



Cytogenetics and Crop Improvement Studies in Pigeonpea [*Cajanus cajan* (L.) Millsp.]: A Review

S.G.P. Karthikeya Reddy, S.K. Verma, Shubham Kumawat, Satvinder Singh, Amit Kumar

10.18805/ag.R-2552

ABSTRACT

Pigeonpea has $2n=2x=22$ chromosomes with length ranging from $5.73\pm1.15\ \mu\text{m}$ to $10.92\pm2.69\ \mu\text{m}$. The largest long arm of chromosome pair (q) was $6.22\pm1.05\ \mu\text{m}$, while the shortest measured $3.37\pm0.83\ \mu\text{m}$. The longest short arm chromosome pair was $4.70\pm1.65\ \mu\text{m}$, while the shortest measured $2.37\pm0.43\ \mu\text{m}$. Metacentric and submetacentric chromosomal shapes exist in pigeonpea, but metacentric dominating. Pigeonpea's karyotypic formula is $2n=2x=9m+2sm$. In pigeonpea, regardless of maturity groups, the main and foremost goal is to breed for higher yield/area/time. Resistance to diseases including wilt, sterility mosaic, phytophthora and alternaria blights, as well as insect pests like pod borers and pod flies, is being bred for. Resistance to abiotic stresses like drought tolerance can be achieved by osmotic adjustment (OA), dehydration tolerance and relative water content (RWC). Reduced Na and Cl translocation from root to stem, osmoprotectants and the optimal leaf area index (LAI) for salinity tolerance. Many outstanding varieties has been developed in pigeonpea through germplasm selection, pedigree breeding, mutation breeding and Heterosis breeding.

Key words: Heterosis, Karyotype, Metacentric, Mutation, Pedigree, Submetacentric.

India is the world's top producer and user of red gram. India is the world's leader in pigeonpea cultivation with an area (5.58 mha) and production (4.29 mt) (FAO 2020). Pigeonpea productivity in India has increased by 11.63 percent in the last five years, from 693 kg/ha (2009-2013) to 806 kg/ha (2014-2020). The red gram is a high-protein staple meal. It has a protein content of roughly 22%, almost three times that of grains. Red gram is primarily ingested as a split pulse known as Dal, which is an important addition to a cereal-based diet. In the average Indian diet, the major ingredients are Dal-Chawal (pulse-rice) or Dal-Roti (pulse-wheat bread). Because the essential amino acids complement each other, when wheat or rice is coupled with red gram, the biological value increases dramatically. Lysine, riboflavin, thiamine, niacin and iron are abundant in pigeonpea (ICMR 1971).

Nutritional values of edible portion per 100 g of pigeonpea.

Crop	Energy (Cal)	Protein (g)	Fat (g)	Ca (mg)	Fe (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vit-A (mcg)
Red gram	335	22.3	1.7	7.3	5.8	0.45	0.19	2.9	132

Red gram contributes to soil fertility by increasing soil physical qualities and fixing atmospheric nitrogen in a symbiotic relationship with *Rhizobium* bacteria in the root nodules, in addition to being an essential source of human food and animal feed. Intercropping with different crops (cotton, sorghum, pearl millet, moong, urd, maize, soybean, groundnut) to increase production and maintain soil fertility is possible with the red gram. It is good for dry land farming and is commonly used as an intercrop with other crops due to its drought resistance. Its exact origin is a point of contention, since some claim it originated in India, while

Department of Genetics and Plant breeding, G.B Pant University of Agriculture and Technology, Pantnagar-263 145, Uttarakhand, India.

Corresponding Author: S.G.P. Karthikeya Reddy, Department of Genetics and Plant breeding, G.B Pant University of Agriculture and Technology, Pantnagar-263 145, Uttarakhand, India. Email: sgpkreddy@gmail.com

How to cite this article: Reddy, S.G.P.K., Verma, S.K., Kumawat, S., Singh, S. and Kumar, A. (2022). Cytogenetics and Crop Improvement Studies in Pigeonpea [*Cajanus cajan* (L.) Millsp.]: A Review. Agricultural Reviews. DOI: 10.18805/ag.R-2552.

Submitted: 21-05-2022 **Accepted:** 14-10-2022 **Online:** 12-11-2022

others believe it originated in Africa. *Cajanus* originated in Hindustan, according to Vavilov (1928). According to Van Der Maesen (1980), the crop's origins are in India. India is regarded as the primary source of diversity, while Africa is regarded as the secondary source.

Cytogenetics of pigeonpea

The cytogenetics study was conducted on November 2017 until May 2018 at Plant Physiology and Biotechnology Laboratory, Sebelas Maret University and Plant Breeding Laboratory Mendel Room, Gadjah Mada University. The results revealed that the pigeonpea has $2n=2x=22$ chromosomes, with chromosomal length ranging from $5.73\pm1.15\ \mu\text{m}$ to $10.92\pm2.69\ \mu\text{m}$. Metacentric and submetacentric chromosomal shapes exist in pigeonpea's, with metacentric dominating. $2n=2x=9m+2sm$ is the pigeonpea's karyotypic formula (Yuniastuti *et al.*, 2021).

Chromosome number

Plant breeding programmes requires genetic information. One of these is karyotype analysis, which involves examining the number, shape and length of chromosomes.

