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Jai Kumar

Deptt. of FPU, Faculty of Forestry, BAU, Ranchi, Jharkhand, India

Animesh Sinha

Head, Division of Forest genetics & Tree Breeding, IFP, Ranchi, Jharkhand, India

Ravinder Raj Lal

CTO, IFP, Lalgutwa, Ranchi, Jharkhand, India

Priyashi

Deptt. of Pharmaceutical Sciences & Technology, BIT, Mesra, Ranchi, Jharkhand, India

Corresponding Author: Jai Kumar Deptt. of FPU, Faculty of Forestry, BAU, Ranchi, Jharkhand, India

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Qualitative characterization and diversity assessment in Kalmegh (Andrographis paniculata Wall. Ex Ness) germplasm

Jai Kumar, Animesh Sinha, Ravinder Raj Lal and Priyashi

Abstract

Keeping the importance of characterization and evaluation of the M&APs germplasm, an experiment was undertaken with 25 germplasm of Kalmegh collected across India from its natural habitat to screen out the existing diversity through its qualitative parameters under the climatic and edaphic conditions of Jharkhand at BAU, Ranchi. Plant growth habit of all accession was found erect. Leaf colour of 15 Kalmegh germplasm was pale green, green for 5 germplasm and dark green for 5 germplasm. Leaf surface of all germplasm was found glabrous. Leaf shape of 15 Kalmegh germplasm was found lanceolate, for 9 germplasm it was narrowly ovate and for 1 it was oblanceolate. Branching pattern of 5 germplasm were low branched, 10 had medium branched and rest 10 showed profusely branched. Corolla pubescence of 11 germplasm was slightly pubescent and rest 14 had densely pubescent. Seed colour of 5 germplasm was brown, 10 were yellowish brown and 10 were yellow in colour. Seed shape of all accession was ovoid and inflorescence type was lax panicle. Flower color of all germplasm was white with dark purple streaks. Capsule shape of 22 germplasm was found lanceolate and rest 3 was oblong in nature. From the findings, large diversity was recorded in the collected Kalmegh germplasm, which will be useful for Plant Breeders for its crop improvement.

Keywords: Kalmegh, Andrographis paniculata, characterization, evaluation, genetic diversity

Introduction

Adequate characterization for agronomic, morphological, physiological and developmental traits of crop is necessary to classify it in small and well-organized sector that will facilitate advanced utilization of germplasm by breeders in crop improvement program. To achieve this, germplasm accessions of crops are characterized over the years for qualitative characters (McMillan and Salhuana, 1983)^[10] with the objectives to describe accession, establish their diagnostic characteristics and identify duplicates and estimate the extent of variation in the collection (Frankel, 1986)^[5]. Characterization of a crop is being done at various crop stages like seedling, vegetative, early reproductive, late reproductive, harvest and post-harvest stages (Pandey and Mandal, 2010)^[14]. Germplasm variability documentation based on basic passport and characterization data for major morphological characters and developed primarily to make genetic diversity available to researchers have limited value unless this is evaluated extensively for traits of economic importance. According to Frankel (1983)^[4], an essential prerequisite for a species to survive against environmental pressures is the availability of a pool of genetic diversity and in the absence of that extinction would appear inevitable. Determining how much genetic diversity exists in a species and explaining this diversity in terms of its origin, organization and maintenance are thus of fundamental significance in the

application of genetic principles to conservation. Therefore a great deal of information on morphological, physiological and genetic parameters is necessary before the observed pattern of variation may be interpreted (Sabu, 2006) ^[16].

