K, Yano S, Yamada Y (1982) The selection of cultured plant cell lines producing high levels of biotin. *Phytochemistry* 21: 513-516
61. Yew C, Balakrishnan B, Balakrishnan J, Subramaniam S (2010) The effect of cytokinins on in vitro shoot length and multiplication of *Hymenocallis littoralis*. *J Med Plants Res* 4: 2641-2646
62. Yokoya NS, Handro W (1996) Effects of auxins and cytokinins on tissue culture of *Grateloupia dichotoma* (Gigartinales, Rhodophyta). *Hydrobiologia* 326: 393-400.
63. Zuzarte M, Dinis A, Cavaleiro C, Salgueiro L, Canhoto J (2010) Trichomes, essential oils and in vitro propagation of *Lavandula pedunculata* (Lamiaceae). *Ind. Crops. Prod* 32:580-587.

Table 1: Media combinations and their effect on growth and development in *Lavandula angustifolia* Mill

Sl No	Media composition	<i>In-vitro</i> establishment and multiplication of cultures.	Callogenesis	Caulogenesis	Rhizogenesis
1	0.5MS ₀	Least growth response	-NA-	Least response	Better response
2	0.5MS ₀ +0.50 ppm IBA	-NA-	No response	-NA-	Best response
3	0.5MS ₀ +0.50 ppm IAA	-NA-	Better response	-NA-	-NA-
4	0.5MS ₀ +0.50 ppm IAA+0.50 ppm IBA	-NA-	Better response	-NA-	-NA-
5	MS ₀	75% of the explants completely recovered from initial inoculation stress within 2 weeks.	-NA-	Low response	Good response
6	MS ₀ +1 ppm BAP	Moderate growth response	-NA-	Moderate response	-NA-
7	MS ₀ +2 ppm BAP	Moderate growth response	-NA-	Good response	-NA-
8	MS ₀ + 0.25 ppm Kin	Moderate growth response	-NA-	Low response	-NA-
9	MS ₀ + 0.50 ppm Kin	Good growth response	-NA-	Moderate response	-NA-
10	MS ₀ +1 ppm BAP+ 0.25 ppm Kin	Better growth response	-NA-	Better response	-NA-
11	MS ₀ +1 ppm BAP+ 0.50 ppm Kin	Best overall growth response for fastest multiplication in minimum time.	-NA-	Best response	-NA-
12	MS ₀ +2 ppm BAP+ 0.25 ppm Kin	Better growth response	-NA-	Better response	-NA-
13	MS ₀ +2 ppm BAP+ 0.50 ppm Kin	Good growth response	-NA-	Good response	-NA-
14	MS ₀ +5 ppm 2,4-D+ 0.25 ppm Kin	-NA-	Best response in minimum time with apical shoot explants.	-NA-	-NA-
15	MS ₀ +0.50 ppm IAA	-NA-	Better response	-NA-	-NA-
16	MS ₀ +0.50 ppm IBA	-NA-	No response	-NA-	Good response
17	MS ₀ +0.50 ppm IAA+0.50 ppm IBA	-NA-	Better response	-NA-	-NA-

Where, -NA- = Not Applicable

Table 2: Descriptive features of morpho-chemo-metric traits of *L. angustifolia* Mill

Features	Morphometric traits						Chemometric traits														
	T1	T2	T3	T4	T5	T6	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Mean ± Standard Error	24.86 ± 0.60	16.59 ± 0.98	14.59 ± 1.19	29.08 ± 1.15	2.11 ± 0.09	17.73 ± 0.88	23.59 ± 3.39	1.03 ± 0.55	11.64 ± 1.14	8.29 ± 0.82	4.62 ± 0.37	2.68 ± 0.33	1.30 ± 0.35	1.23 ± 0.59	13.90 ± 1.85	0.39 ± 0.06	3.05 ± 1.09	2.67 ± 0.65	0.77 ± 0.29	3.39 ± 1.87	0.01 ± 0.01
Standard Deviation	2.83	4.59	5.59	5.39	0.46	4.12	15.93	2.59	5.34	3.87	1.74	1.57	1.64	2.74	8.66	0.26	5.15	3.03	1.39	8.77	0.04
Sample Variance	8.03	21.11	31.25	29.15	0.21	16.99	253.73	6.74	28.36	14.97	3.04	2.47	2.68	7.53	75.07	0.07	26.51	9.16	1.93	76.87	0.002
Count	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22

Chemometric traits are : F1=Eucalyptol(%), F2= endo-Borneol(%), F3= 3-Carene(%), F4= Camphene(%), F5= α-Pinene(%), F6= p-Cymene(%), F7= o-Cymene(%), F8= Cyclobutane,1,2-dicyclopropyl-(%), F9= Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-(%), F10= Linalool(%), F11= D-Limonene(%), F12= (+)-2-Bornanone(%), F13= α-Phellandrene(%), F14= β-Phellandrene(%), F15= Linalyl acetate(%).

Table 3: ANOVA for six morphometric traits of *L. angustifolia* Mill. somaclones indicating significant variation between groups

Source of variation	Sum of Squares	Degrees of freedom	Mean Sum of Squares	F- value
Between groups	9556.33	5.00	1911.26***	107.43
Within groups	2241.59	126.00	17.79	
Total	11797.92	131.00		

Where, *** = $p < 0.001$.

Table 4: Essential oil constituents in somaclones of *L. angustifolia* Mill

Treatments	Concentrations (%) of essential oil constituents from leaves of lavender treatments.															Total %	Number of highest Constituent(s)/Treatment
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15		
SC1	0.00	0.00	<u>22.52</u>	5.74	3.26	3.10	0.00	0.00	9.48	0.50	0.00	0.48	2.83	0.00	0.00	47.91	1
SC2	20.50	0.00	12.30	11.32	6.06	4.15	0.00	0.00	19.89	0.39	0.00	1.68	0.78	0.00	0.00	77.07	0
SC3	13.15	0.00	16.01	6.61	3.39	3.65	0.00	0.00	10.30	0.00	0.00	0.55	1.32	<u>27.39</u>	0.00	82.37	1
SC4	0.00	0.00	14.41	6.09	3.25	3.11	0.00	0.00	17.28	0.29	0.00	0.83	1.13	0.00	0.00	<u>46.39</u>	0
SC5	11.35	0.00	15.05	0.00	<u>8.62</u>	0.00	<u>5.60</u>	0.00	8.92	0.20	<u>17.42</u>	1.43	0.68	0.00	0.00	69.36	3
SC6	22.47	1.49	0.00	<u>16.35</u>	0.00	0.00	2.78	0.00	0.00	0.44	5.05	<u>15.00</u>	0.61	0.00	0.00	64.19	2
SC7	10.54	0.00	18.15	9.65	5.22	3.84	2.29	<u>0.00</u>	20.86	<u>1.04</u>	0.00	1.93	1.01	0.00	0.00	74.53	1
SC8	22.70	4.11	16.66	3.12	2.98	1.24	1.99	<u>0.00</u>	0.00	0.41	0.00	0.00	1.24	24.32	<u>0.19</u>	78.96	1
SC9	8.82	0.00	18.28	8.47	5.59	2.40	1.39	<u>0.00</u>	12.85	0.43	0.00	0.90	1.19	22.93	0.00	83.25	0
SC10	15.04	0.00	15.29	7.93	4.63	3.57	0.00	<u>8.61</u>	19.51	0.47	0.00	1.93	<u>6.09</u>	0.00	0.00	83.07	2
SC11	21.31	0.00	8.28	10.85	5.02	1.34	2.78	0.00	25.29	0.86	7.57	3.39	0.00	0.00	0.00	86.69	0
SC12	39.91	0.00	15.00	9.39	5.39	<u>4.72</u>	0.00	0.00	8.14	0.19	0.00	1.87	0.00	0.00	0.00	84.61	1
SC13	14.91	0.00	8.91	11.27	5.63	1.35	2.88	0.00	21.15	0.48	13.41	3.42	0.00	0.00	0.00	83.41	0
SC14	29.69	0.00	3.84	11.01	5.45	4.37	0.00	0.00	22.92	0.38	5.68	4.62	0.00	0.00	0.00	<u>87.96</u>	0
SC15	18.46	0.00	7.24	12.34	6.23	4.51	0.00	0.00	23.87	0.71	9.73	3.92	0.00	0.00	0.00	87.01	0
SC16	41.51	0.00	8.48	6.26	5.08	4.34	0.00	0.00	11.58	0.22	0.00	1.74	0.00	0.00	0.00	79.21	0
SC17	20.83	0.00	10.60	13.19	5.93	1.48	3.07	7.45	17.95	0.38	0.00	3.76	0.00	0.00	0.00	84.64	0
SC18	21.62	0.00	7.26	10.88	5.03	4.05	0.00	0.00	<u>25.56</u>	0.55	8.28	3.61	0.00	0.00	0.00	86.84	1
SC19	41.53	0.00	12.44	4.81	3.99	3.00	0.00	4.57	9.66	0.27	0.00	1.02	0.00	0.00	0.00	81.29	0
SC20	28.32	0.00	10.27	9.52	5.43	0.00	3.21	6.39	20.68	0.26	0.00	2.58	0.00	0.00	0.00	86.66	0
Control 1	<u>59.45</u>	7.59	7.28	3.98	2.57	1.31	2.59	0.00	0.00	0.00	0.00	2.42	0.00	0.00	0.00	87.19	1
Control2	56.91	<u>9.38</u>	7.67	3.70	2.98	3.40	0.00	0.00	0.00	0.00	0.00	1.64	0.00	0.00	0.00	85.68	1

