Department of Botany, University of Jammu, Jammu

Progress Report of Research and Seed Grant

Title of the project: Assessment of morphological and molecular variability in wild *Carissa spinarum* L. from Jammu Province

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Introduction:

The genus *Carissa* belongs to tribe Carisseae and sub-tribe Carissinae of family Apocynaceae. It comprises of 36 species distributed in the tropical and sub-tropical regions of the world. *Carissa spinarum* is a thorny shrub of 2-3 m height and bears ovate leathery leaves. It produces white, bisexual flowers and ovoid berries which are green when unripe and turn black on ripening. This species is largely valued for its fruits which contain good amount of organic nutrients, minerals, iron, vitamins, polyphenols, and tannins. They also possess therapeutic properties such as anti-arthritic, anti-microbial, anti-helminthic, anti-diabetic and hepatoprotective. Besides, hard twigs are used as tooth brush and green leaves as fodder to browsing animals. Its robust wood is also used for timber, in live hedging and crafting handles of agricultural tools.

Habitat:

In total, fifteen *C. spinarum* populations have been screened. Of these, six belong to Kathua (Chhan Morian, Dinga Amb, Jandhi, Kootah, Mandli and Saiswan), three to Jammu (Akhnoor, Ban, Chouki Choura), two each to Samba (Mansar and Sandhi), Udhampur (Battal Ballian and Phalata) and one one each to Rajouri (Sunderbani) and Reasi (Bhamla). At these locations, plants were found growing near water channel (Fig. 2a), amidst the stones (Fig. 2b), in a crop field (Fig. 2c), along roadside (Fig. 2d), as a hedge plant (Fig. 2e) and amongst the Pine trees (Fig. 2f).

Phenology: (Fig. 1)

Plants of *C. spinarum* are evergreen shrub or small tree. This plant begins to bear buds and blooms in the final week of March and continues until the end of June. In July, when the monsoon season begins, flowering ends. Second blossoming occurs in August and September. Fruit development begins in middle of October, and fruit matures in November in most of the studied populations. Some fruits (5–10%) begin to ripe in December, while others ripen after March. In places like Mandli (Kathua) and Sunderbani (Rajouri), the flowering season is delayed by about one month. The phenological events are shown in Fig. 1.

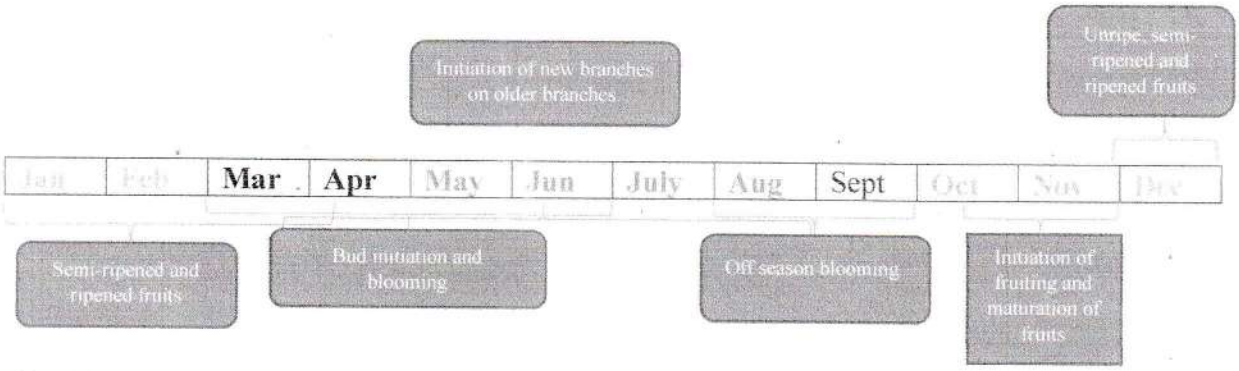

Fig. 1: Vegetative, flowering and fruiting phases of *C. spinarum*

Fig. 2: *C. spinarum* plants growing (a) near the water channel, (b) amidst the stones, (c) in a crop field, (d) along the roadside, (e)as a hedge plant, (f) amongst the *Pinus* trees

