BIOLOGY OF CAJANUS CAJAN (PIGEONPEA)

Phase II Capacity Building Project on Biosafety

Ministry of Environment, Forest and Climate Change
Government of India
Biology of *Cajanus cajan* (Pigeonpea)

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under UNEP/GEF supported Phase II Capacity Building Project on Biosafety

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Message

I am happy to learn that the Ministry of Environment, Forest & Climate Change (MoEFCC) as part of the initiative under the UNEP GEF supported “Phase II Capacity Building Project on Biosafety” has developed eight crop specific biology document on Chickpea, Mustard, Papaya, Pigeon-pea, Potato, Rubber, Sorghum, and Tomato.

I am happy to note that the documents have been prepared with support from seven research institutions namely Indian Institute of Pulses Research, Directorate of Rapeseed and Mustard Research, Indian Institute of Horticulture Research, Central Potato Research Institute, Rubber Research Institute of India, Indian Institute of Millets Research and Indian Institute of Vegetable Research.

While Bt cotton is the only genetically modified (GM) crop approved for commercial cultivation in India, there are several crops under various stages of research, development and field trials. The present set of crop specific biology documents aims to provide scientific baseline information of a particular plant species that can be used as credible source of information for conducting safety assessment of GM plants.

I would like to congratulate all those who were involved in preparing these documents and those involved in steering this initiative.

I am confident that these biology documents will serve as a valuable tool for regulators, scientists, crop developers, policymakers, academicians and other stakeholders who are involved in the safety assessment of GM plants. I am also hopeful that baseline information provided in the biology document would further enhance awareness on biosafety aspects of GM crops.

(Pракश Javadekar)
India is an agriculture based economy with abundance of genetic base, diverse agro-climatic zones and highly qualified manpower which provides a rich scope for technological advances in agricultural biotechnology. The shortage of healthy seeds/planting material, lack of disease resistant clones, crop damage by insects, pests etc. have often affected the Indian agricultural economy adversely and therefore the role of new technologies assumes significant importance for Indian economy.

With significant advances in the field of agricultural biotechnology the regulatory system has to deal with multiple crops integrated with multiple traits. In order to streamline the process of safety assessment, the Ministry of Environment, Forest & Climate Change (MoEF&CC) under the UNEP-GEF supported “Phase II Capacity Building Project on Biosafety” has prepared a set of crop specific biology documents namely Chickpea, Mustard, Papaya, Pigeon-Pea, Potato, Rubber, Sorghum, Tomato with support from six Indian Council of Agriculture Research (ICAR) institutions and Rubber Research Institute of India.

The biology documents provides an overview of baseline biological information of a particular plant species such as taxonomy, the centres of origin, its related species including wild relatives, general description of their morphology, reproductive biology, biochemistry, potential for gene introgression, biotic and abiotic interactions. Such species specific information is expected to serve as a guiding tool for use in risk assessment of genetically modified (GM) plants.

The documents has been prepared through a consultative approach and comments received from several organizations have been extremely useful in validating this
document. I express my deep appreciation for the support provided by Indian Institute of Pulses Research, Directorate of Rapeseed and Mustard Research, Indian Institute of Horticulture Research, Central Potato Research Institute, Rubber Research Institute of India, Indian Institute of Millets Research and Indian Institute of Vegetable Research in preparing these documents. I would also like to congratulate Dr. Ranjini Warrier, Advisor, (MoEFCC) and Dr. O.P. Govila (Former Professor, Department of Genetics, IARI) for their sincere efforts and the consultative approach adopted in finalizing the biology documents.

I am confident that these crop specific biology documents would be of immense value for researchers, regulators and industry in planning for the safety assessment of GM crops.

Hem Pande
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1. INTRODUCTION
1.1 Classification and Nomenclature

Pigeonpea [Cajanus cajan (L.) Millsp.] belongs to the genus - Cajanus, subtribe - Cajaninae, tribe - Phaseoleae, order - Fabales, family - Fabaceae and sub-family Faboideae. Several edible beans like Lablab, Dolichos, Phaseolus, Vigna and Cajanus come under tribe Phaseoleae but in the sub-tribe Cajaninae, only one species, Cajanus cajan has been domesticated and cultivated. The species belonging to Cajaninae have peculiar vesicular glands on the leaves, calyx and pods which deposit a sticky substance on their surface. It is the second most important pulse crop grown in India.