Keeping in view the importance of diversity in M&APs germplasm, systematic research trial namely "Assessment of morphological, biochemical and molecular variability in Kalmegh (Andrographis paniculata Wall. Ex Ness) germplasms" was carried out at Research farm of BAU, Ranchi under the climatic and edaphic conditions of Jharkhand through its qualitative parameters. Andrographis paniculata is a member of the Acanthaceae family, belongs to small tribe Andrographideae of the subfamily Acanthoideae. Kalmegh is an important indigenous medicinal plant commonly known as 'King of Bitters' (Gomathinayagam et al., 2009) [6] found throughout tropical & sub-tropical asia (Kataky and Handique, 2010) [7]. Native populations of Andrographis paniculata are spread throughout India and Sri Lanka which perhaps represent the centre of origin and diversity of the species (Padua et al., 1999) [13]. Kalmegh is found wild and weedy in the plains throughout India and in the undergrowth of evergreen, pine and deciduous forests from the Himalayan foothills southwards into Sri Lanka (Norman and Bunyapraphatsara, 1992, Lattoo et al., 2008)^{[12,} ^{8]}. Synchronization of anther dehiscence and stigma receptivity in Kalmegh along with the flower structure in which intimate proximity of stigma with the two-celled anthers supports the plant to be a hermaphroditic, selfpollinated, self-compatible and habitual in breeder (Lattoo et *al.*, 2006) ^[9], as a consequence the rate of cross-pollination as well as genetic diversity is very low. Major problem of Kalmegh for its judicious selection in crop improvement program is the lack of genetic background data. As Kalmegh is poorly characterized, it is important at the beginning of breeding program, to discriminate among available genotypes thus to establish the level of genetic diversity and thereby identifying the most suitable materials for crossing (Wijarat et al., 2011)^[20].

Materials and Methods

Location of the experimental site: The experimental site was located at Research Farm, Birsa Agricultural University, Kanke, Ranchi, located in the plateau region of Jharkhand. Geographically, it is located at 23°26'30" N latitude and 85°18'20" E longitude in Chhotanagpur plateau, situated in north eastern part of India and at an altitude of between 646 m above the mean sea level. The soil of the site is lateritic, developed from granite-gneiss, sandy loam in texture, sedentary in nature and well drained with low water holding capacity and poor consistency.

Climatic conditions of the experimental site: The general climate of the region is classified as 'sub humid mega thermal' with mean daily temperature of about 24.2 °C. The region is characterized by three distinct seasons namely summer, rainy and winter seasons. The mean relative humidity is about 70.88% with its range from 57.0 to 92.0% in the area. The monsoon breaks out in the middle of June and last till mid-October. The average annual rainfall of this area is approximately 1400mm which is mostly erratic, punctuated with occasional dry spells. The mean wind velocity and evaporation varies from 3.6 to 4.4 km/hr and 130 to 140 mm, respectively.

Experimental materials: The experimental materials comprised of twenty five genotypes of Kalmegh, for which seeds were collected from its natural habitat across six states of India and NBPGR, New Delhi including wild and cultivated varieties. Out of 25 Kalmegh accessions, 4 each were collected from Jharkhand, Chhattisgarh, Madhya Pradesh, Karnataka, 6 from Orissa, 2 from NBPGR and one from Gujarat. Care was taken to maintain a geographical distance of about 8-10 km between each collection site in order to reduce genetic similarity in accessions and to minimize sampling error while interpreting the data. While collecting plants from their natural habitats stratified random sampling method (Moss and Guarino, 1995)^[11] was followed. Collected seed samples were germinated, raised in polytubes and maintained under identical growing conditions in experimental area and used for analyzing qualitative variations.

 Table 1: Details of Andrographis paniculata germplasm used as experimental materials

Sl. No.	State/ Institution	Germplasm	Status
1.	Jharkhand	T ₁ -JHAP ₁ , T ₂ -JHAP ₂ , T ₃ -JHAP ₃ , T ₄ -JHAP ₄	Wild
2.	Orissa	T5 - OAP1, T6 - OAP2, T7 - OAP3, T8 - OAP4, T9 - OAP5, T10 - OAP6	Wild
3.	Chhattisgarh	T ₁₁ - CHAP ₁ , T ₁₂ - CHAP ₂ , T ₁₃ - CHAP ₃ , T ₁₄ - CHAP ₄	Wild
4.	Madhya Pradesh	T15 - MPAP1, T16 - MPAP2, T17 - MPAP3, T18 - MPAP4	Wild
5.	Karnataka	T19 - KAP1, T20 - KAP2, T21 - KAP3, T22 - KAP4	Wild
6.	NBPGR, New Delhi	T ₂₃ - IC 111286, T ₂₄ - IC 471890	Cultivated
7.	Gujarat	T ₂₅ - GAP ₁	Wild