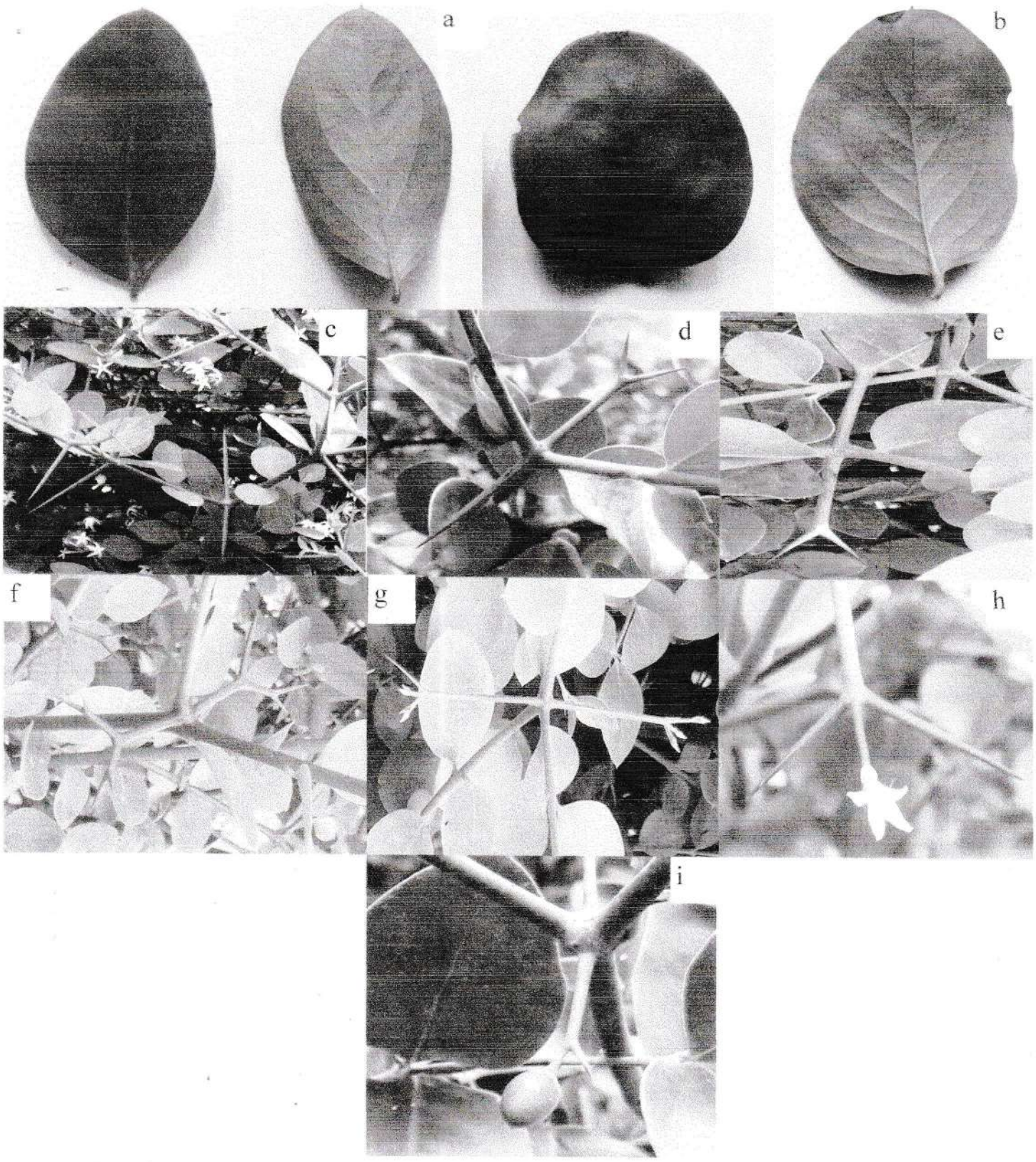

Fig. 3: *C. spinarum* - (a) dorsal and ventral side of lanceolate leaf, (b) dorsal and ventral side of ovate leaf, (c) two straight thorns arising at a node, (d) one straight and other bifurcated thorn arising at a node, (e) two forked thorns arising at a node, (f) two bifurcated thorns showing branches at second level, (g) 2-3 buds arising in the axils, (h) solitary flower borne between two thorns (i) fruit borne adjacent to thorn

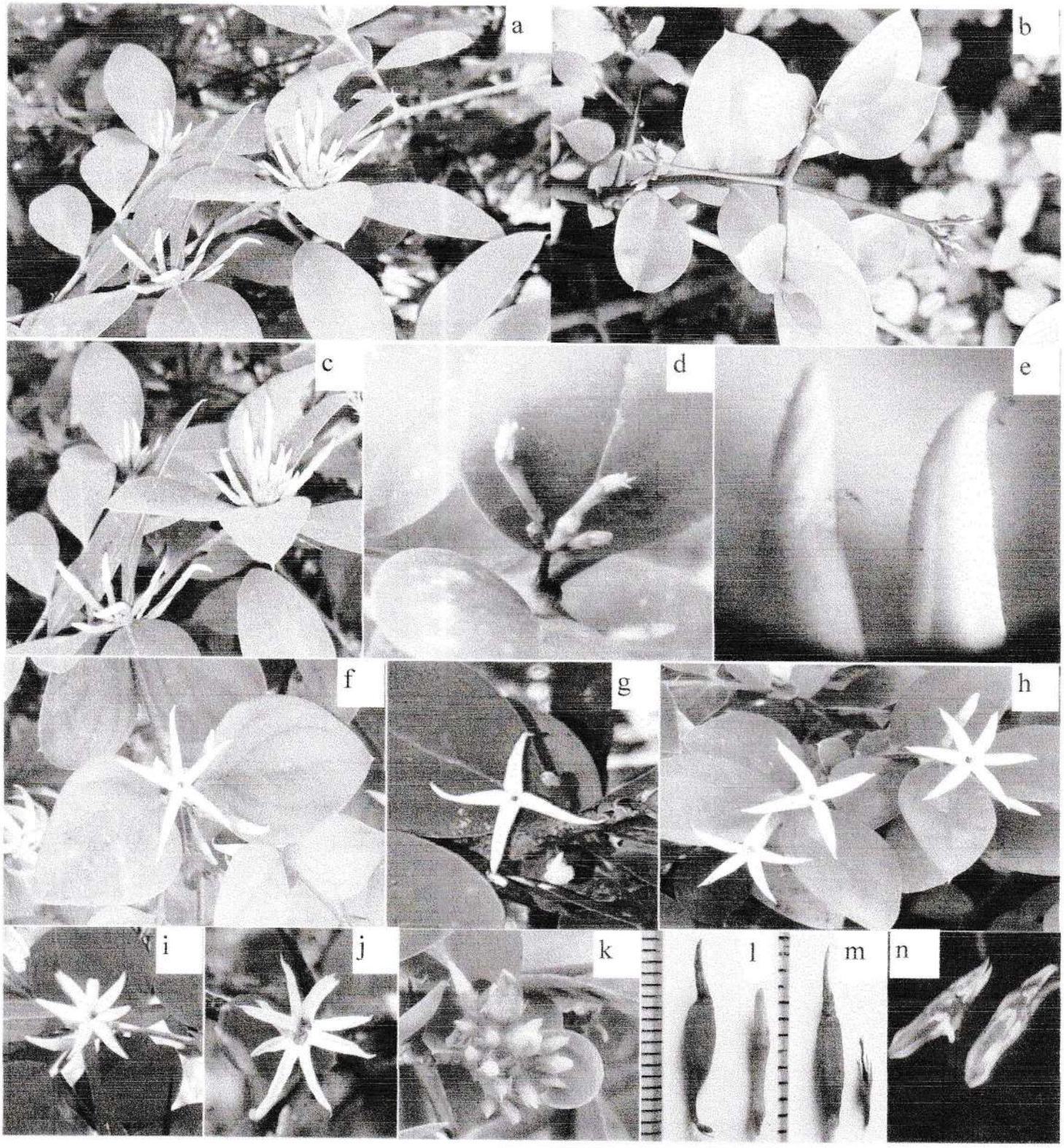

Fig. 4: *C. spinarum*, (a) inflorescence having short peduncle, (b) inflorescence having long peduncle, (c) inflorescence having white coloured corolla lobes, (d) inflorescence having pink tinged corolla lobes, (e) curved and straight corolla lobes, (f) flower with 5 petals, (g) flower with 4 petals, (h) flower with 4, 5 and 6 petals, (i) flower with 7 petals, (j) fused flower, (k) some floral buds showing abnormal growth due to insects infestation, (l) abnormal floral bud (red) and normal floral bud (green), (m)abnormal floral bud with dying off corolla lobes and pistil , (n) larvae of insects present within the floral buds