Where, SC= Somaclones, F1=Eucalyptol, F2= endo-Borneol, F3= 3-Carene, F4= Camphene, F5= alpha.-Pinene, F6= p-Cymene, F7= o-Cymene, F8= Cyclobutane, 1,2-dicyclopropyl-, F9= Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-, F10= Linalool, F11= D-Limonene, F12= (+)-2-Bornanone, F13= .alpha.-Phellandrene, F14= beta.-Phellandrene, F15= Linalyl acetate. Underlined numbers indicate highest values while underlined & italicized number indicate lowest values in the range.

Table 5: ANOVA for fifteen chemometric traits of *L. angustifolia* Mill. somaclones indicating significant variation between groups

Source of Variation	Sum of Squares	Degrees of freedom	Mean Sum of Squares	F- value
Between Groups	13290.41	14	949.315***	27.969
Within Groups	10691.50	315	33.941	
Total	23981.92	329		

Where, *** = $p < 0.001$.

Table 6: Regression table for best fit model related to chemometric traits of *L. angustifolia* Mill.

Regression Statistics	Chemometric traits														
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Multiple r	0.953	0.917	0.987	0.962	0.945	0.965	0.969	0.872	0.985	0.927	0.902	0.991	0.877	0.766	0.823
r ²	0.909	0.841	0.974	0.926	0.892	0.932	0.938	0.761	0.971	0.859	0.814	0.981	0.769	0.587	0.678
Adjusted r ²	0.725	0.523	0.921	0.778	0.676	0.796	0.815	0.282	0.913	0.578	0.441	0.944	0.309	-0.239	0.033
Standard Error	8.349	1.792	1.495	1.823	0.991	0.709	0.704	2.326	2.552	0.168	3.850	0.719	1.155	9.758	0.039
Observations	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
Model Rank(s)	-	-	2nd	-	-	-	-	-	3rd	-	-	1st	-	-	-

Where, Chemometric traits are : F1=Eucalyptol, F2= endo-Borneol, F3= 3-Carene, F4= Camphene, F5= alpha.-Pinene, F6= p-Cymene, F7= o-Cymene, F8= Cyclobutane, 1,2-dicyclopropyl-, F9= Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-, F10= Linalool, F11= D-Limonene, F12= (+)-2-Bornanone, F13= .alpha.-Phellandrene, F14= beta.-Phellandrene, F15= Linalyl acetate.

Table 7: ANOVA for twentyone (T1-T6 and F1-F15) morpho-chemo-metric traits of *L. angustifolia* Mill. somaclones indicating significant variation between groups.

Source of Variation	Sum of Squares	Degrees of freedom	Mean Sum of Squares	F- value
Between Groups	37012.519	20	1850.626***	63.104
Within Groups	12933.097	441	29.327	
Total	49945.616	461		

Where, *** = $p < 0.001$.

Table 8: Comparison of efficacy among three post hoc tests simplified through Set Theory.

Sl No.	Set :	Set notation :	Number of significant pairs(n) :	Information:
1.	Tukey-Kramer post-hoc test	T	n(T)=124	59.05% significant pairs.
2.	Scheffe's post-hoc test	S	n(S)=207	98.57% significant pairs.
3.	Bonferroni corrected post-hoc T test	B	n(B)=145	69.05% significant pairs.
4.	Common significant pairs between T, S and B	$T \cap S \cap B$, black zone	$n(T \cap S \cap B)=121$	-
5.	Common significant pairs between T and S	$T \cap S$, orange and black zone	$n(T \cap S)=124$	-
6.	Common significant pairs between S and B	$S \cap B$, violet and black zone	$n(S \cap B)=145$	-
7.	Common significant pairs between T and B	$T \cap B$, blue and black zone	$n(T \cap B)=121$	$(T \cap B) = (T \cap S \cap B) = 121$
8.	Common significant pairs only between T and S	Orange zone	$n(T \cap S) - n(T \cap S \cap B) = 124 - 121 = 3$	-
9.	Common significant pairs only between S and B	Violet zone	$n(S \cap B) - n(T \cap S \cap B) = 145 - 121 = 24$	-
10.	Common significant pairs only between T and B	Blue zone	$n(T \cap B) - n(T \cap S \cap B) = 121 - 121 = 0$	ϕ
11.	Number of elements of only T	Yellow zone	$n(T) - \{n(T \cap B) \cup n(S \cap T)\} = 0$	ϕ
12.	Number of elements of only S	Red zone	$n(S) - \{n(T \cap S) \cup n(S \cap B)\} = 59$	-
13.	Number of elements of only B	Green zone	$n(B) - \{n(T \cap B) \cup n(S \cap B)\} = 0$	ϕ

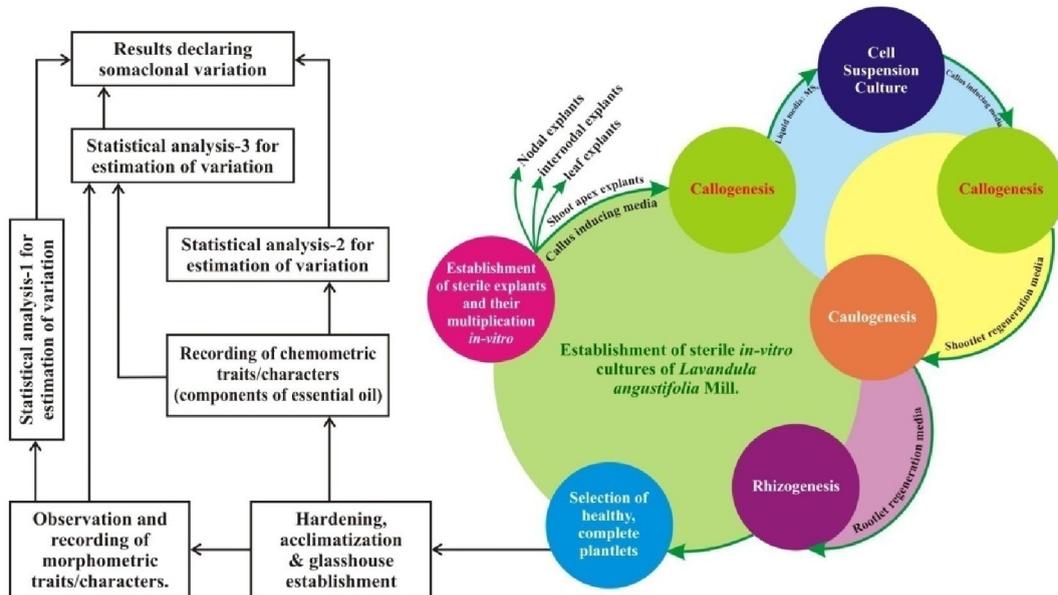
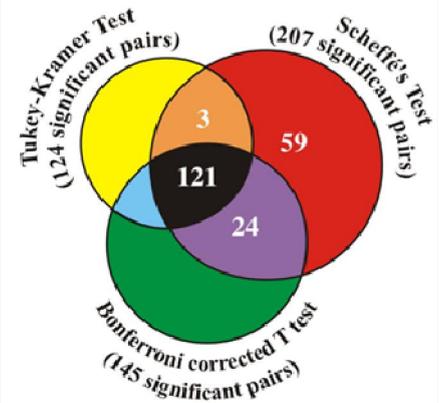


Figure 1: A schematic representation of the present study.