Morphological (Qualitative) parameters of *Andrographis paniculata* **germplasm:** It was recorded as per NBPGR format (Singh *et al.*, 2003) ^[18] at different growth stages. Thirteen qualitative traits were selected for morphological diversity among Kalmegh germplasm like plant habit, plant growth habit, surface of first pair of leaves, leaf surface, leaf shape, leaf colour, branching pattern, seed colour, seed shape, inflorescence type, corolla pubescence, flower colour and capsule shape. Details of parameters and the time of collection of data was

- Plant habit (was recorded at late vegetative stage)-Annual, Biennial, Perennial
- Plant growth habit (was recorded at full bloom stage)-Erect, Spreading, Others
- Surface of first pair of leaves (was recorded at the time of initiation of germination)-Hairy, Non-hairy
- Branching pattern (was recorded at late vegetative stage)-Branched, Profusely branched, Others
- Leaf shape (was recorded on the main stem nearest to the primary branching node at flowering stage) - Average of 10 random leaves-Narrowly ovate, Ovate, Oblong, Lanceolate, Oblanceolate
- Leaf surface (was recorded at late vegetative stage)-Glabrous, Hairy, Others
- Leaf colour (was recorded at full bloom stage using RHS colour chart)-Pale green, Green, Dark green, Others
- Inflorescence type (was recorded when the inflorescence are completely visible)-Lax panicle, Compact panicle, Others

- Flower colour (was recorded when the inflorescence are completely visible using RHS colour chart)-White, White with dark-purple streaks, Purple, Others
- Corolla pubescence (was recorded when the flowers are fully opened)-Slightly pubescent, Densely pubescent, Others
- Seed shape (was recorded on matured and dried seeds)-Ovoid, Oblong, Sub-quadrate, Others
- Seed colour (was recorded on matured and dried seeds using Munsell Colour Chart)-Yellow, Yellowish-brown, Brown, Others
- Capsule shape (was recorded when capsules are fully matured)-Linear, Oblong, Lanceolate, Others

Results and Discussion

Morphological (Qualitative) parameters of Kalmegh germplasm: Parameters such as plant habit, plant growth habit, surface of first pair of leaves, leaf surface, leaf shape, leaf colour, and branching pattern of different Kalmegh germplasm was collected and presented in Table 2

Plant habit and plant growth habit of Kalmegh germplasm: The plant habit of all the 25 Kalmegh germplasm was observed as annual with their life span varied from 225.34 days (KAP₄) to 295.67 days (OAP₆). It was observed that occurrence of rain even at end of cropping period rejuvenates the plants growth once again and several branching was found emerged from base of the plant. The plant growth habit of all the Kalmegh accessions was found erect, however as per Annual report, DMAPR, (2014-15), out of 295 accessions of Kalmegh at Raipur, 166 accessions were semi erect type, 67 were erect type and 64 were spreading type and plant growth habit was erect in DMAPR AP2, 19 and 24 and dropping in DMAPR AP3 and 21. As per Annual report, DMAPR, (2012-13), plant habit of DMAPR AP11, 16, 19 and 35 were erect type and DMAPR AP21 was trailing type. Sabu (2006) ^[16] found that the distinct morphological characteristics of Kalmegh may be due to certain combinations of genes which had become randomly fixed.

Surface of first pair of leaves of Kalmegh germplasm: One specific feature in different Kalmegh accessions was noticed. The first pair of emerged after germination of seed was found hairy for all the germplasm.