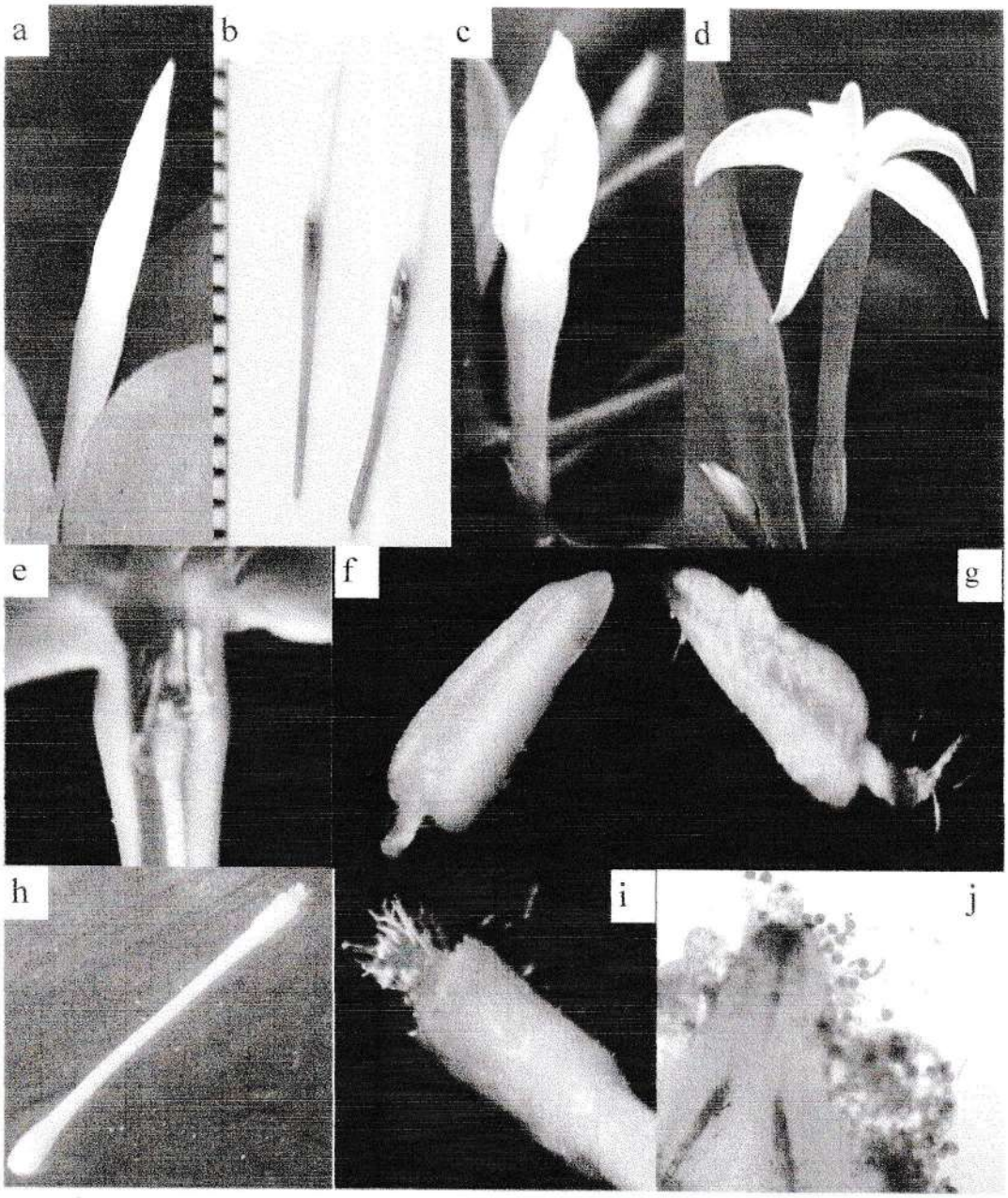

Fig. 5: *C. spinarum*, (a) mature bud, (b) bud cut into two halves to show dehisced anthers, (c) ready to open bud, (d) opened flower showing 5 petals, corolla tube and calyx, (e) position of stigma and anthers within the corolla tube, (f) mature and intact anther with filament, (g) dehisced anther, (h) pistil showing ovary, style and stigma, (i) papillate stigma, (j) germination of pollens on the stigma

Floral Features:

The mature floral buds are with white corolla lobes (Fig. 5a). The dehiscence of anthers occurs before the opening of the floral buds (Fig. 5b). Floral buds show bulging before opening (Fig. 5c). The opened flower usually show 5 petals and are white in colour, corolla tube is green in colour and sepals are ovate, acuminate and ciliate (Fig. 5d). An open flower shows 5 anthers at the neck of the corolla tube and stigma is present just below the anthers (Fig. 5e). The Figs. 5f-g shows undehisced and dehisced anther. Pistil, papillate stigma and germination of pollens on the stigma are shown in Figs. 5h-j.