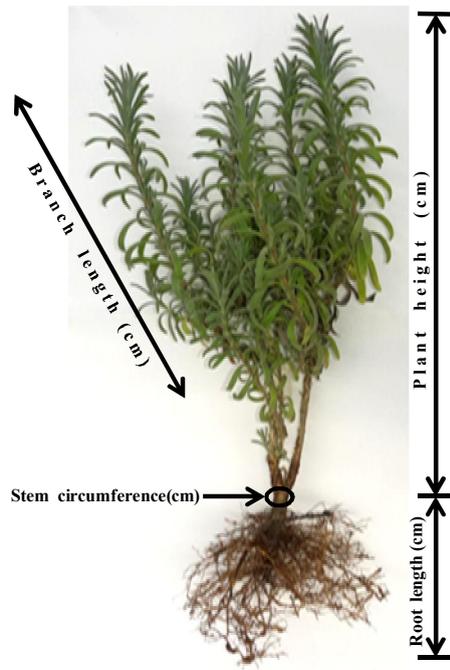
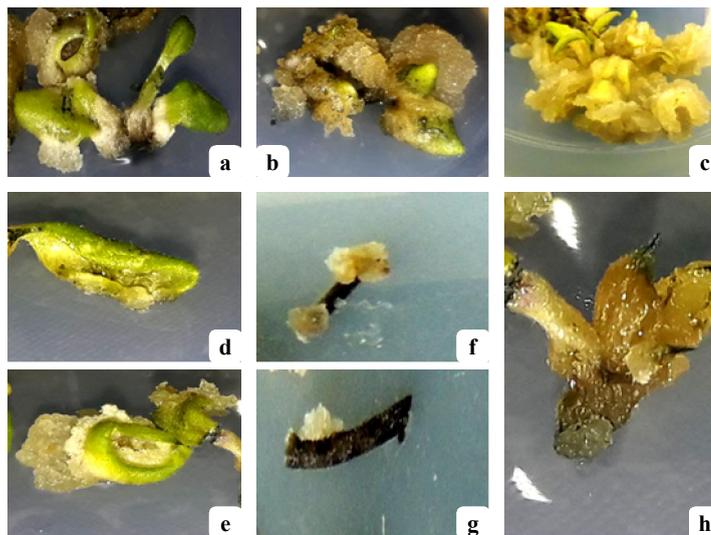
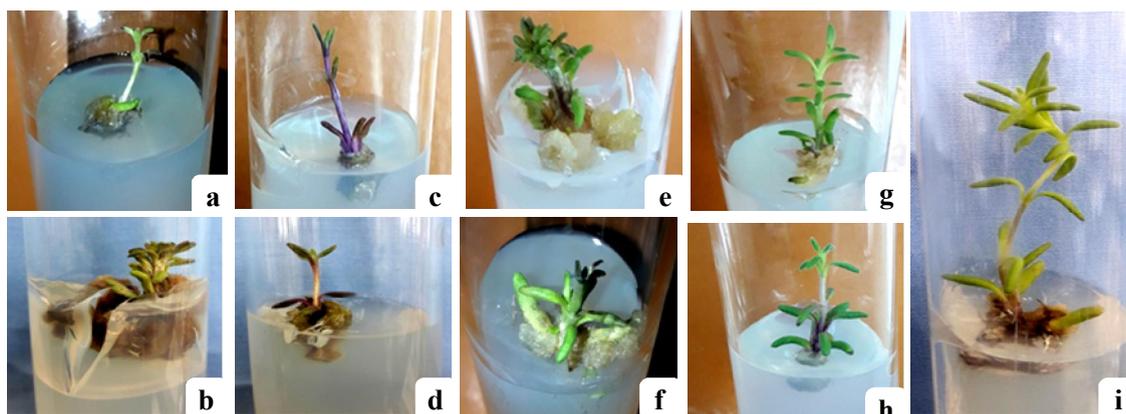


Figure 2: Some of the morphometric traits of *L. angustifolia* Mill. considered in our study.



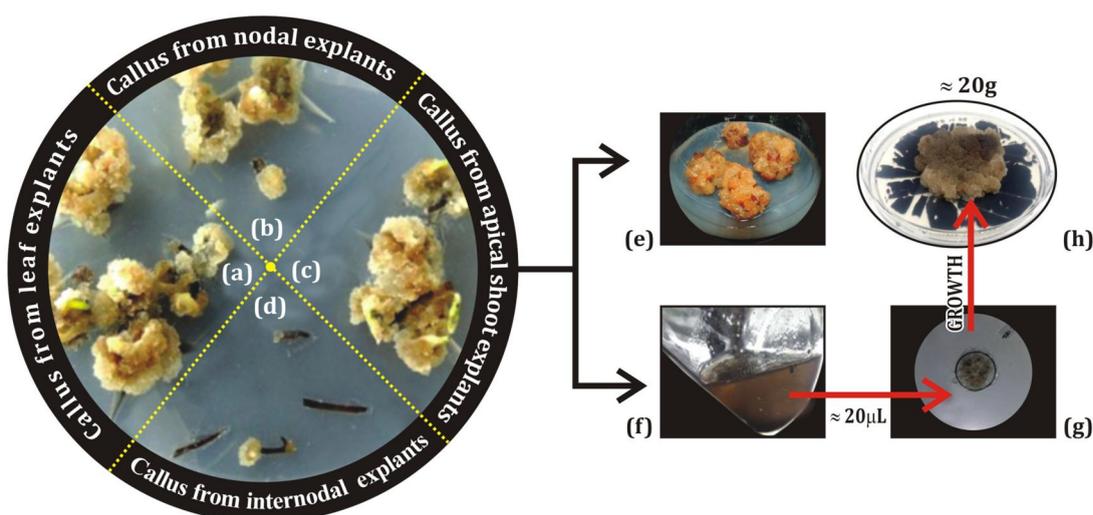
(a)-(c) apical shoot explants giving rise to vigorous callus, (d)-(e) leaf explants giving rise to callus, (f)-(g) internodal explants giving rise to inconspicuous callus and (h) nodal explant giving rise to callus.

Figure 3: Different stages of callogenesis of *L. angustifolia* explants.



(a)-(b) $0.5MS_0+0.5$ ppm IAA+0.5 ppm IBA media composition, (c)-(d) $0.5MS_0+0.5$ ppm IBA media composition, (e)&(g) $MS_0+0.5$ ppm IAA media composition, (f) $0.5MS_0+0.5$ ppm IAA media composition, (h)-(i) $MS_0+0.5$ ppm IAA+0.5 ppm IBA media composition gave rise to callus.

Figure 4: Callogenic response in some media combinations on apical shoot explants of *L. angustifolia*.