The number of chromosomes in the pigeonpea [*Cajanus cajan* (L.) Millsp.] was found to be $2n=2x=22$ as shown in Fig 1. The pigeonpea has $2n=22$ chromosomes (Udensi *et al.*, 2011). When meiotic cell division occurs, chromosome number is typically detected, whereas karyotype can be observed during mitotic cell division (Riesenberg *et al.*, 1987). In early prometaphase, the number of chromosomes could be seen clearly. This phase began when the prophase cell completed its final division and entered early metaphase (Hardianingsih *et al.*, 2015). The chromosome distributed evenly during this phase, allowing its length and shape to be seen clearly. The optimal timing for cell division in each plant was varied and usual. Each plant has its own biological clock (Johansen, 1940). The number and length of chromosomes are more stable than physical characteristics, making it easier to identify plant relatives.

Chromosomes length and shape

The long arm (q), short arm (p), length total (q+p) and long arm and short arm ratio (q/p) are all ways to assess chromosomal length. The long arm and short arm ratio were used to determine the pigeonpea's chromosome shape as shown in Table 1. (Parjanto *et al.*, 2003); (Yuniastuti *et al.*, 2018).

The average of chromosomal pair was used to compute chromosome measurement. The length of the long and short arms was utilized to calculate the chromosomal arm ratio. The pigeonpea's chromosomal shape will be used to arrange the karyotype formula:

Table 1: Chromosome shape based on long arm and short arm ratio.

Chromosome shape	Arm ratio ($r=q/p$) μm
Metacentric (m)	1.0 \leq 1.7
Submetacentric (sm)	1.7 \leq 3.0
Acrocentric (t)	3.0 \leq 7.0
Telocentric (T)	> 7.0

The chromosomal length of the pigeonpea [*Cajanus cajan* (L.) Millsp.] ranges from $5.73 \pm 1.15 \mu m$ to $10.92 \pm 2.69 \mu m$ on average. Every species' chromosome length was diverse and varied. It was between 0.2 and 50 μm (Suryo, 2003). The chromosome length will be shorter as the number of chromosomes increases. The pigeonpea chromosome has the largest total length of $11.16 \pm 3.16 \mu m$ and the smallest total length of $5.73 \pm 2.35 \mu m$. The average length of a pigeonpea chromosomal pair was $8.14 \pm 3.49 \mu m$. The largest long arm of chromosome pair (q) was $6.22 \pm 1.05 \mu m$, while the shortest measured $3.37 \pm 0.83 \mu m$. The longest short arm chromosome pair was $4.70 \pm 1.65 \mu m$, while the shortest measured $2.37 \pm 0.43 \mu m$.

The average of pigeonpea arm ratio chromosome was 1.50 μm . According to Table 1, the typical chromosomal shape of pigeonpea was metacentric, with some being submetacentric. Table 2 shows that pigeonpea chromosomes have 9 metacentric chromosome pairs and 2 submetacentric chromosomal pair. Submetacentric chromosome pairs 3 and 8 were found. While chromosomes 1, 2, 4, 5, 6, 7, 9, 10 and 11 were metacentric. It was discovered that the pigeonpea chromosome has 18 metacentric chromosomes and 4 submetacentric chromosomes. The chromosomal shape of the *Cajanus* genus revealed that it is metacentric and submetacentric (Deodikar and Thakar, 1956). The metacentric shape of the chromosome arm ratio is nearly one.

Karyotype

Karyotype or karyogamic arrangement based on length and shape similarity. Karyotype arranged from the longest long arm to the shortest one and paired with the chromosome pair. Fig 2 showed that the karyotype of the pigeonpea was made up of 11 chromosome sets, each of which had two chromosomes. Based on chromosome number and shape, the pigeonpea karyotype formula was $2n=18m+4sm$, where m= metacentric and sm= submetacentric. The Table 2 revealed that metacentric chromosome pairs were 9 and submetacentric chromosome pairs were 2. Karyotype arrangement was determined by observing chromosomal

Table 2: The average of chromosome arm ratio of pigeonpea [*Cajanus cajan* (L.) Millsp.].

Chromosome pair	Chromosome length= ($X \pm SD$) μm			Ratio $r=q/p$ ($X \pm SD$)	Chromosome shape
	Total (q+p)	Long Arm (q)	Short Arm (p)		
1	10.92 ± 2.69	6.22 ± 1.05	4.70 ± 1.65	1.40 ± 0.34	Metacentric
2	9.85 ± 2.77	5.80 ± 1.66	4.05 ± 1.14	1.43 ± 0.10	Metacentric
3	8.65 ± 4.99	5.55 ± 3.21	3.10 ± 1.82	1.85 ± 0.39	Submetacentric
4	9.30 ± 5.04	5.28 ± 2.75	4.02 ± 2.31	1.34 ± 0.12	Metacentric
5	8.77 ± 6.87	5.10 ± 3.90	3.67 ± 2.98	1.42 ± 0.26	Metacentric
6	7.57 ± 3.12	4.55 ± 1.82	3.02 ± 1.35	1.53 ± 0.21	Metacentric
7	7.03 ± 1.97	4.20 ± 1.06	2.83 ± 0.91	1.50 ± 0.11	Metacentric
8	6.82 ± 2.03	4.40 ± 1.61	2.42 ± 0.53	1.82 ± 0.48	Submetacentric
9	7.47 ± 4.62	4.07 ± 2.41	3.40 ± 2.21	1.22 ± 0.10	Metacentric
10	7.43 ± 3.16	4.03 ± 1.68	3.40 ± 1.48	1.20 ± 0.04	Metacentric
11	5.73 ± 1.15	3.37 ± 0.83	2.37 ± 0.43	1.42 ± 0.26	Metacentric

length, number and shape (Elrod and Stansfield, 2002). Neither chromosome number nor chromosome shape were employed to diagnose chromosome aberration using the karyotype arrangement (Ramadhani *et al.*, 2011). To explain taxonomy between species, evolution and polyploidy process, karyo morphology investigation was required (Tabur *et al.*, 2012).