Table 2: Morphometric details of floral traits of *C. spinarum*

Populations	No. of cymes	No. of buds	Petals length (mm)	Petals width (mm)	Sepals length (mm)	Sepals width (mm)	Pedicel length (mm)	Corolla tube length (mm)	Pistil length (mm)
Kootah	11.66±0.45 (9.0-15)	10.71±0.29 (6.0-14)	7.93±0.04 (7.3-8.5)	2.26±0.03 (2.0-2.6)	3.35±0.04 (3.0-4.0)	1.62±0.03 (1.0-2.0)	2.29±0.03 (2.0-2.6)	10.26±0.06 (9.0-10.5)	8.80±0.07 (8.0-10)
Dinga Amb	10.73±0.34 (9.0-14)	10.64±0.38 (6.0-15)	8.32±0.16 (7.5-10)	1.76±0.04 (1-2)	2.70±0.03 (2.4-3.0)	1.49±0.00 (1.0-2.0)	1.76±0.03 (1.5-2.0)	9.32±0.17 (9.0-10)	8.84±0.17 (8.0-9.5)
Mandli	13.6±0.46 (12-17)	10.26±0.30 (5.0-16)	8.13±0.03 (8.0-8.5)	2.06±0.03 (1.5-2.5)	3.02±0.03 (2.5-3.5)	1.59±0.03 (1.0-2.0)	3.12±0.04 (2.8-3.5)	10.11±0.03 (9.5-10.5)	8.58±0.03 (8.0-9.5)
Chhan Morian	10.73±0.44 (9-14)	10.42±0.26 (5.0-14)	7.09±0.03 (6.5-7.5)	2.11±0.03 (1.5-2.5)	2.38±0.05 (2.0-2.6)	1.60±0.02 (1.0-2.0)	2.19±0.03 (2.0-2.5)	9.19±0.04 (9.0-10)	8.32±0.05 (8.0-9.0)
Sandhi	11.93±0.41 (10-15)	9.97±0.33 (4.0-15)	8.17±0.03 (8.0-8.5)	2.23±0.03 (2.0-2.5)	3.21±0.03 (3.0-3.5)	1.29±0.03 (1.0-2.0)	2.18±0.03 (2.0-2.5)	9.15±0.03 (9.0-9.5)	8.15±0.03 (8.0-9.0)
Chouki Choura	11.8±0.52 (9.0-15)	10.62±0.44 (5.0-15)	7.29±0.03 (7.0-7.6)	1.77±0.06 (1.5-2.0)	2.00±0.09 (1.0-3.0)	1.22±0.07 (1.0-2.0)	1.24±0.03 (1.0-1.5)	8.49±0.05 (8.0-9.0)	7.25±0.05 (7.0-8.0)
Bhamla	10.26±1.06 (4.0-17)	7.44±0.23 (3.0-10)	5.96±0.04 (5.7-6.5)	1.59±0.02 (1.5-2.0)	2.15±0.02 (2.0-2.5)	1.72±0.02 (1.0-2.0)	1.68±0.03 1.5-2.0)	7.95±0.03 (7.5-8.5)	7.15±0.02 (7.0-8.0)
Phalata	12.53±0.72 (8.0-16)	12.11±0.30 (4.0-15)	6.32±0.19 (5.5-8.5)	1.78±0.03 (1.5-2.2)	2.07±0.02 (1.9-2.5)	1.34±0.03 (1.0-2.0)	2.14±0.02 (1.9-2.5)	7.83±0.03 (7.5-8.5)	7.10±0.03 (7.0-8.0)
Battal Ballian	14.26±0.39 (9.0-18)	9.71±0.27 (6.0-13)	7.81±0.03 (7.0-8.5)	2.19±0.03 (1.8-2.5)	3.01±0.04 (2.5-3.5)	1.32±0.03 (1.0-2.0)	2.18±0.02 (1.9-2.5)	9.14±0.03 (9.0-9.5)	7.86±0.03 (7.0-8.5)
Sunderbani	13.46±0.83 (9.0-19)	9.84±0.38 (7.0-15)	8.21±0.03 (8.0-8.5)	2.11±0.03 (1.9-2.6)	2.25±0.03 2.0-3.0)	1.79±0.03 (1.0-2.0)	2.18±0.03 (1.9-2.4)	9.35±0.03 (9.0-10)	8.20±0.03 (8.0-9.5)
Saiswan	11.26±0.48 (10-14)	10.75±0.43 (4.0-14)	7.26±0.03 (7.0-7.60)	2.28±0.03 (2.0-2.5)	3.13±0.06 (2.5-3.5)	1.86±0.03 (1.0-2.0)	2.14±0.03 (1.8-2.4)	9.3±0.04 (9.0-9.5)	8.07±0.03 (8.0-8.5)
Jandhi	12.26±0.43 (10-15)	10.26±0.37 (4.0-15)	7.15±0.04 (6.5-7.5)	2.20±0.03 (2.0-2.5)	3.19±0.03 (2.8-3.5)	2.19±0.03 (2.0-3.0)	2.15±0.03 (2.0-2.5)	8.47±0.07 (8.0-9.0)	7.09±0.03 (7.5-8.5)
Mansar	13.2±0.40 (10-16)	12.22±0.36 (6.0-15)	7.34±0.03 (7.0-7.7)	1.93±0.05 (1.8-2.5)	3.22±0.03 (3.0-3.5)	2.14±0.03 (2.0-2.5)	2.13±0.03 (2.0-2.5)	9.20±0.03 (9.0-9.5)	7.95±0.02 7.5-8.5)
Akhnoor	12.8±0.54 (9.0-15)	11.55±0.32 (6-15)	7.30±0.03 (7.0-7.5)	2.15±0.03 (2.0-2.5)	3.1±0.03 (2.7-3.4)	2.04±0.02 (1.5-2.5)	2.09±0.02 (1.9-2.4)	9.18±0.03 (9.0-9.5)	7.82±0.03 (7.5-8.5)
Bann	10.46±0.55 (8.0-15)	11.95±0.28 (7.0-15)	7.4±0.04 (7.0-8.0)	2.22±0.03 (2.0-2.6)	2.72±0.04 (2.5-3.1)	2.12±0.03 (2.0-2.5)	2.06±0.02 (1.9-2.4)	9.12±0.04 (9.0-9.5)	8.01±0.03 (7.5-8.5)

Floral visitors:

Many floral visitors have been found on the flowers of the *C. spinarum* viz., *Apis mellifera* (Fig. 6a), *Amata phegea* (Fig. 6b), *Pieris canidia* (Fig. 6c), *Delias eucharias* (Fig. 6d), Hover fly (Fig. 6e), *Camponotus* spp. (Fig. 6d) etc.