Leaf surface and leaf shape of Kalmegh germplasm: Leaf surface of all the 25 Kalmegh germplasm was found glabrous. The leaf shape of 15 Kalmegh accessions was observed as Lanceolate i.e. leaf shape like a lance head, tapering to a point at each end. This feature was observed for all the germplasm

of Jharkhand, Chhattisgarh, Madhya Pradesh, NBPGR and Gujarat. The leaf shape of 5 germplasm of Orissa and 4 germplasm of Karnataka was narrowly ovate in shape i.e. less broader at the base and more tapering. The leaf shape of one germplasm of Orissa (OAP₆) was observed as Oblanceolate i.e. having a round apex and tapering base. Anonymous (2009) ^[3] reported lanceolate leaves, acute at both ends in case of HIM (J)-90. As per Annual report, DMAPR, (2014-15), leaf lamina shape was linear lanceolate in DMAPR AP3, lanceolate in DMAPR AP6, elliptical in DMAPR AP48, ovate-elliptical to ovate-lanceolate in DMAPR AP1, 18 and 19, ovate in DMAPR AP24.

Leaf colour of Kalmegh germplasm: The leaf colour of 15 Kalmegh accessions was observed as pale green, while green colour of leaves was observed with 5 germplasm. The leaf colour of 5 germplasm namely JHAP₁, JHAP₄, CHAP₁, CHAP₄ and MPAP₁ was observed as deep green. Sabu (2006) ^[16] emphasized variations in colour of the leaves due to presence of chlorophylls, carotenoids and anthocyanins. He mentioned that increase of anthocyanin synthesis towards maturity, a proportionate decrease in total chlorophyll and carotenoid content occurred resulted in reddish-brown or pink coloured leaves and stem mostly towards their maturity. Sharma *et al.*, (2009) ^[17] found the leaf colour in KI_2 was comparatively distinct (light green) compared to other 15 Kalmegh germplasm of Chhattisgarh and adjoining states (dark green). Anonymous (2009) ^[3] reported pale yellow green leaves for AK-1, light green leaves for CIM-Megha and light green leaves for KI5. As per Annual report, DMAPR, (2012-13), DMAPR AP6 and 19 had light green colour leaves and DMAPR AP3 and 42 had dark green leaves. Valdiani et al., (2014) [19] found high harmony between geographical distributions of Kalmegh with their morphological demonstration. As per Annual report, DMAPR, (2014-15) leaf colour was dark green in DMAPR AP3 and yellowish green in DMAPR AP19.

Branching pattern of Kalmegh germplasm: The branching pattern of 5 Kalmegh accessions of Orissa was observed as low branched, 10 germplasm of different states showed medium branched. 10 germplasm of Jharkhand, Chhattisgarh and Madhya Pradesh showed profusely branched. As per Annual report, DMAPR, (2014-15), branching pattern was open in DMAPR AP13 and 22 & closed in DMAPR AP18 and 19. Ravindran *et al.*, (1990) ^[15] reported that morphological variation depends on seasonal or developmental changes that affect individuals in a population regardless of genotype.

Treatments	Plant habit	Plant growth habit	Surface of first pair of leaves	Leaf surface	Leaf shape	Leaf colour	Branching pattern	
T_1 (JHAP ₁)	Annual (255.84 days)	Erect	Hairy	Glabrous	Lanceolate	Dark green	Profusely branched	
T ₂ (JHAP ₂)	Annual (250.34 days)	Erect	Hairy	Glabrous	Lanceolate	Green	Profusely branched	
T ₃ (JHAP ₃)	Annual (251.34 days)	Erect	Hairy	Glabrous	Lanceolate	Green	Medium branched	
T ₄ (JHAP ₄)	Annual (256.67 days)	Erect	Hairy	Glabrous	Lanceolate	Dark green	Medium branched	
T5 (OAP1)	Annual (262.17 days)	Erect	Hairy	Glabrous	Narrowly ovate	Green	Low branched	
T ₆ (OAP ₂)	Annual (265.50 days)	Erect	Hairy	Glabrous	Narrowly ovate	Pale green	Low branched	
T7 (OAP3)	Annual (268.16 days)	Erect	Hairy	Glabrous	Narrowly ovate	Pale green	Low branched	
T ₈ (OAP ₄)	Annual (260.17 days)	Erect	Hairy	Glabrous	Narrowly ovate	Green	Low branched	
T9 (OAP5)	Annual (264.50 days)	Erect	Hairy	Glabrous	Narrowly ovate	Pale green	Low branched	
T10 (OAP6)	Annual (295.67 days)	Erect	Hairy	Glabrous	Oblanceolate	Pale green	Medium branched	
T ₁₁ (CHAP ₁)	Annual (263.34 days)	Erect	Hairy	Glabrous	Lanceolate	Dark green	Medium branched	