An ideogram is a chromosome diagrammatical of one species that is used to compare another species' karyotype (Sastrosumarjo *et al.*, 2006). Ideograms comprise chromosome number, shape and length measurements for each arm (long and short) and are displayed in a Fig 3. There was a chromosome with varying arm lengths. It could be caused by manually paired karyotype arrangement based on arm ratio and chromosomal shape.

Genetics of important characters

Crop improvement programme

Floral biology of pigeonpea

Pigeonpea flowers are zygomorphic, borne on terminal or auxiliary racemes and are usually yellow with occasional variations in color. The tubular calyx has five gamosepalous sepals. The standard, wing and keel petals make up the corolla, which is bright yellow in color and papilionaceous. It contains ten stamens with bright or dark yellow anthers in a diadelphous state. A lengthy style is linked to a thicker, incurved and enlarged stigma, indicating that the ovary is superior. With an average of 20% cross-pollination, pigeonpea is an often cross-pollinated crop (Bisen and Sheldrake, 1981).

Selfing in pigeonpea

Selfing is done by covering an individual inflorescence, a branch of a plant, or a whole plot. When a large amount of seed is required, groups of plants are covered with bee-proof cages.

Bagging

Three types of selfing bags are used for pigeonpea (Gupta *et al.*, 1981).

Table 3: Qualitative inheritance.

Genetics of trait	Contrasting characters	Description	Reference
Growth habit	Spreading vs erect	Spreading branch habit (Sbr) is dominant over erect branching (sbr).	(Dcruz and Deokar, 1970).
	Determinate vs Indeterminate	Determinate monogenic recessive to indeterminate	(Saxena and Sharma, 1990)
Stem characters	Stem color: purple vs green	Purple (Pst) is dominant to green (pst).	(Narkhede <i>et al.</i> , 1980)
	Height: Tall vs Dwarf	Dwarfness controlled by single recessive gene	(Marekar <i>et al.</i> , 1978)
Leaf characters	Shape	Lanceolate leaflets (Lit) monogenic dominant to short leaflets (Lst). Two genes (Bd1ba and Bd1bb) controls broad leaflets which is dominant to narrow	(Dcruz and Deokar 1970) (Kolhe <i>et al.</i> , 1972)
	Petiole size	Long petiole (Lpt) is dominant to short petiole (lpt)	(Ghatge and kolhe, 1984)
	Foliate number	Trifoliate (Tf) is dominant to multifoliate	(Patil <i>et al.</i> , 1972)
	Leaf structure	Gene for normal leaf (Nh); Gigas leaf with crinkled surface (nh).	(Pokle, 1976)
Flower characters	Male sterility	Translucent anthers governed by single recessive gene <i>ms</i> .	(Saxena <i>et al.</i> , 1983)
Seed characters	Seed coat color	Monogenic inheritance of seed coat color (3brown:1white).	(Dcruz <i>et al.</i> , 1974)
Disease resistance	Wilt	Resistance to wilt showed a single gene dominant to susceptibility	(Pawar and Mayee, 1986).
	Sterility mosaic disease	SMD was governed by four nonallelic genes that were independent of one another. Resistance required the existence of at least one dominant and one recessive gene.	(Singh <i>et al.</i> , 1983)
	Phytophthora blight	Resistance to phytophthora blight was reported to be governed by a single recessive (pd1) gene.	(Sharma <i>et al.</i> , 1982)
	Alternaria blight	Alternaria blight was controlled by a single recessive (abr1) gene.	(Sharma 1982) (Singh <i>et al.</i> , 1988)

1. Glassine bag (13 cm × 8 cm) to cover an individual inflorescence.
2. A small-muslin-cloth bag (60 cm × 20 cm) to cover a flowering branch.
3. A large-muslin-cloth bag (135 cm × 90 cm) to cover an entire plant.

Emasculation and crossing techniques

Emasculation

In pigeonpea, emasculation is required for artificial hybridization. For emasculation, the buds that are most likely to discharge pollen for pollination the next day are chosen. These buds are about 66 percent the size of mature buds

and are closed tightly. The corolla of such a blossom is a bright golden color with no greenish tint to it. Two buds on one inflorescence are optimal for emasculation and two to ten buds on a branch can be emasculated. All other buds are removed (Sharma and Green, 1980).

Emasculation steps

Pollination

Pollination can be done soon after emasculation since the stigma of pigeonpea is receptive before anthesis. Between 800 and 1000 pollen-source buds are harvested each day. These buds are maintained on moist filter papers or in covered petridishes.