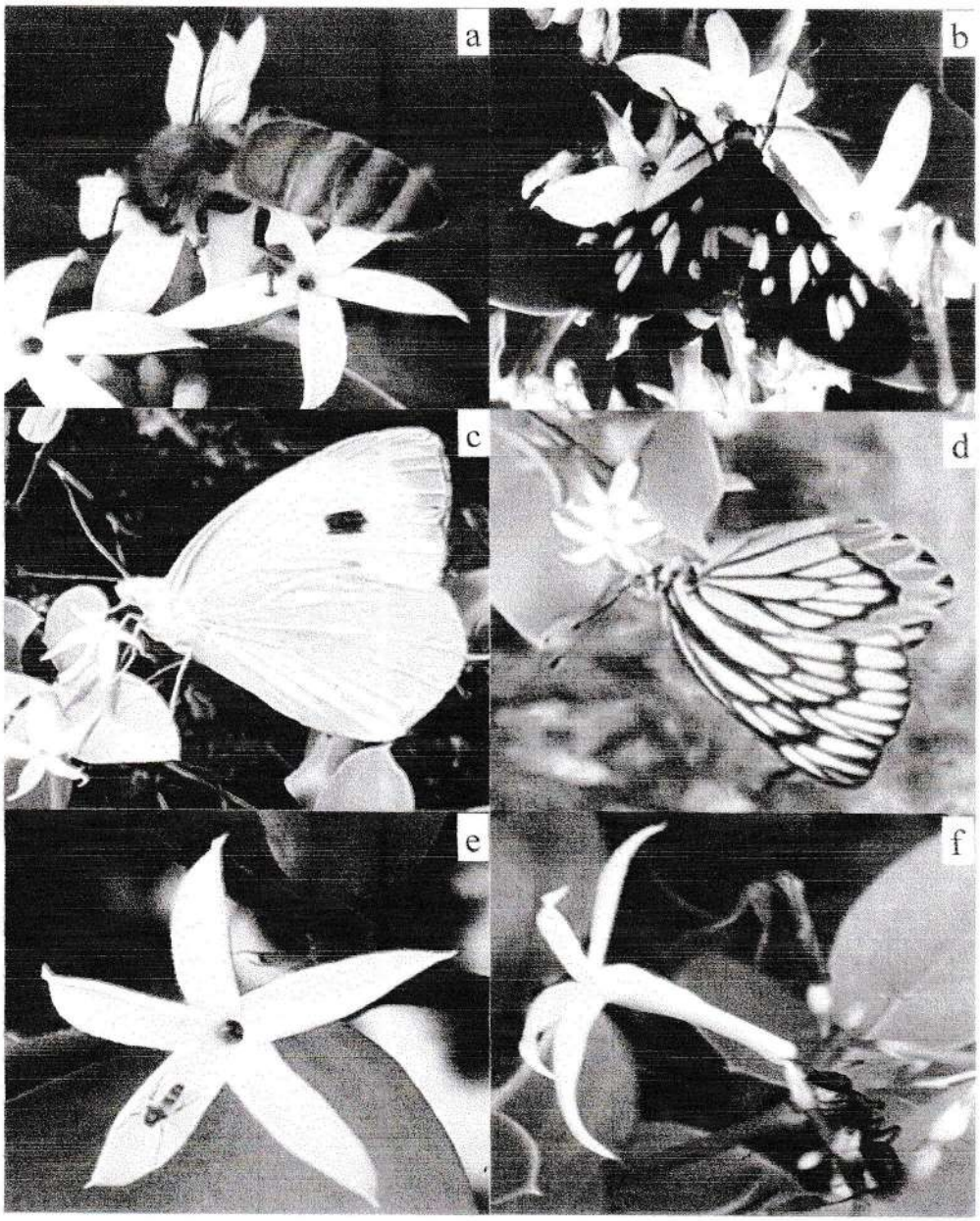

Fig. 6: *C. spinarum*, flower visitors - (a) *Apis mellifera*, (b) *Amata phegea*, (c) *Pieris canidia*, (d) *Delias eucharis*, (e) Hover fly, (f) *Camponotus* spp.

At a node, two thorns appear that are distinct in type based on their shapes. Most of the time, both thorns are straight at a node (Fig. 3c). Occasionally, one thorn is straight and the other is bifurcated. Rarely both thorns are bifurcated (Figs. 3d,e). Trifurcated thorns were also seen at several nodes (Fig. 3f). Certain thorns develop buds and flowers at their ends (Figs. 3g,h), which eventually turn into fruits (Fig. 3i).

In the studied populations, thorn number per 12 inch of branchlet varies from 19.6 (Dinga Amb) to 28.8 (Battal Ballian). Thorn length also varies 2.56 cm (Mandli) to 4.38 cm (Phalata) (Table 1).

Morphological variations in *C. spinarum*

Inflorescence:

In *C. spinarum*, inflorescence is terminal as well as axillary cymes which forms 3-15 buds having short as well as long peduncle (Figs. 4a-b). Buds with white and pink corolla lobes are present (Figs. 4c-d). Straight as well as curved corolla lobes (Fig. 3e) are present in the morphologically analysed plants from the study area. As per the perusal of Table 2, number of cymes per 12 inch of branchlet were maximum in Battal Ballian (14.26) population whereas minimum in Bhamla (10.26) population. The number of buds per cyme were maximum in Mansar (12.22) population whereas minimum in Bhamla (7.44) population. Maximum (8.32 mm) and minimum (5.96 mm) petal length was noted in the flowers of Dinga Amb and Bhamla populations. Petal width was maximum (2.28 mm) in Saiswan population whereas it was minimum (1.59 mm) in Bhamla population. Sepal length was maximum (3.35 mm) in Kootah population and minimum (2.00 mm) in Chouki Choura population. Sepal width was maximum (2.19 mm) in Jandhi population and minimum (1.22 mm) in Chouki Choura population. Pedicel length was maximum (3.12 mm) in Mandli population and minimum (1.24 mm) in Chouki Choura population. In so far as corolla tube length is concerned, it was maximum (10.26 mm) in Kootah population and minimum (7.95 mm) in Bhamla population. The pistil length was maximum (8.84 mm) in Dinga Amb population and minimum (7.09 mm) in Jandhi population.

Flowers are short-stalked, sweetly scented having green sepals and white petals which are fused upto half. Sepals (narrowly) are ovate, 2.0-3.5 mm long, 1.5-2.0 mm wide, acuminate and ciliate. There is variations in the number of petals per flower. Most flowers have 5 petals whereas 4,6 or 7 petals per flower have been noted occasionally (Figs. 4f-j). In some inflorescences, few buds show unusual growth and become large in size with pink corolla tubes and lobes (Fig. 4k). These buds remain closed and their corolla lobes and pistil wither with passage of time (Fig. 4l). The abnormal growth of these buds might be due to insect infestation which is shown by the presence of 3-4 larvae in the floral buds (Fig. 4m).

Leaves and thorns:

C. spinarum is an erect thorny shrub, with bifurcated branches, 1-3 m in height. Plants are evergreen which produce leaves nearly throughout the year. Leaves are opposite, lanceolate or ovate in shape with glossy appearance from above surface and dull green shade at below surface. Leaves are leathery with reticulate venation (Figs. 3a,b). Leaves have entire margin and exudes white latex, when plucked from the stem. The studied populations show variation in leaf-related traits. Maximum number of leaves per 12 inch of branchlet were borne by the plants of Battal Ballian population whereas minimum number of leaves were shown in the plants of Bhamla population. Studied populations also differ in leaf size. Measuring 4.12×3.08 cm, leaves of Kootah population are largest whereas those of Phalata are the smallest (2.94×1.80). Petiole length ranges from 2.59-3.86 mm in the studied populations (Table 1).