Callus formation in progress from (a) leaf explants, (b) nodal explants, (c) apical shoot explants, (d) internodal explants, (e) Callus formation complete from apical shoot explants, which is used for other experiments not included in this study, (f) Callus from apical shoot explants used to develop cell suspension culture in liquid MS_0 medium (no agar and no PGRs), (g) 20µl of cell suspension culture transferred and evenly distributed on $MS_0 + 5.00$ ppm 2,4-D + 0.25 ppm Kin media composition, (h) a mass of callus (20 g) obtained from cell suspension culture.

Figure 5: Callogenic response of different explants in $MS_0 + 5.00$ ppm 2,4-D + 0.25 ppm Kin media composition of *L. angustifolia*.

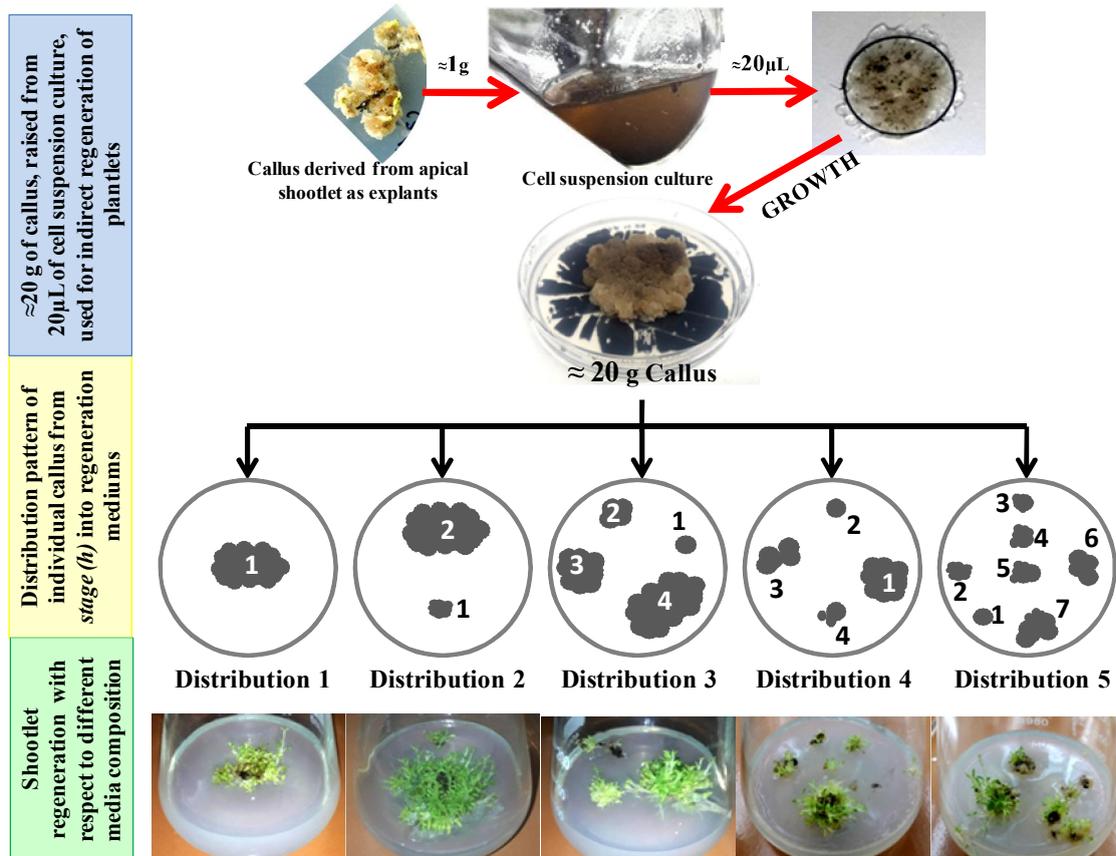
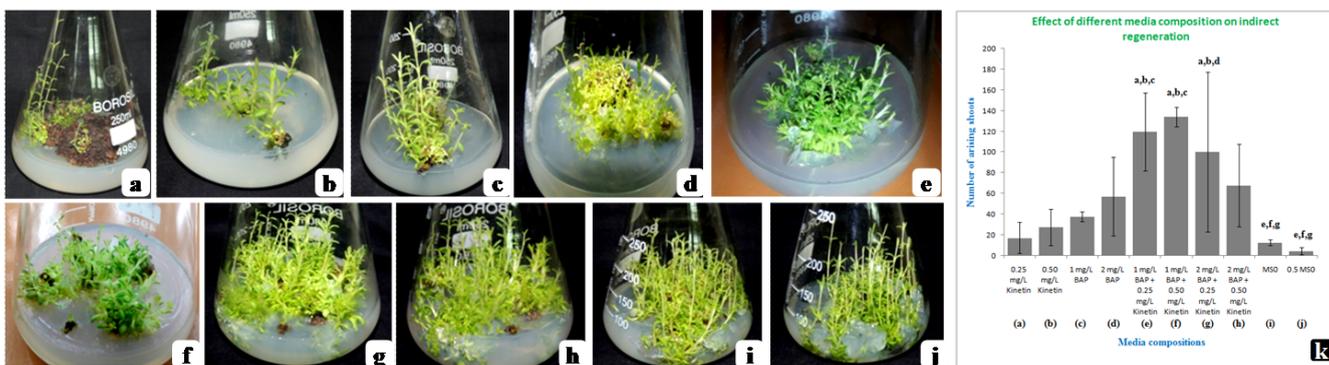
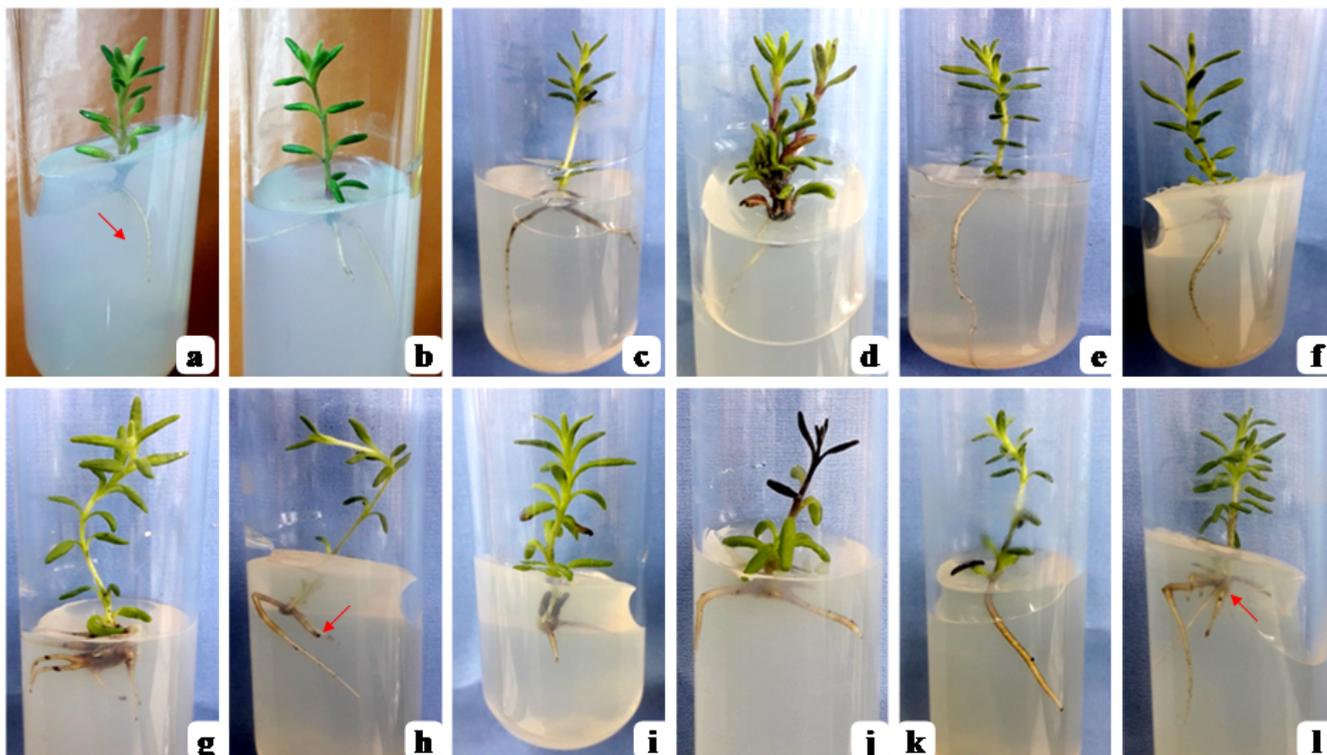


Figure 6: Caulogenic response in different distribution pattern in shoot inducing media composition of *L. angustifolia*.