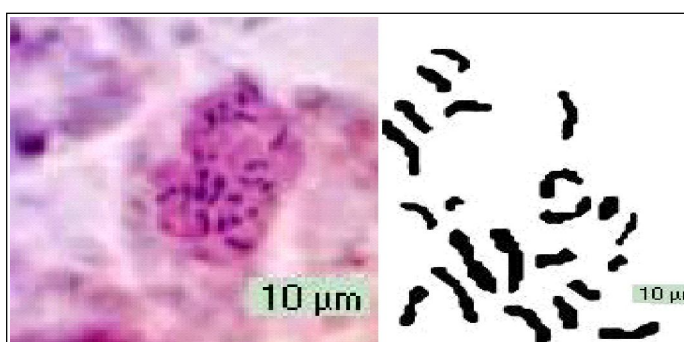


Fig 1: Chromosome of pigeonpea [*Cajanus cajan* (L.) Millsp.].

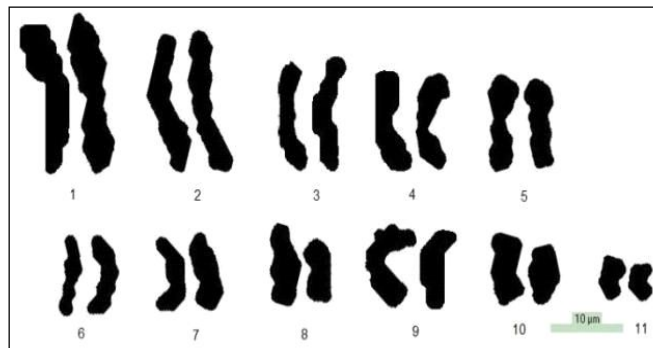


Fig 2: Karyotype of pigeonpea [*Cajanus cajan* (L.) Millsp.].

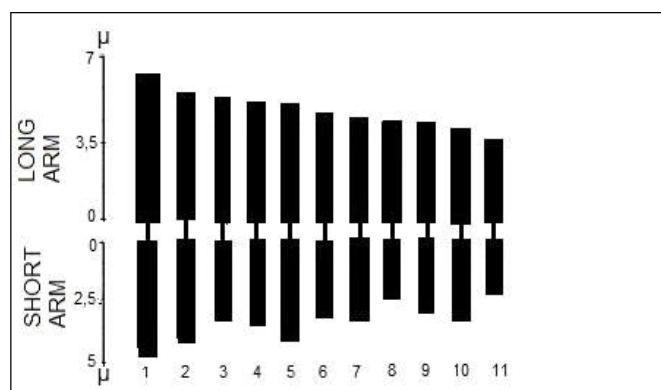


Fig 3: Ideogram of pigeonpea [*Cajanus cajan* (L.) Millsp.].

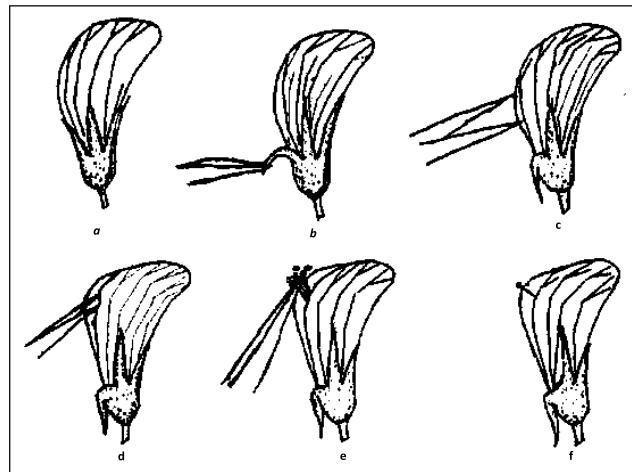


Fig 4: Steps of emasculation procedure.

- a. Hold the bud firmly between the thumb and middle finger with the support of the index finger so that the keel of the standard is facing towards the emasculator (Fig 4a).
- b. Remove or push down the sepal covering the keel (Fig 4b).
- c. Open the corolla by inserting one tip of the forceps at the base of the keel. Now move the forceps upward to the tip of the standard (Fig 4c).
- d. Press the bud with a slight pressure until it opens. This makes the anthers visible (Fig 4d).
- e. With the forceps remove the anthers from the staminal column (Fig 4e).
- f. Count the anthers or examine the bud with a magnifying glass to ensure that all the anthers or their broken parts are completely removed (Fig 4f).



Fig 5: Dodder multiplying on pigeonpea plant.



Fig 7: Larvae and pupae of pod fly in pods.



Fig 6: Pod borer larvae feeding on pigeonpea flower.



Fig 8: Adult pod fly on leaf and flower buds.

Pollen is viable for 42 hours in the bud when kept at 25-28°C and 50% relative humidity. When maintained in a refrigerator for 11 days at 10°C and 37% relative humidity, such buds can contain viable pollen (Prasad *et al.*, 1977).

Remove the pollen-source bud's corolla, leaving the staminal columns and anthers exposed. Hold the bloom in one hand and rub the anthers against the stigma of an emasculated bud to transfer pollen. Two or three emasculated flowers can be pollinated by a single blossom (Sharma and Green, 1980).

Pod development

After pollination, the pods appear one week later and develop in 15-20 days. The seed is ready to harvest after 30-35 days of physiological maturation (Rao, 1974).

Harvesting of pods

30-35 days after pollination, the seed is harvested at physiological maturity. It should be dried to a low moisture content (about 6%) and stored in clearly labelled containers with parentage, season and year of crossing on them.