Table 1: Morphometric details of vegetative traits of *C. spinarum*

Populations	Plant height (m)	Leaf no.	Leaf length (cm)	Leaf width (cm)	Petiole length (mm)	Thorn number	Thorn length (cm)
Kootah	3.09±0.11 (2.86-3.23)	81.33±1.75 (82-104)	4.12±0.06 (3.6-5.4)	3.08±0.02 (2.1-3.5)	3.55±0.06 (3.0-4.5)	23.46±0.66 (20-28)	3.36±0.02
Dinga Amb	2.14±0.02 (2.1-2.2)	78.66±1.13 (70-86)	2.96±0.06 (2.7-3.7)	1.94±0.04 (1.5-2.5)	2.59±0.07 (2.0-3.5)	19.6±0.55 (16-22)	3.75±0.07 (3.3-4.5)
Mandli	3.1±0.14 (2.85-3.35)	87.46±1.30 (80-94)	4.39±0.03 (4.0-4.9)	2.28±0.01 (2.0-2.5)	3.49±0.06 (3.0-4.0)	27.33±0.60 (24-32)	2.56±0.03 (2.3-3.0)
Chhan Morian	2.47±0.09 (2.35-2.65)	81.06±1.49 (76-92)	3.49±0.04 (3.0-4.0)	2.56±0.01 (2.2-2.8)	3.09±0.04 (2.5-3.5)	21.46±0.98 (14-28)	3.28±0.03 (2.9-3.5)
Sandhi	1.93±0.19 (1.67-2.31)	76.26±1.36 (66-86)	3.34±0.02 (3.0-3.6)	2.55±0.03 (2.0-3.0)	3.18±0.03 (3.0-3.5)	21.73±0.64 (18-24)	3.38±0.02 (3.0-3.6)
Chouki Choura	1.37±0.09 (1.12-1.52)	84.93±1.66 (76-94)	4.04±0.09 (3.4-5.1)	2.9±0.04 (2.5-3.4)	3.86±0.12 (3.1-5.12)	22.8±0.97 (20-28)	3.28±0.03 (2.8-3.5)
Bhamla	1.33±0.08 (1.24-1.50)	72.4±1.95 (60-86)	3.20±0.03 (2.5-3.4)	2.10±0.02 (1.7-2.3)	3.14±0.12 (2.0-4.0)	20.26±0.95 (16-26)	3.17±0.02 (2.8-3.5)
Phalata	1.51±0.32 (1.10-2.15)	85.06±1.55 (74-94)	2.94±0.03 (2.7-3.3)	1.8±0.01 (1.6-2.3)	2.94±0.04 (2.5-3.5)	23.46±0.83 (18-28)	4.38±0.03 (4.0-4.7)
Battal Ballian	2.68±.0.13 (2.43-2.89)	134±1.47 (125-145)	4.13±0.02 (3.9-4.5)	2.3±0.03 (2.0-2.6)	3.15±0.03 (3.0-3.5)	28.8±0.57 (24-32)	4.16±0.02 (3.7-4.3)
Sunderbani	1.73±0.04 (1.65-1.80)	74.5±1.53 (68-80)	3.58±0.02 (3.4-3.9)	2.4±0.01 (2.2-2.7)	3.16±0.03 (3.0-3.5)	22.53±0.86 (18-28)	4.19±0.02 (4.0-4.4)
Saiswan	2.72±0.20 (2.4-3.1)	80.33±1.23 (74-90)	4.12±0.05 (3.4-4.6)	2.4±0.02 (2.0-2.7)	3.13±0.03 (3.0-3.5)	24.13±0.45 (20-26)	4.09±0.06 (3.0-4.5)
Jandhi	2.75±0.07 (2.61-2.88)	80.46±1.72 (71-94)	4.00±0.06 (3.3-4.8)	2.4±0.01 (2.1-2.5)	3.16±0.03 (2.5-3.5)	23.73±0.38 (22-26)	4.1±0.05 (3.0-4.4)
Mansar	2.11±0.08 (1.95-2.25)	79.53±1.37 (70-84)	4.20±0.05 (3.6-4.8)	2.23±0.02 (2.1-2.6)	3.11±0.04 (2.2-3.5)	23.46±0.53 (20-26)	3.42±0.03 (3.0-3.8)
Akhnoor	1.70±0.04 (1.64-1.80)	88.53±1.11 (82-94)	3.64±0.3 (3.4-4.5)	2.22±0.03 (2.0-2.6)	3.16±0.03 (3.0-3.5)	24.26±0.43 (20-26)	3.80±0.05 (3.5-4.5)
Bann	1.56±0.04 (1.48-1.64)	102.93±1.25 (96-110)	3.80 ±0.05 (3.4-4.8)	2.34±0.02 (2.0-2.6)	3.04±0.02 (3.0-3.5)	22.26±0.64 (18-26)	4.23±0.04 (3.5-4.5)

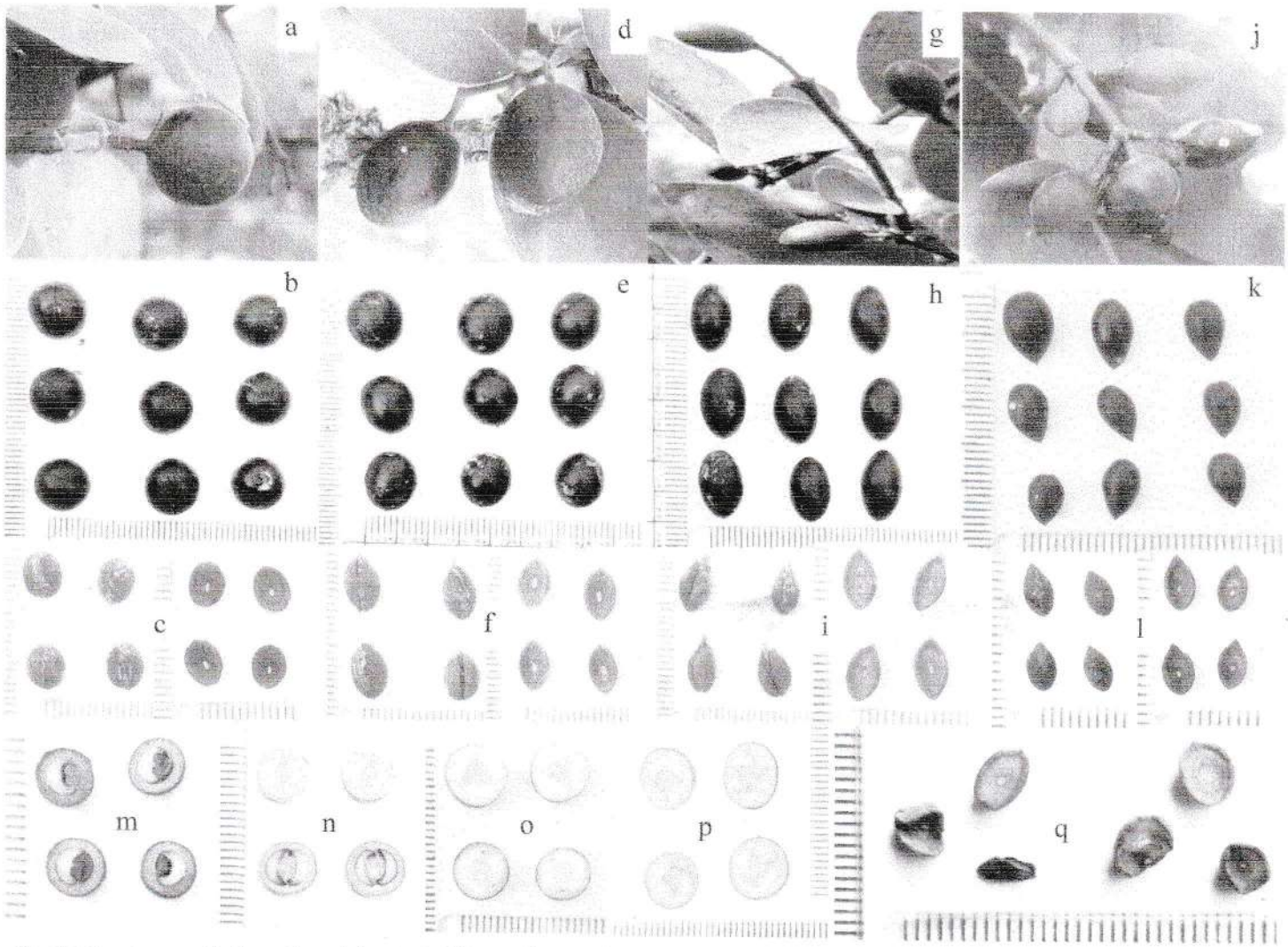

Fig. 7: *C. spinarum*, fruits and seeds having different shapes - (a-c) round, (d-f) oval, , (g-i) oblong, (j-l) conical; (m-p) horizontally cut fruits showing one, two, three and four seeds; (q) deformed seeds present in 3-4 seeded fruits