(a) 0.50 MS₀, (b) MS₀, (c) MS₀ + 0.25 ppm Kin, (d) MS₀ + 0.50 ppm Kin, (e) MS₀ + 1.00 ppm BAP, (f) MS₀ + 2.00 ppm BAP, (g) MS₀ + 1.00 ppm BAP + 0.25 ppm Kin, (h) MS₀ + 1.00 ppm BAP + 0.50 ppm Kin, (i) MS₀ + 2.00 ppm BAP + 0.25 ppm Kin, (j) MS₀ + 2.00 ppm BAP + 0.50 ppm Kin, (k) an estimation of the number of shoots arising from different shoot regeneration media. The mean number of shoots from five replicates per medium combination are represented by respective columns and the error bars depict respective SD. The different letters above error bars indicate a statistically significant difference as per Tukey-Kramer procedure with significance level at $p < 0.05$.

Figure 7: Caulogenic response in different media composition of *L. angustifolia*.



(a) $0.50 MS_0 + 0.50 \text{ ppm IBA}$, (b) $0.5 MS_0$, (c) $0.5 MS_0$, (d) MS_0 , (e) $0.5 MS_0$, (f) MS_0 , (g) $MS_0 + 0.50 \text{ ppm IBA}$, (h) $0.50 MS_0 + 0.50 \text{ ppm IBA}$, (i) $MS_0 + 0.50 \text{ ppm IBA}$, (j) $0.5 MS_0$, (k) $0.5 MS_0$, (l) $0.50 MS_0 + 0.50 \text{ ppm IBA}$.

Figure 8: Rhizogenesis in different media composition of *L. angustifolia* leading to the formation of complete plantlets. Red arrows indicates development of primary branches in roots.

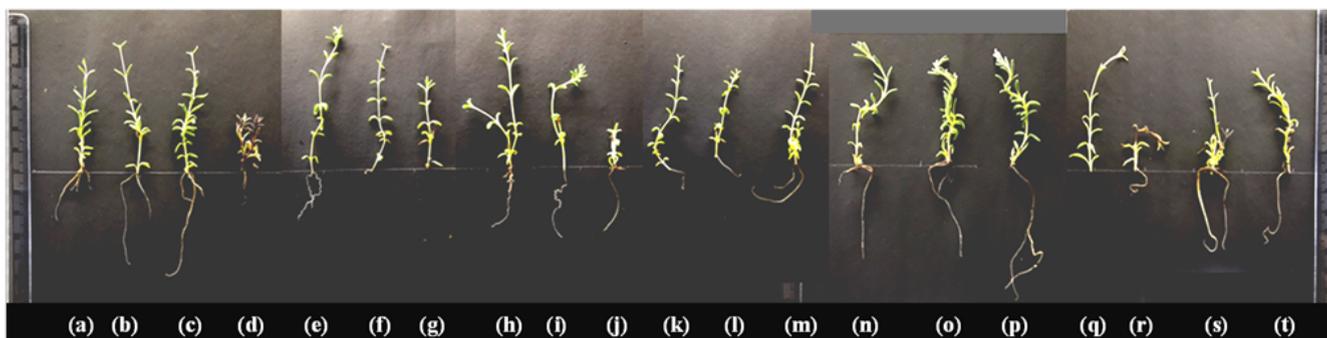
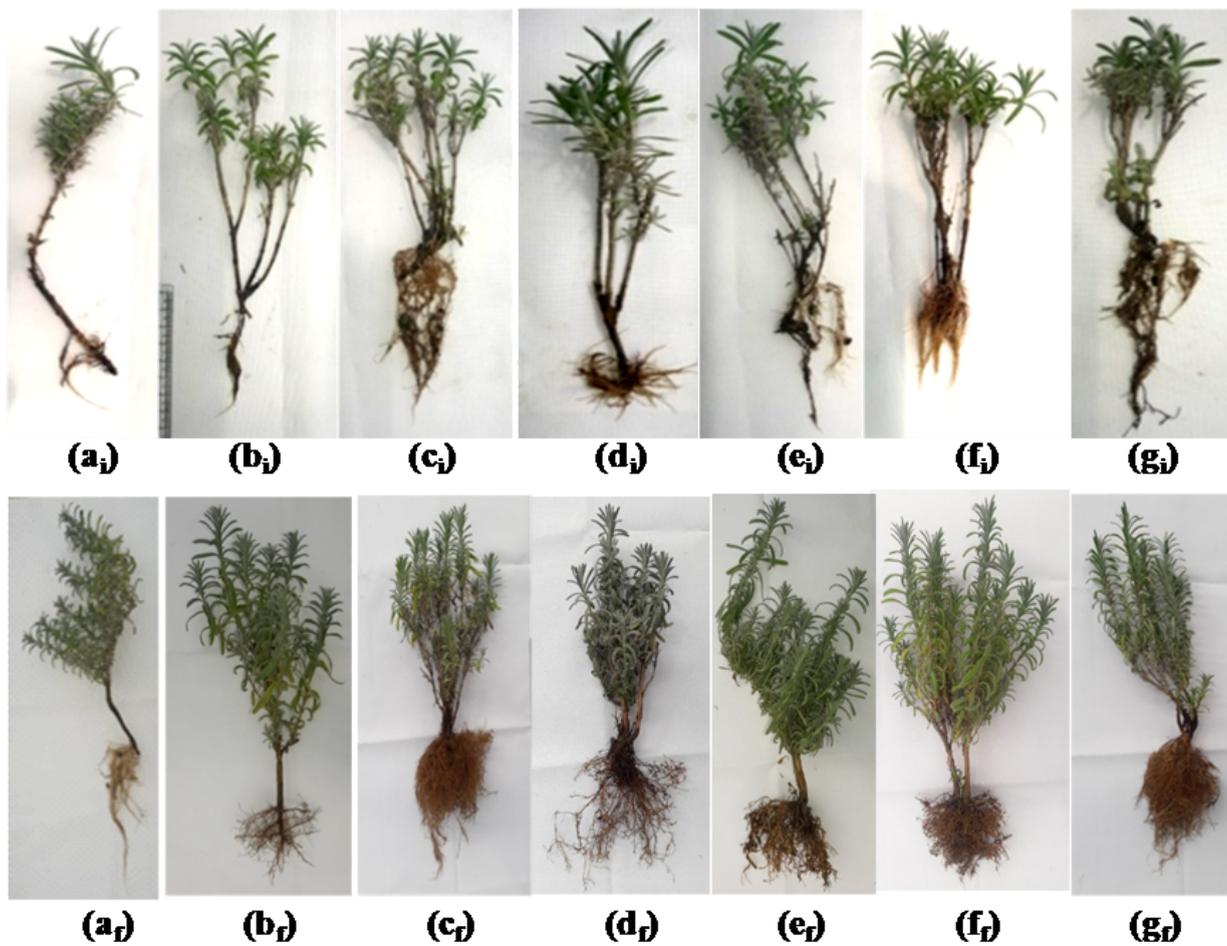


Figure 9: Some of the complete plantlets of *L. angustifolia* (somaclones) ready to go through acclimatization and hardening stress.