Table 2: Morphological (Qualitative) parameters of Kalmegh germplasm

T12 (CHAP2)	Annual (260.00 days)	Erect	Hairy	Glabrous	Lanceolate	Green	Medium branched	
T ₁₃ (CHAP ₃)	Annual (262.33 days)	Erect	Hairy	Glabrous	Lanceolate	Pale green	Profusely branched	
T ₁₄ (CHAP ₄)	Annual (260.17 days)	Erect	Hairy	Glabrous	Lanceolate	Dark green	Profusely branched	
T ₁₅ (MPAP ₁)	Annual (287.83 days)	Erect	Hairy	Glabrous	Lanceolate	Dark green	Profusely branched	
T ₁₆ (MPAP ₂)	Annual (259.33 days)	Erect	Hairy	Glabrous	Lanceolate	Pale green	Profusely branched	
T ₁₇ (MPAP ₃)	Annual (275.83 days)	Erect	Hairy	Glabrous	Lanceolate	Pale green	Profusely branched	
T ₁₈ (MPAP ₄)	Annual (257.34 days)	Erect	Hairy	Glabrous	Lanceolate	Pale green	Profusely branched	
T19 (KAP1)	Annual (230.17 days)	Erect	Hairy	Glabrous	Narrowly ovate	Pale green	Medium branched	
T ₂₀ (KAP ₂)	Annual (230.67 days)	Erect	Hairy	Glabrous	Narrowly ovate	Pale green	Medium branched	
T ₂₁ (KAP ₃)	Annual (228.84 days)	Erect	Hairy	Glabrous	Narrowly ovate	Pale green	Medium branched	
T ₂₂ (KAP ₄)	Annual (225.34 days)	Erect	Hairy	Glabrous	Narrowly ovate	Pale green	Medium branched	
T ₂₃ (IC 111286)	Annual (281.00 days)	Erect	Hairy	Glabrous	Lanceolate	Pale green	Medium branched	
T ₂₄ (IC 471890)	Annual (264.67 days)	Erect	Hairy	Glabrous	Lanceolate	Pale green	Profusely branched	
T ₂₅ (GAP ₁)	Annual (265.50 days)	Erect	Hairy	Glabrous	Lanceolate	Pale green	Profusely branched	

Parameters such as seed colour, seed shape, inflorescence type, corolla pubescence, flower colour and capsule shape of different Kalmegh germplasm was collected and presented in Table 3

Seed colour and seed shape of Kalmegh germplasm: The seed colour of 10 germplasm was observed as yellowish as revealed by "Munshell colour chart". Again the seed colour of 10 germplasm was observed as yellowish brown while the seed colour of 5 germplasm was observed as brownish. The seed shape of all the 25 Kalmegh germplasm was observed as ovoid i.e. egg shaped.

germplasm: Inflorescence type of all the Kalmegh germplasm was observed as "lax panicle". The corolla pubescence i.e. covered with fine short hairs of 11 germplasm was observed as lightly pubescent and rest 14 had densely pubescent.

Flower colour and capsule shape of Kalmegh germplasm: The flower colour of all the 25 Kalmegh germplasm was observed as "White with dark purple streaks". The capsule shape of 22 Kalmegh germplasm was observed as "lanceolate", while three had "oblong" in nature i.e. two long sides and two short sides and in which all the angles are at right angle. Flowers showed characteristic pigmentation and brownish spots on petals.