Breeding objectives

The main goal of pigeonpea breeding, regardless of maturity groups, is to breed for higher yield / area / time. Because a large G X E interaction influences pigeonpea phenology and reproductive development, productivity stability is crucial. Stress caused by diseases like wilt, sterility mosaic, Phytophthora and Alternaria blights, as well as insect pests like pod borers and pod flies, all have an impact on output stability. As a result, resistance to these biotic agents must be incorporated into the variety. Pigeonpea is subjected to extremes of moisture stress because it is mostly grown under rainfed conditions. Plant types with some degree of thermo-insensitivity are necessary for the North East Plain Zone since the crop is exposed to low temperatures throughout the winter months.

Breeding for biotic stresses

Diseases (Phytophthora blight, Sterility mosaic disease, Fusarium wilt and Alternaria blight), insect pests [Pod borer (Fig 6), Pod fly (Fig 7 and 8), Maruca scale insect, *etc.*], nematodes [Root knot nematode and Cyst nematode] and parasitic weeds [Dodder (Fig 5)] all offer biotic challenges to pigeonpea. The resistance/tolerance of pigeonpea germplasm to these pests and diseases has been tested. Gene(s) for conferring resistance/tolerance (particularly against infections) have been found and integrated. As a result, various disease-resistant pigeonpea cultivars have been released. A variety of morphological and biochemical markers have been proposed as the basis of host plant resistance to pod fly. Pod wall thickness, trichome density, reducing and non-reducing sugars, total phenols, tannins and crude fibers have been observed to be negatively associated ($r = -0.83^{**}$ to -0.97^{**}) with pod fly infestation.

Breeding for abiotic stresses

Throughout its life cycle, pigeonpea plants are exposed to a variety of abiotic challenges, the most important of which are moisture, temperature, photoperiod and mineral-related factors. While water logging impacts plant growth by lowering the rate of oxygen diffusion between the soil and the atmosphere and altering the physical and chemical qualities of the soil, drought and high temperatures mostly damage long duration pigeonpea cultivars, causing them to mature prematurely. Similarly, cold temperatures cause the conversion of intracellular water to ice, resulting in cell shrinkage, wilting and plant death. Pigeonpea plants are affected by soil salinity due to osmotic stress and difficulty with mineral nutrient uptake. Some areas in North Eastern Plain Zone and Central Zone have acidic soils with problems of aluminium toxicity. A number of morphological and physiological markers have been identified as a result of genetic enhancement for resistance to various stresses. These include osmotic adjustment (OA), dehydration tolerance and relative water content (RWC) for drought tolerance, Reduced Na and Cl translocation from root to stem, osmoprotectants and the optimal leaf area index (LAI) for salinity tolerance, aluminium exclusion from roots for Aluminium toxicity tolerance and lenticels development, increased root biomass and the presence of adventitious roots for excess moisture tolerance.

Breeding methods

Because pigeonpea [*Cajanus cajan* (L.) Millsp.] is often cross-pollinated, breeding techniques similar to those used for other autogamous crops were applied to improve its genetics. Direct introduction, pure line selection from germplasm, hybridization followed by pedigree selection and mutation breeding were among the techniques used. The following section summarizes the main plant breeding achievements made in the past.

Germplasm selection

Initially, landraces were collected from farmers' fields and the potential landraces were directly released as cultivars from these genetic pools. Most of the centres were involved in the gathering and evaluation of heterogeneous land races in the beginning. Individual plant selections were used to separate high-yielding pure lines with suitable agronomic characteristics from the rest. This resulted in the release of a variety of cultivars in the early (UPAS 120, AL 15, *etc.*), medium (BDN 1, BDN 2, No.148, Hy3C, LRG 30, C 11, Maruthi and the like) and late maturity groups (Bahar, Narendra Arhar 1, MA 3, T 7, *etc.*). Many of these types are still grown and have significant acreage in the areas where they were adopted. Initially, the focus was mostly on increasing yield.

Pedigree breeding

In the second phase of pigeonpea breeding, hybridization and pedigree selection were used to improve certain

qualities. Among the characteristics investigated, early maturity, seed size, pod size, plant type, disease resistance and yield (Saxena *et al.*, 2016). To achieve this, hybridization between parents with diverse genetic backgrounds was performed. The pedigree approach was mostly utilised to deal with separating generations. Only a few hybridized kinds exist: Pragati, Jagriti, Manak, Pusa 33 (in the early group), BSMR 175, Asha, BSMR 736 (in the medium group), DA 11, Pusa 9 and MA 6 (in the late group). Pedigree breeding has resulted in the release of 89 pigeonpea cultivars since 1960. Among them, few popular varieties were listed in Table 4. In addition, 455 advanced breeding lines were nominated for the National Coordinated Trials of the Indian Council of Agricultural Research (ICAR). These facts demonstrate the widespread use of pedigree breeding in pigeonpea.

Mutation breeding

Induced mutagenesis has been employed in the development of commercial pigeonpea cultivars to examine the spectrum of variation and to use valuable variants. Mutagen sensitivity has been found to vary by genotype. α -rays, diethyl sulphonate (DES), ethyl methane sulphonate

(EMS), sodium azide, hydrazine, hydroxyl amine, streptomycin is among the most regularly utilised. The first mutant breeding studies in pigeonpea were limited to determining the effective doses of various chemical and physical mutagens, as well as recording the generated genetic variation for various morphological features. Treatments using ethyl methane sulfonate ($C_3H_8SO_3$), fast neutrons and gamma rays were found to be effective in developing meaningful diversity, although their utility in breeding high yielding cultivars was restricted. Pigeonpea has showed results in genetic enhancement for production, earliness, seed size and disease resistance through mutations. (Pawar *et al.*, 1991). The achievements obtained through mutation breeding in pigeonpea were listed in Table 5.