(a_i - g_i) somaclones during intermediate stage of growth and development, (a_f - g_f) same somaclones during final stage of growth and development.

*Figure 10: Some representative somaclones of *L. angustifolia* under acclimatization and hardening stress.*

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	T1	T2	T3	T4	T5	T6	
F1	1.000																					
F2	0.667**	1.000																				
F3	-0.495**	-0.230**	1.000																			
F4	-0.183**	-0.403**	-0.481**	1.000																		
F5	-0.213**	-0.470**	0.209**	-0.058**	1.000																	
F6	0.059**	-0.158**	0.145**	0.074**	0.127**	1.000																
F7	-0.104**	0.029**	-0.080**	-0.062**	0.243**	-0.864**	1.000															
F8	0.020**	-0.185**	0.061**	0.113**	0.120**	-0.187**	0.087**	1.000														
F9	-0.432**	-0.664**	-0.055**	0.493**	0.581**	0.289**	-0.099**	0.205**	1.000													
F10	-0.504**	-0.450**	0.077**	0.490**	0.200**	0.083**	0.100**	-0.042**	0.604**	1.000												
F11	-0.223**	-0.219**	-0.320**	0.076**	0.459**	-0.291**	0.482**	-0.278**	0.289**	0.230**	1.000											
F12	0.055**	-0.025**	-0.725**	0.680**	-0.401**	-0.324**	0.234**	-0.036**	-0.073**	0.177**	0.267**	1.000										
F13	-0.436**	-0.149**	0.505**	-0.154**	-0.123**	0.112**	-0.209**	0.396**	0.008**	0.117**	-0.243**	-0.179**	1.000									
F14	-0.220**	0.049**	0.402**	-0.236**	-0.161**	-0.051**	-0.056**	-0.181**	-0.289**	-0.187**	-0.240**	-0.293**	0.142**	1.000								
F15	-0.013**	0.265**	0.211**	-0.299**	-0.211**	-0.205**	0.094**	-0.100**	-0.358**	0.022**	-0.132**	-0.197**	0.076**	0.533**	1.000							
T1	0.022**	0.212**	0.158**	-0.390**	-0.253**	0.219**	-0.378**	0.025**	-0.291**	-0.204**	-0.182**	-0.092**	0.380**	-0.068**	0.011**	1.000						
T2	-0.192**	0.258**	-0.014**	-0.075**	-0.174**	0.040**	-0.015**	-0.358**	-0.137**	0.189**	0.102**	0.112**	0.174**	0.272**	0.263**	0.427**	1.000					
T3	0.238**	0.124**	-0.442**	0.044**	0.283**	0.174**	-0.031**	-0.041**	0.259**	0.058**	0.364**	0.025**	-0.307**	0.028**	0.036**	-0.180**	0.223**	1.000				
T4	-0.169**	-0.116**	0.087**	0.170**	0.268**	-0.237**	0.353**	0.267**	0.121**	0.133**	-0.060**	0.047**	0.172**	0.090**	0.059**	-0.145**	0.412**	0.049**	1.000			
T5	-0.196**	0.228**	0.325**	-0.370**	-0.348**	-0.444**	0.293**	-0.206**	-0.519**	-0.151**	-0.168**	-0.083**	0.051**	0.263**	0.503**	0.310**	0.310**	-0.519**	0.076**	1.000		
T6	-0.207**	0.046**	0.217**	-0.287**	0.267**	0.302**	-0.220**	-0.044**	0.153**	-0.170**	0.041**	-0.339**	0.308**	-0.086**	-0.050**	0.499**	0.456**	0.316**	0.101**	0.101**	1.000	

Figure 11: Correlation between twenty one traits of *L. angustifolia* (somaclones).
 Where, Morphometric traits are included in the area DEFD: T1= Plant height, T2= Number of branches, T3=Root length, T4=Plant fresh weight, T5=Eucalyptol, T6= Stem circumference, T7= endo-Borneol, T8= 3-Carene, T9= Camphene, T10= alpha.-Pinene, T11= p-Cymene, T12= o-Cymene, T13= Cyclobutane, T14= 1,2-dicyclopropyl-, T15= Bicyclo[2.2.1]heptan-2-ol, T16= 1,7,7-trimethyl-, T17= D-Limonene, T18= (+)-2-Bornanone, T19= .alpha.-Phellandrene, T20= beta.-Phellandrene, T21= Linalyl acetate.; Area BCDFB includes correlation coefficients encompassing morphometric and chemometric traits;
 *** = p < 0.01; Gradual transformation of green colour to red colour indicates highly positive significant values to highly negative significant values respectively.

Figure 11: Correlation between twenty one traits of *L. angustifolia* (somaclones).

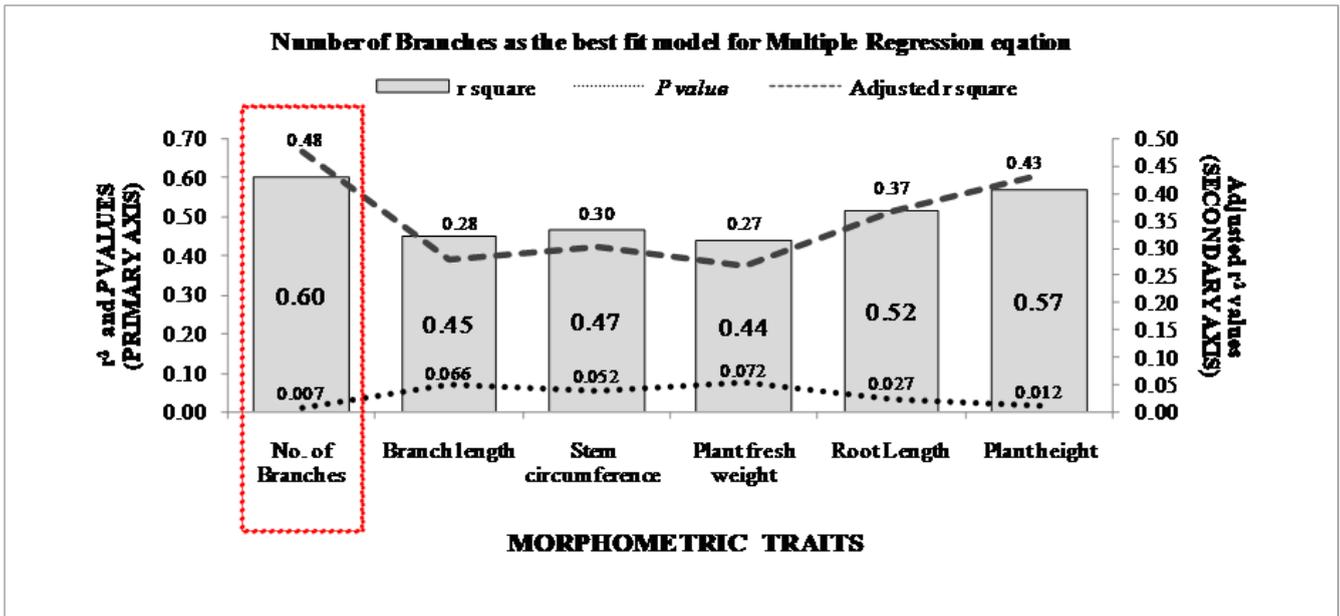
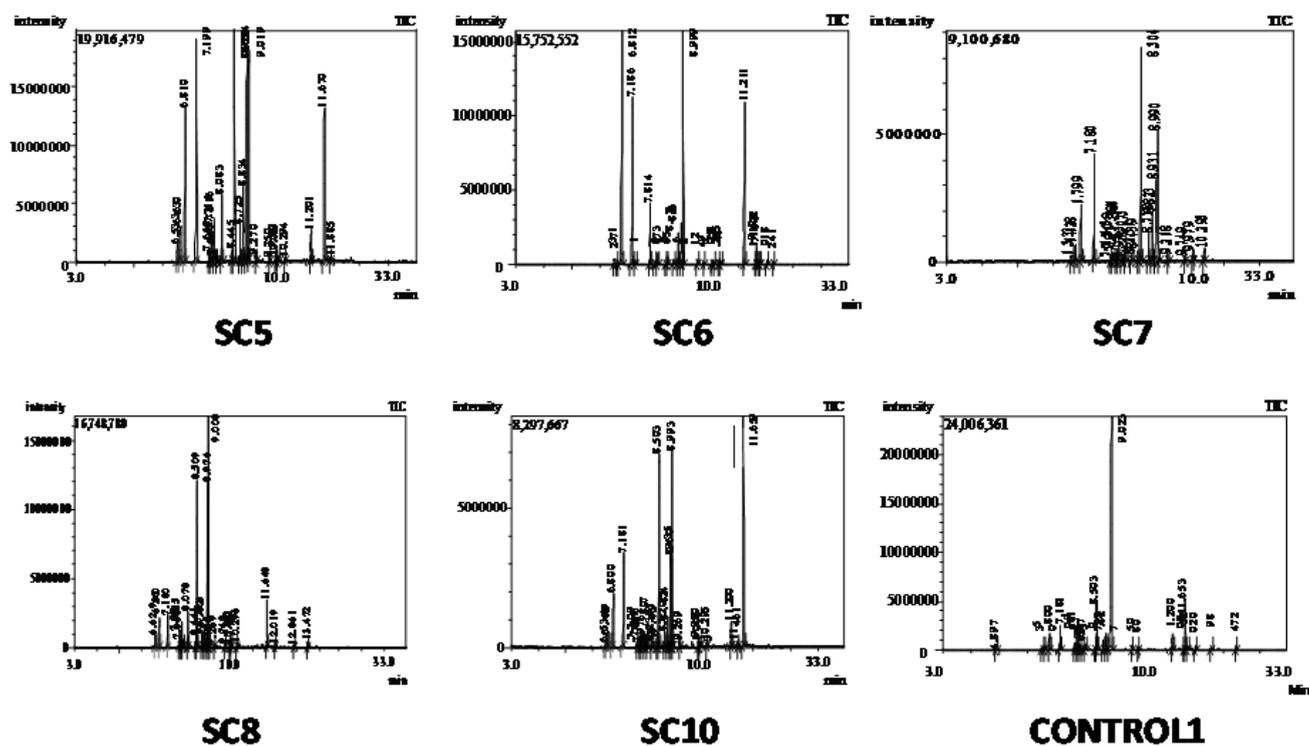