Inflorescence type and flower cole	our of Kalmegh
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Treatments Seed colour		Seed	Inflorescence	Corolla	Flower colour	Capsule
T_1 (IHAP ₁)	Yellowish brown (HUF7 5YR5/6)	Ovoid	Lax panicle	Densely pubescent	White with dark nurnle streaks	I anceolate
T_2 (JHAP ₂)	Yellowish brown (HUE7.5YR5/6)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T ₃ (JHAP ₃)	Yellow (HUE7.5YR4/6)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T ₄ (JHAP ₄)	Yellow (HUE7.5YR4/4)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T ₅ (OAP ₁)	Yellow (HUE7.5YR5/4)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Lanceolate
T ₆ (OAP ₂)	Yellowish brown (HUE7.5YR5/8)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T ₇ (OAP ₃)	Brown (HUE7.5YR6/6)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T ₈ (OAP ₄)	Yellowish brown (HUE7.5YR5/6)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Lanceolate
T ₉ (OAP ₅)	Yellowish brown (HUE7.5YR5/6)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T ₁₀ (OAP ₆)	Yellowish brown (HUE7.5YR5/8)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Oblong
T ₁₁ (CHAP ₁)	Yellow (HUE7.5YR4/6)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Lanceolate
T ₁₂ (CHAP ₂)	Yellow (HUE7.5YR4/6)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T13 (CHAP3)	Yellow (HUE7.5YR5/4)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T ₁₄ (CHAP ₄)	Yellow (HUE7.5YR4/4)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T ₁₅ (MPAP ₁)	Brown (HUE7.5YR6/8)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T ₁₆ (MPAP ₂)	Yellowish brown (HUE7.5YR5/8)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Lanceolate
T ₁₇ (MPAP ₃)	Yellowish brown (HUE7.5YR5/8)	Ovoid	Lax panicle	Slightly pubescent	White with dark purple streaks	Lanceolate
T ₁₈ (MPAP ₄)	Brown (HUE7.5YR6/8)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Lanceolate
T19 (KAP1)	Yellow (HUE7.5YR5/4)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Lanceolate
T ₂₀ (KAP ₂)	Yellow (HUE7.5YR5/4)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Lanceolate
T ₂₁ (KAP ₃)	Yellowish brown (HUE7.5YR5/6)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Lanceolate
T ₂₂ (KAP ₄)	Yellow (HUE7.5YR5/4)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Lanceolate
T ₂₃ (IC 111286)	Brown (HUE7.5YR6/8)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Oblong
T ₂₄ (IC 471890)	Brown (HUE7.5YR6/6)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Oblong
T ₂₅ (GAP ₁)	Yellowish brown (HUE7.5YR5/8)	Ovoid	Lax panicle	Densely pubescent	White with dark purple streaks	Lanceolate

Table 3: Morphological (Qualitative) parameters of Kalmegh germplasm

Conclusion

The plant habit of all the 25 Kalmegh germplasm was observed as annual while plant growth habit was found erect. Leaf surface of all the 25 Kalmegh germplasm was found glabrous. The leaf shape of 15 Kalmegh accessions of Jharkhand, Chhattisgarh, Madhya Pradesh, NBPGR and Gujarat was observed as lanceolate, while for germplasm of Orissa and Karnataka it was narrowly ovate. The leaf shape of one germplasm of Orissa (OAP_6) was observed as Oblanceolate. The leaf colour of 15 Kalmegh accessions was observed as pale green, green for 5 germplasm and deep green for 5 germplasm namely JHAP₁, JHAP₄, CHAP₁, CHAP₄ and MPAP₁. The branching pattern of 5 Kalmegh accessions has low branched, 10 germplasm had medium branched and 10 germplasm had profusely branched. The seed colour of 10 germplasm was observed as yellowish, for 10 germplasm it was yellowish brown and for 5 germplasm it was brownish. The seed shape of all the 25 Kalmegh germplasm was observed as ovoid. Inflorescence type of all the Kalmegh germplasm was observed as "lax panicle". The corolla pubescence of 11 germplasm was observed as lightly pubescent and rest 14 had densely pubescent. The flower colour of all the 25 Kalmegh germplasm was observed as "White with dark purple streaks". The capsule shape of 22 Kalmegh germplasm was observed as "lanceolate", while three had "oblong" in nature.

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