Hybrid breeding

As a result of many efforts, several genetic male-sterile lines have been found. These lines' general combining ability (GCA) and specific combining ability (SCA) were evaluated. All of these efforts culminated in the production and release of the first commercial genetic male-sterility (GMS) hybrid,

Table 4: List of some of the popular pigeonpea varieties developed by pedigree and selection schemes that are still preferred for cultivation despite having released more than 10 years earlier.

Variety	Release year	Pedigree	Important traits
ICPL 87119 (Asha)	1993	C 11 \times ICPL 6	Resistant to FW and SMD
TJT 501	2009	ICPL 84008 \times TT 6	Early maturity
Narendra Arhar 1 (NDA 88-2)	1997	Selection from Faizabad (Uttar Pradesh)	Compact, indeterminate
MalviyaChamatkar (MAL 13)	2005	(MA 2 \times MA 166) \times Bahar	Spreading, indeterminate, tolerant to wilt, pod borer and SMD
UPAS 120	1976	Selection	Early maturity
LRG 41	2007	Selection from Chilakaluripetin Guntur (Andhra Pradesh)	Resistant to FW and SMD
Jawahar Tur (JKM 189)	2007	ICPL 87119 \times Plant 142	Moderately resistant to wilt, SMD and phytophthora blight
Maruti (ICP8863)	1986	Selection	Indeterminate, semi-spreading, wilt resistant
Bahar	1986	Selection from Motihari (Bihar)	Compact, resistant to SMD
BSMR 736	1996	CTP 7217 \times No 148	Resistant to FW and SMD

Table 5: Pigeonpea varieties released through mutation breeding.

Variety	Year of release	Mutagen treatment	Salient features
Vishakha-1(TT 6)	1976	T21 treated with fast neutrons	35% increase in seed size over parent variety
Co 3	1977	Co 1 treated with 0.6% EMS	High yield, bold seed
Co 5	1984	Co 1 treated with 16 kRY-ray	Early maturity, drought tolerant
TAT 5	1984	T21 treated with 1.5 kR fast neutrons	Early maturity, large seed size (>17 g/100 seeds)
TAT 10	1985	Developed from a cross of mutants, TT 2 \times TT 9	Extra-early maturity, medium-large grain
Pusa 855	1993	Mutant of T21	Early, indeterminate, medium bold grain
Co 6	1993	Mutant of SA 1	Indeterminate, tolerant to pod borer
TT 401	2007	ICPL84008 \times TT 6	-
TJT 501	2008	ICPL84008 \times TT 6	Tolerant to pod borer and pod fly

Table 6: Pigeonpea GMS hybrids released in India.

Hybrids	Parents	Released by	Year	Characteristics	Recom. area
ICPH8	ms Prabhat × ICPL161	ICRISAT, Hyderabad	1991	GMS-based early hybrid, 41% yield advantage over the check	Andhra Pradesh
PPH4	ms Prabhat × AL 688	PAU, Ludhiana	1994	GMS-based early hybrid, 14% yield advantage over the check	Punjab
CoPH1	ms T21 × ICPL 87109	TNAU, Coimbatore	1994	GMS-based early hybrid, 22% yield advantage over the check	Tamil Nadu
CoPH2	ms Co5 × ICPL 83027	TNAU, Coimbatore	1997	GMS-based early hybrid, 35% yield advantage over the check	Tamil Nadu
AKPH 4101	ms Prabhat × AK 101	PDKV, Akola	1997	GMS-based early hybrid, 64% yield advantage over the check	Maharashtra
AKPH 2022	Akms 2 × AK-22	PDKV, Akola	1998	GMS-based early hybrid, 35% yield advantage over the check	Maharashtra
GTH1	GT 288A × GTR-11	SDAU, Sardar Krushinagar	2007	CMS-based hybrid of medium maturity, 25% yield advantage over the check	Central Zone
ICPH 2671	ICPA 2043 × ICPR 2671	ICRISAT, Hyderabad	2010	CMS-based hybrid of medium maturity, 41% yield advantage over the check	Madhya Pradesh

ICPH8, in 1991. It's an indeterminate hybrid that was created by crossing male-sterile Prabhat (determinate) with ICPL161 (indeterminate). It showed a significant (>40%) advantage over their parents and the check variety (UPAS 120). Following then, five more GMS-based hybrids were released. (Table 6). However, due to inherent weaknesses in the male-sterility system and commercial seed production of such hybrids, none of these hybrids could ever find a position in farmers' fields. Breeders, on the other hand, learnt a lesson from such hybrids and moved their focus to cytoplasmic male sterility (CMS)-based hybrids.