Figure 12: Regression analysis with six morphometric traits and identification of the best regression model.



Highest concentration of essential oil components present in leaves of some treatments(SC5-8) and control1.					
Control1	SC5	SC6	SC7	SC8	SC10
<i>Eucalyptol</i> RT: 9.023 Peak# 15	<i>α-pinene</i> RT : 6.810 Peak# 3	<i>Camphene</i> RT : 7.186 Peak# 4	<i>Linalool</i> RT :10.295 Peak# 21	<i>Linalyl acetate</i> RT : 12.861 Peak# 22	<i>α-phellandrene</i> RT : 8.439 Peak# 11
	<i>o-cymene</i> RT : 8.725 Peak# 12 RT : 8.834 Peak#13	<i>(+)-2-Bornanone</i> RT : 11.211 Peak# 21			<i>Cyclobutane, 1,2-dicyclopropyl-</i> RT : 8.935 Peak# 15
	<i>D-Limonene</i> RT : 8.966 Peak# 14				

Figure 13: HS-GCMS chromatograms of somaclones(SC) 5,6,7,8,10 and control1 of *L. angustifolia* representing variation in essential oil profile.

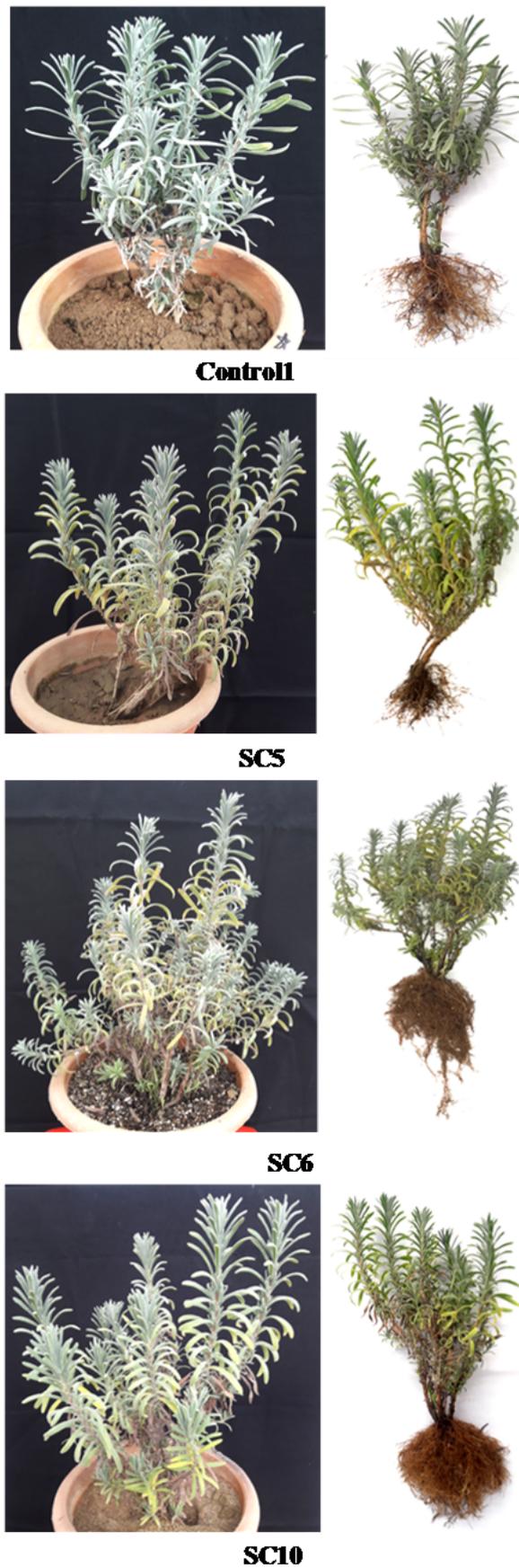
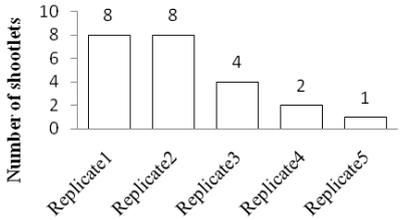
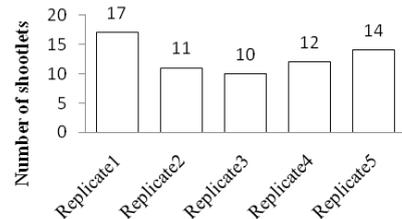
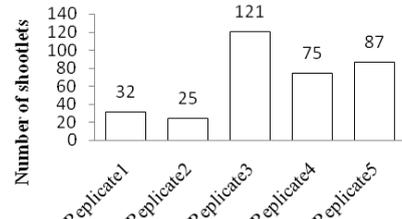
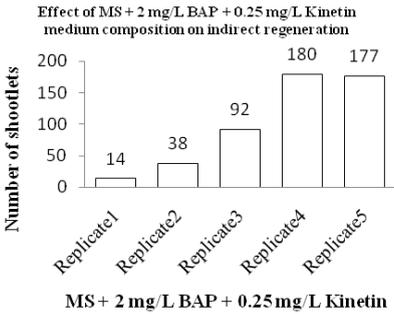
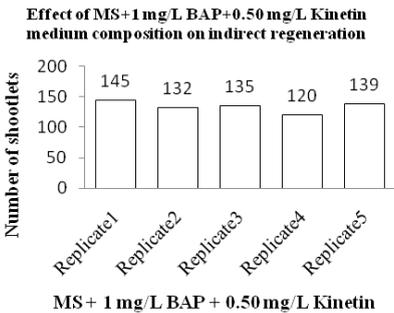
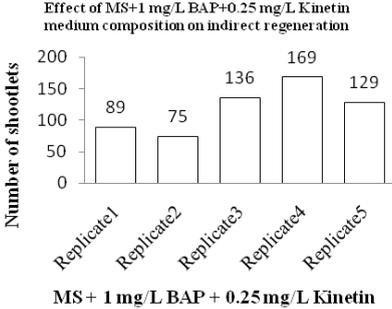
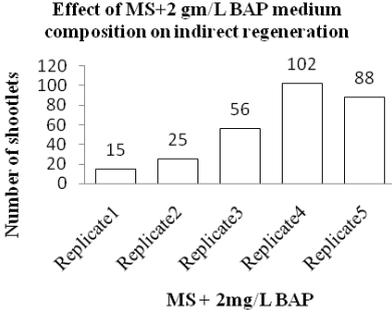
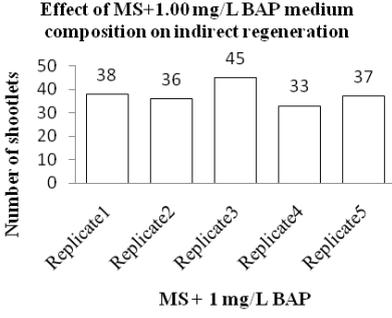