Fruits and seeds:

The fruits of *C. spinarum* are oblong, oval, round, or conical in shape and contain one to five seeds (Figs. 7a-p). The fruits are green at first, then turn reddish-brown and finally black. The ripened black fruits are tasty and have a high nutritional content. Three to five seeded fruits have deformed seeds (Fig. 7q). The Battal Ballian population bears the highest number of fruits (94.83) per 12-inch branchlet, whereas the Bhamla population had produces lowest number (32.66). The Bann population has maximum values for fruit length (7.96 mm) and breadth (6.56 mm), as well as seed length (6.95 mm) and width (4.7 mm). The fruits of the Bhamla population have the minimum length of 6.26 cm. The Saiswan plant bear fruits of least width (4.53 mm). The Mandli plants differentiate the smallest seeds of length (4.96 mm), whereas the Dinga amb plants bear seed of smallest width (2.56 mm) (Table 3).

In so as fruit related traits are concerned, the maximum weight of 30 fruits per population (12.60 gram) is seen in the Sunderbani population. The fruits of the Chhan Morian population have maximum pulp weight of 8.46 grams. In fruits from the Sunderbani population, the maximum seed weight of 3.55 gm is found. The maximum septum weight (0.65 gram) is noted in the fruits belonging to the Bann population. In the Jandhi population, the weight of the seed plus septum reached its maximum (4.21 gm). In the Chhan Morian population, the pulp to seed plus septum ratio reached its maximum of 2.82.

Table 3: Quantitative data of fruit and seed related traits

Populations	Fruit no. (n=30)	Fruit length (mm)	Fruit width (mm)	Seed length (mm)	Seed width (mm)
Kootah	74.1±1.88 (70-95)	6.68±0.10 (6.0-7.5)	5.66±0.09	5.1±0.08 (4.0-6.0)	2.85±0.08
Dinga Amb	72.93±1.80 (54-84)	7.21±0.11 (6.5-7.5)	5.51±0.08 (5.0-6.0)	5.55±0.09 (5.0-6.5)	2.56±0.07 (2.0-3.5)
Mandli	64.9±1.41 (51-76)	6.38±0.12 (6.0-7.0)	5.18±0.10 (4.0-6.0)	4.96±0.14 (4.5-5.5)	3.08±0.09 (2.5-4.5)
Chhan Morian	75.2±1.30 (64-82)	7.56±0.08 (7.0-8.0)	5.56±0.09 (5.0-7.0)	6.31±0.09 (5.5-6.5)	3.53±0.07 (2.5-4.5)
Sandhi	70.56±1.80 (55-92)	7.65±0.11 (7.0-8.2)	6.1±0.11 (5.5-7.5)	6.3±0.11 (5.5-7.0)	3.66±0.10 (3.5-5.0)
Chouki Choura	48.96±1.55 (32-65)	7.06±0.13 (6.5-7.5)	6.46±0.14 (6.0-7.0)	5.88±0.14 (5.0-6.0)	3.28±0.08 (3.0-4.5)
Bhamla	32.66±1.54 (22-48)	6.26±0.15 (6.0-7.0)	5.4±0.11 (4.0-6.0)	4.96±0.12 (4.0-6.0)	3.95±0.12 (2.8-4.5)
Phalata	84.9±2.75 (57-115)	6.93±0.12 (5.0-7.5)	6.42±0.12 (4.0-6.5)	5.73±0.12 (4.0-6.5)	2.88±0.06 (2.0-4.0)
Battal Ballian	94.83±2.32 (75-117)	7.03±0.13 (6.0-7.5)	5.98±0.12 (5.0-6.5)	6.0±0.13 (4.5-6.5)	4.18±0.11 (3.0-5.0)
Sunderbani	80±1.87 (64-96)	7.46±0.13 (6.5-8.0)	6.21±0.09 (5.5-7.5)	5.93±0.12 (5.0-6.0)	3.71±0.07 (3.0-5.0)
Saiswan	73. ±1.17 (65-84)	7.13±0.09 (6.5-8.0)	4.53±0.10 (4.0-6.5)	5.56±0.09 (5.5-7.0)	2.66±0.05 (2.0-4.0)
Jandhi	76.7±1.68 (63-86)	7.83±0.12 (7.0-9.0)	5.55±0.16 (5.5-7.5)	5.58±0.15 (5.0-7.0)	3.28±0.11 (2.5-5.0)

Mansar	71.73±2.11 (41-87)	7.38±0.15 (6.0-8.5)	5.43±0.11 (4.5-6.5)	5.76±0.15 (5.0-7.0)	3.68±0.08 (3.0-4.5)
Akhnoor	70.4±2.29 (48-92)	7.9±0.13 (6.0-8.5)	6.28±0.13 (5.0-6.5)	6.71±0.13 (5.0-7.0)	4.53±0.08 (3.0-4.5)
Bann	68.46±1.47 (50-80)	7.96±0.14 (6.0-9.0)	6.56±0.12 (5.5-7.5)	6.95±0.13 (5.0-7.5)	4.7±0.10 (3.5-5.5)

Table 4: Quantitative data of fruit and seed related traits

Populations	Fruit weight (gm) (n=30)	Pulp weight (gm)	Seed weight (gm)	Septum weight (gm)	Seed+septum weight (gm)	Pulp to seed+septum ratio
Kootah	10.68	6.73	3.55	0.40	3.95	1.70
Dinga Amb	10.31	6.92	2.83	0.56	3.39	2.04
Mandli	9.35	6.47	2.53	0.35	2.88	2.24
Chhan Morian	11.45	8.46	2.51	0.48	2.99	2.82
Sandhi	8.03	5.41	2.14	0.48	2.62	2.06
Chouki Choura	6.60	4.40	1.60	0.36	1.96	2.24
Bhamla	7.37	4.70	2.25	0.42	2.67	1.76
Phalata	9.51	6.76	2.22	0.53	2.75	2.45
Battal Ballian	10.01	6.82	2.69	0.50	3.19	2.13
Sunderbani	12.60	8.42	3.66	0.52	4.18	2.01
Saiswan	9.13	6.67	2.04	0.42	2.46	2.71
Jandhi	11.36	7.15	3.82	0.39	4.21	1.69
Mansar	9.30	5.92	2.83	0.55	3.38	1.75
Akhnoor	10.64	7.65	2.64	0.35	2.99	2.55
Bann	9.64	6.83	2.16	0.65	2.81	2.43