CMS based hybrids

Initially, breeders looked for the CMS system in farmed germplasm, but their efforts were fruitless. Following that, they created other CMS systems using wild pigeonpea relatives. In the last few years, five similar systems have been discovered. These are (i) A_1 cytoplasm from *Cajanus sericeus*, (ii) A_2 cytoplasm from *Cajanus scarabaeoides*, (iii) A_3 cytoplasm from *Cajanus volubilis*, (iv) A_4 cytoplasm from *Cajanus cajanifolius*, (v) A_5 cytoplasm from cytoplasm of cultivated species, *Cajanus cajan*, (vi) A_6 cytoplasm from *Cajanus lineatus*, (vii) A_7 cytoplasm from *Cajanus platycarpus* and (viii) A_8 cytoplasm from *Cajanus reticulatus*. The use of A_2 cytoplasm led in the discovery of a medium maturity hybrid GTH 1 (GT 288A × GTR 11) in 2004, but the announcement was postponed for another three years. Over the normal check variant, it has showed a yield advantage of over 25%. This hybrid, on the other hand, has yet to be grown on a large scale. Out of these, A_4 cytoplasm has been promising because of its stability under various agro-climate zones and availability of good maintainers and restores. The F_1 hybrids developed from this CMS source produce

excellent pollen load and pod set. ICRISAT is now using A_4 cytoplasm to generate commercially viable hybrids in large quantities. In Madhya Pradesh, one such hybrid, ICPH 2671, has already been released. It's also an A_4 cytoplasm-based medium-duration hybrid. In the F_1 generation, it showed a higher degree of yield advantage (42 per cent over Maruthi) and uniform fertility restoration. The preliminary results have proven beyond a shadow of a doubt that hybrid technology is capable of improving pigeonpea yield (Kumar *et al.*, 2016). Due to FW and SMD resistance, OUAT (Odisha University of Agriculture and Technology) introduced ICPH 3762 (A_4) later that year, which had a yield advantage of 20%-67% over local controls. In 2015, Professor Jayashankar Telangana State Agriculture University, Hyderabad released ICPH 2740, which exceeded the national check Asha by 42 percent (Kumar *et al.*, 2016). The development of highly vigorous, disease-resistant pigeonpea hybrids resulted in a rise in pigeonpea production.

Current limitations

Breeding in pigeonpea has always been the biggest bet for breeders. The inherent crop specific constraints are detailed below:

Long generation turnover time

The length of time it takes to complete one seed-to-seed generation limits pigeonpea breeding attempts. It's mostly owing of the plants' tight short-day flowering requirements. As a result, breeding a new cultivar takes 10-12 years. In pigeonpea, photo-sensitivity is connected to maturity genes, therefore creating a long-lasting photo-insensitive cultivar are out of the question (Saxena and Tickle, 2015).

Natural cross-pollination

Natural out-crossing to the range of 25%-30% in pigeonpea is a prevalent characteristic of this crop. The presence of insects and nectar glands at the base of flowers aids this process (Saxena *et al.*, 2016). Because breeding is almost always done in open fields, the crop is subjected to unrestricted insect visits, resulting in undetected cross-hybridization of individual plants, which causes inefficiencies in pedigree breeding by lowering the breeding value of the selected plants. Surprisingly, pigeonpea breeders always used pedigree breeding while generating new cultivars, despite recognizing the negative impacts of natural cross-pollination in the crop.

Low harvest index

Harvest index is regarded as a reliable indication of crop grain productivity. This variable is linked to the efficiency with which plants transport dry materials to growing grains. Because the pigeonpea is a perennial plant, it causes indeterminacy in the plants, resulting in massive biomass and a great number of blooms under ideal growing conditions. Because just a little amount of photosynthates is transferred to developing seeds, there is a lot of flower drop, low yield and low harvest indices (0.2-0.3) (Chauhan *et al.*, 1995).

Limited genetic diversity

More than 13,000 accessions make up the primary gene pool of pigeonpea germplasm and this collection demonstrates significant phenotypic variability for both quantitative and qualitative aspects (Bohra *et al.*, 2010; Reddy *et al.*, 2005). However, the same cannot be said for diversity at the molecular level (Bohra *et al.*, 2011; Bohra *et al.*, 2017; Odeny *et al.*, 2007; Yang *et al.*, 2006). According to these experts, the amount of genetic diversity in the secondary gene pool is significantly more than in the primary gene pool. According to (Kumar *et al.*, 2003) just a small percentage of germplasm from the primary gene pool was employed by pigeonpea breeders over the last half-century and this could be one of the important factors responsible for low productivity of new cultivars. Pigeonpea breeders have traditionally ignored the genetic diversity seen in the secondary gene pool for a variety of reasons. This could be due to various reasons, including a lack of resources, poor inter-specific hybridization success and selection troubles caused by the existence of significant linkage drag. (Saxena *et al.*, 2018).

Poor response to selection for seed yield

A review of the performance data gathered over the years from a number of national co-ordinated trials in India. Although substantial genetic breakthroughs have been made through breeding in terms of inherited features, productivity gains have lagged well behind predictions (Green *et al.*, 1981; Ramanujam and Singh, 1981). According to (Swaminathan, 1973) this failure was due to

poor selection efficiency as well as physiological and management constraints. It was interpreted by (Chauhan *et al.*, 1995) as the result of intrinsically inadequate carbohydrate partitioning (Green *et al.*, 1981) hypothesised that genotype-environment interactions for seed yield in pigeonpea were highly large even at the micro (single plant) level. Such interactions cause a lot of non-heritable variability in individual plants, which leads to low seed yield heritability.

Linkage drag

Transferring target genes into cultivated varieties is frequently hampered by a close association between desirable features and unfavorable plant/ seed attributes. For example, selecting a high protein, productive phenology and high yield after transferring high protein genes from *C. scarabaeoides* and *Cajanus albicans* to the cultivated type took 12-14 generations. (Saxena and Sawargaonkar, 2014).