Figure 14: The resultant putative somaclonal variants (SC5, SC6 and SC10) and Control1 of *L. angustifolia* in potted and exposed condition.

Supplementary Table : Caulogenesis

SI No	Particulars	Details		Histogram
1	Media composition	0.50% MS		<p>Effect of 0.5% MS medium composition on indirect regeneration</p>  <p>0.50% MS + 0.00 PGRs</p>
	PGR	Nil		
	Culture duration (weeks)	03		
	Mean of regeneration	4.60±3.29		
	Characteristics of the culture	a. callus ceased to grow, browning of callus. b. frequency of regeneration is least. c. survival rate of regenerants is poorest. d. yellow leaflets with internodal elongation in shootlets.		
2	Media composition	MS		<p>Effect of MS basal medium composition on indirect regeneration</p>  <p>MS + 0.00 PGRs</p>
	PGR	Nil		
	Culture duration (weeks)	03		
	Mean of regeneration	12.80±2.77		
	Characteristics of the culture	a. callus giving rise to healthy shootlets. b. frequency of regeneration is poor. c. survival rate of regenerants considerable. d. green leaflets with healthy shootlets.		
3	Media composition	MS		<p>Effect of MS + 2 mg/L BAP + 0.50 mg/L Kinetin medium composition on indirect regeneration</p>  <p>MS + 2 mg/L BAP + 0.50 mg/L Kinetin</p>
	PGR	2.00 mg/l BAP + 0.50 mg/l Kinetin		
	Culture duration (weeks)	03		
	Mean of regeneration	68.00±39.89		

	Characteristics of the culture	<ul style="list-style-type: none"> a. callus giving rise to healthy shootlets. b. frequency of regeneration is good. c. survival rate of regenerents more considerable. d. green leaflets with healthy shootlets. e. leaflets densely arranged in lower part of the shootlets and internodal elongation in shootlets. 														
4	Media composition	MS		<p>Effect of MS + 2 mg/L BAP + 0.25 mg/L Kinetin medium composition on indirect regeneration</p>  <table border="1"> <caption>Number of shootlets for MS + 2 mg/L BAP + 0.25 mg/L Kinetin</caption> <thead> <tr> <th>Replicate</th> <th>Number of shootlets</th> </tr> </thead> <tbody> <tr> <td>Replicate1</td> <td>14</td> </tr> <tr> <td>Replicate2</td> <td>38</td> </tr> <tr> <td>Replicate3</td> <td>92</td> </tr> <tr> <td>Replicate4</td> <td>180</td> </tr> <tr> <td>Replicate5</td> <td>177</td> </tr> </tbody> </table>	Replicate	Number of shootlets	Replicate1	14	Replicate2	38	Replicate3	92	Replicate4	180	Replicate5	177
	Replicate	Number of shootlets														
	Replicate1	14														
	Replicate2	38														
	Replicate3	92														
Replicate4	180															
Replicate5	177															
PGR	2.00 mg/l BAP + 0.25 mg/l Kinetin															
Culture duration (weeks)	03															
Mean of regeneration	100.20±76.86															
Characteristics of the culture	<ul style="list-style-type: none"> a. callus giving rise to healthy shootlets. b. abundant healthy regenerents. c. primary branching observed. d. green leaflets with healthy shootlets. e. leaflets densely arranged in lower part of the shootlets and internodal elongation in shootlets. 															
5	Media composition	MS		<p>Effect of MS + 1 mg/L BAP + 0.50 mg/L Kinetin medium composition on indirect regeneration</p>  <table border="1"> <caption>Number of shootlets for MS + 1 mg/L BAP + 0.50 mg/L Kinetin</caption> <thead> <tr> <th>Replicate</th> <th>Number of shootlets</th> </tr> </thead> <tbody> <tr> <td>Replicate1</td> <td>145</td> </tr> <tr> <td>Replicate2</td> <td>132</td> </tr> <tr> <td>Replicate3</td> <td>135</td> </tr> <tr> <td>Replicate4</td> <td>120</td> </tr> <tr> <td>Replicate5</td> <td>139</td> </tr> </tbody> </table>	Replicate	Number of shootlets	Replicate1	145	Replicate2	132	Replicate3	135	Replicate4	120	Replicate5	139
	Replicate	Number of shootlets														
	Replicate1	145														
	Replicate2	132														
	Replicate3	135														
Replicate4	120															
Replicate5	139															
PGR	1.00 mg/l BAP + 0.50 mg/l Kinetin															
Culture duration (weeks)	03															
Mean of regeneration	134.20±9.31															
Characteristics of the culture	<ul style="list-style-type: none"> a. callus giving rise to very healthy shootlets. b. most abundant healthy regenerents. c. primary branching observed more. d. green leaflets with healthy shootlets. e. no internodal elongation in shootlets. 															

6	Media composition	MS		<p>Effect of MS+1 mg/L BAP+0.25 mg/L Kinetin medium composition on indirect regeneration</p>  <p>MS + 1 mg/L BAP + 0.25 mg/L Kinetin</p>
	PGR	1.00 mg/l BAP + 0.25 mg/l Kinetin		
	Culture duration (weeks)	03		
	Mean of regeneration	119.60±37.83		
	Characteristics of the culture	<p>a. callus giving rise to very healthy shootlets.</p> <p>b. abundant healthy regenerents.</p> <p>c. primary branching less frequent.</p> <p>d. green leaflets with healthy shootlets.</p> <p>e. leaflets arranged normally over shootlets with no internodal elongation in the later.</p>		
7	Media composition	MS		<p>Effect of MS+2 gm/L BAP medium composition on indirect regeneration</p>  <p>MS + 2mg/L BAP</p>
	PGR	2.00 mg/l BAP		
	Culture duration (weeks)	03		
	Mean of regeneration	57.20±38.00		
	Characteristics of the culture	<p>a. callus giving rise to very healthy shootlets.</p> <p>b. abundant regenerents with delayed response</p> <p>c. green leaflets with healthy shootlets.</p> <p>d. leaflets arranged normally over shootlets with no internodal elongation in the later.</p>		
8	Media composition	MS		<p>Effect of MS+1.00 mg/L BAP medium composition on indirect regeneration</p>  <p>MS + 1 mg/L BAP</p>
	PGR	1.00 mg/l BAP		
	Culture duration (weeks)	03		
	Mean of regeneration	37.80±4.44		
	Characteristics of the culture			