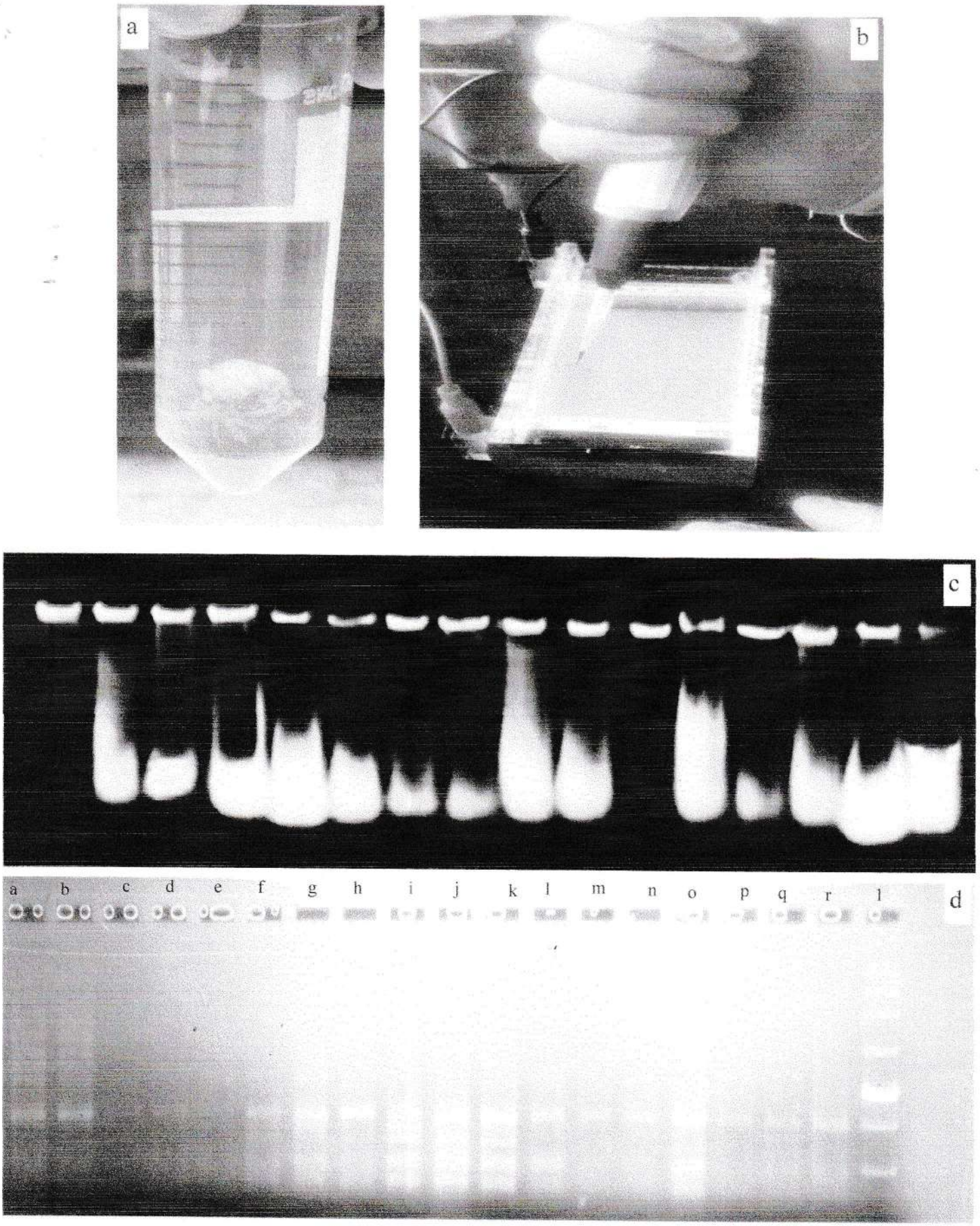

Fig. 8: *C. spinarum*, (a) DNA spool, (b) loading of gel, (c) genomic DNA in the gel, (d) bands of DNA after PCR amplification of 3 populations i.e. a-f Kootah, g-l-Dinga Amb, m-r-Mandli along with DNA ladder in column 1.

Molecular studies

Genetic diversity parameters of *C. spinarum* species were determined by using ISSR (Inter Simple Sequence Repeats) markers. The procedure undertaken involves isolation of genomic DNA from young leaves, purification and quantification of the isolated DNA. This was followed by optimisation of the PCR program, screening of different primers to select those giving good results, amplification using the selected ones, scoring number of bands on the gel and their analysis using various softwares.

DNA has been isolated by using Doyle and Doyle protocol. The quality and quantity of DNA has been determined by running DNA samples on 0.8% agarose gel and using a nanodrop spectrophotometer respectively.

Presently 6 primers have been screened viz. UBC 844, UBC 850, UBC 846, UBC 807, UBC 809, UBC 825 by using Gradient PCR with Tm 48.4°C, 39.3°C, 46°C, 45°C, 47°C, 48°C respectively. Genomic DNA has been amplified using PCR machine by preparing PCR reaction mixture using genomic DNA, primers, MgCl2, reaction buffer, dNTPs and Taq polymerase. The obtained products were loaded into wells of 1.4% agarose gel and electrophoresed. The marked genetic variability has been noted in number and position of bands in the gel. Further, analysis is in process.

Papers communicated:

Deep A and Sharma G "Intra-specific variability in frequency of meiocytes showing partial desynapsis and multivalent formation in underutilised *Carissa spinarum* L. from Jammu Shivalik: putative causes and consequences" communicated in Journal Nucleus

Utilization Certificate

1. **Title of the project:** Assessment of morphological and molecular variability in wild *Carissa spinarum* L. from Jammu Province
2. **Sanction Order no.:** Reappropriated order no: RA/23/4281-90 dated 5/12/2023 (original order no: RA/23/7260-67 dated 23/01/2023)
3. **Name of the PI:** Dr. Geeta
4. **Department:** Department of Botany, University of Jammu
5. **Total Project Cost:** Rs. 2,00,000
6. **Statement of Expenditure:**

Head	Total grant released	Total expenditure incurred (INR)	Unspent Balance (INR)
Equipment (Repair)	1,12,130/-	1,12,130/-	0
Consumables/ Chemicals/Glassware	82,920/-	82,821/-	99/-
Contingency	4950/-	4950/-	0
Total	2,00,000/-	1,99,901/-	99/-

Certified that out of Rs. 2,00,000 (Two lakhs only) of Grant-in-aid, sanctioned vide order no. reappropriated order no: RA/23/4281-90 dated 5/12/2023 (original order no: RA/23/7260-67 dated 23/01/2023) during the year 2023 in favour of Dr. Geeta (PI), a sum of 1,99,901/- has been utilized for the purpose of research for which it was sanctioned and that balance of Rs. 99/- remained unutilised.

Geeta 14/1/2023

Signature
PI

Assistant Prof.
Dept. of Botany
University of Jammu
JAMMU

Signature
Deputy Registrar

Signature
Joint Registrar (Finance)
University of Jammu