Lack of funds

The lack of systematic public and minimal or no industrial funding support for pigeonpea research and development has resulted in the delayed development of variants with limited genetic advancements in the past. Mr. Bill Gates, the founder of Microsoft Corporation, also stressed the need of private sector funding and support for pigeonpea research and development during his visit to ICRISAT (Varshney *et al.*, 2017).

Future breeding trust

Pigeonpea is a vegetarian protein source for resource-scarce farmers in the tropics. It has drought resistance built in and it can produce even with minimal inputs. There have been major efforts to extend the genetic base and introduce features for biotic stressors and desired abiotic traits. There is increased interest in utilizing additional wild relatives from the secondary gene pool and such initiatives would have a significant impact on expanding the genetic basis of pigeonpea variation and introducing valuable biotic, abiotic and agronomic features. The idea of using wild relatives from the tertiary gene pool to extend the genetic foundation of variation and improve pigeonpea has opened up new horizons. Community effort has boosted the creation of genomic resources and the development of genome-wide markers may pave the way for molecular marker-assisted gene introgressions and breeding.

Temperature-sensitive male sterility system

In a number of crop species, the reversion of male sterility to fertility and vice versa has been seen (Kaul, 1988). Photoperiod, temperature and certain stimuli all affect the expression of genes that control male sterility and fertility. The latter's recent achievement in developing a temperature sensitive male sterility system in pigeonpea (Saxena, 2014) was prompted by the former's recent success in two-line breeding in hybrid rice. When grown at 25°C, such genotypes will stay male sterile, allowing them to be exploited for large-scale F₁ hybrid seed programmes when cross-pollinated by insects. (Saxena, 2014).

Earliness and photo insensitivity

Existing pigeonpea cultivars cannot be employed in preceding or succeeding cropping systems due to their long duration and photosensitivity. As a result, photo insensitivity combined with earliness is a desirable feature for breeders. In this context, super-early pigeonpea has emerged as a novel intervener in pigeonpea breeding, with specified qualities of earliness, photo insensitivity, amazing per-day output, stress escape mechanism and a niche to fit well in wheat-pulse cropping patterns as well as rice fallows (Shruthi *et al.*, 2017). Faster generation turnover, combined with faster trait introgression, makes it easier to examine the genetics of biotic and abiotic stress by rapidly establishing mapping populations (Vales *et al.*, 2012).

Plant type

Pigeonpea has two plant types: Determinate (DT) and Indeterminate (IDT) (Mir *et al.*, 2013). The DT variety is chosen over the IDT type because of its high initial vigour, tolerance to drought and waterlogging and ease of mechanized harvesting. To prevent this uncertainty, (Saxena *et al.* 2017) looked into the inheritance of IDT over DT growth habits. They determined CcTFL1 to be a candidate gene for pigeonpea growth habit using Indel-derived markers to discriminate DT/ IDT lines. Mir *et al.* (2014) identified CcTFL1 as a candidate gene for determinacy, with CcTFL1 accounting for 45-60% of phenotypic variation in determinacy. The mechanism of transition from indeterminate to determinate growth habit in pigeonpea has been revealed utilizing a whole genome scanning method using SNP and DArT tests (Mir *et al.*, 2013); (Mir *et al.*, 2014).

Protein content

Pigeonpea protein content ranges from 20 percent to 22 percent in general. Additive genetic action controls the majority of protein content (Saxena, 2008). Extending hybrid parent research in the direction of breeding high protein A-lines can aid in the development of hybrids with a yield advantage of 25%-30% and a high protein content of 26%-27%. According to (Saxena and Sawargaonkar, 2016) newly produced pigeonpea lines (HPL 2, HPL 7, HPL 8, HPL 24, HPL 25, HPL 26, HPL 28, HPL 40 and HPL 51 exhibit protein levels of 28-30% and yield comparable to cultivars.

Cleistogamous flowers

In varietal breeding, "natural outcrossing," which is beneficial in hybrid breeding, is regarded as a genetic contaminant. Up to 25%-30% of pigeonpea's are outcrossed, according to (Saxena and Sharma, 1990). In this regard, ICRISAT has discovered a new flower feature known as cleistogamy. The insertion of partial cleistogamy into desirable cultivars can help to retain the genetic purity of a variety. With these features in mind, (Saxena *et al.*, 1994) investigated a natural mutant with wrapped flower morphology or cleistogamy. The cleistogamy feature is controlled by a single recessive gene and is relatively straightforward to introduce into commercial lines' backgrounds. ICPL 87154, a partly cleistogamous line

with low natural outcrossing (less than 1%), was produced earlier (Kumar, *et al.*, 2016; Kumar, *et al.*, 2016).

CONCLUSION

Chromosome number of pigeonpea [*Cajanus cajan* (L.) Millsp.] is $2n = 2x = 22$ with chromosome length between 5.70 ± 2.35 μ m until 11.16 ± 3.16 μ m and has 18 metacentric chromosome and 4 submetacentric chromosome. Karyotype formula of pigeonpea [*Cajanus cajan* (L.) Millsp.] is $2n = 9m + 2sm$. Huge variability and plasticity of the pigeonpea crop, provided an opportunity for breeding varieties and hybrids for reducing crop duration, improving seed quality and overcoming the constraints of major diseases like fusarium wilt and sterility mosaic. These milestones have helped to increase the production and area of pigeonpea, in spite of stagnant yield/ha.

Conflict of interest: None.

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