The term ‘pigeonpea’ was coined in Barbados, where its seeds were considered an important pigeon-feed (Gowda et al., 2011). Pigeonpea or red gram or tur is known by several vernacular names in India viz. Tur (Maharashtra and Gujarat), Arhar (Uttar Pradesh, Bihar, Madhya Pradesh), Aral (West Bengal), Kandi (Andhra Pradesh), Harad (Haryana and some parts of western Uttar Pradesh), Rahat (parts of Bihar), Tuvararipppu (Kerala), Kokh-lan (tribes of Tripura), adhaki and tuvarika (Sanskrit). The alternate (Syn.) botanical names of pigeonpea are as follows: Cyrisus cajan L.; C. bicolor DC.; C. flavus DC.; C. indicus Spreng.; C. striatus Bojer (van der Maesen, 1990).

The botanical name, C. cajan (L.) Millsp. has been accepted universally for pigeonpea. The taxonomic position (Van der Maesen 1990; http://www.uniprot.org/taxonomy/3821) of pigeonpea [Cajanus cajan (L.) Millsp.] is as follows in Table 1:

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Fabales</td>
</tr>
<tr>
<td>Family</td>
<td>Fabaceae</td>
</tr>
<tr>
<td>Sub-family</td>
<td>Faboideae</td>
</tr>
<tr>
<td>Tribe</td>
<td>Phaseoleae</td>
</tr>
<tr>
<td>Subtribe</td>
<td>Cajaninae</td>
</tr>
<tr>
<td>Genus</td>
<td>Cajanus</td>
</tr>
<tr>
<td>Species</td>
<td>cajan</td>
</tr>
</tbody>
</table>

De (1974) opined that differences between the two genera Atylosia and Cajanus are in size and vigour of the plants, size and non-shattering of pods and size and number of seeds, which might have resulted due to different forces during domestication. Roy and De (1965) and Van der Maesen (1981) proposed the merger of the two genera (Atylosia and Cajanus) because of their cytological similarity and successful crossing with the diploid species. Van der Maesen (1986) merged the species of Atylosia W. & A. with Cajanus DC on biosystematic grounds. Morphological, cytological, chemical and hybridization data support this merger, even
if the required taxonomic changes are inconvenient (Van der Maesen, 1990). Van der Maesen (1990) indicated *C. ajanifolius* as the most probable progenitor of pigeonpea.


### 1.2 Botanical Description of Pigeonpea

#### 1.2.1 Growth and development

Pigeonpea is adapted to the tropical and subtropical region and it can be grown on marginal land and low fertilizer input, even under drought condition. The growth habit is predominantly indeterminate but some genotypes show determinate growth. The branching pattern varies from erect to spreading. Pigeonpea is a predominantly photoperiod sensitive short day plant and exhibit wide variation in days to flower among genotypes (Gooding, 1962; Spence and Williams, 1972).

#### 1.2.2 The Botanical Features of different plant parts are as follows

**Root**

Pigeonpea has deep tap roots which extend vertically up to 2 meters and spread horizontally through lateral roots. The Root is well developed in upper 60 cm soil profile (Natarajan and Willey, 1980). The root proliferation is correlated with the duration of crop and growth habit (Sheldrake and Narayanan, 1979; Mahta and Dave, 1931).

**Fig. 1: Pigeonpea plant**

**Stem**

An angular and woody stem originates from three ribs starting from the base of each petiole. Starch present in xylem parenchyma and the medullary rays are mobilized to the pod and seed (Sheldrake, 1984). Branching pattern (compact or semi spreading or spreading) is determined by the genetic constitution.

Pigeonpea plant show great plasticity by adjusting its branching behavior depending on the available space between plants.
Leaves
Leaves are spirally arranged, pinnately trifoliate and lanceolate to oblong in shape. The terminal petiole is highly variable and attains a length of 10-20 mm while the lateral petiole is usually 2-3 mm long. Leaf size varies from 6-17 cm; lateral leaflets are smaller than the terminal leaflet which varies from 4-8 cm. Leaves are pubescent due to the presence of simple or glandular hairs (Bisen and Sheldrake, 1981). Figure 1 represents pigeonpea plant.

1.2.3 Reproductive Parts
Inflorescence
The inflorescence is raceme which contain up to ten flowers per panicle and usually two flowers open at a time on a single inflorescence (Sharma and Green, 1980). Flowering is acropetal (in the direction of apex), both within the raceme and on the branch. A single plant can hold up to 915 racemes (Remanandan et al., 1988). The terminal or auxiliary raceme is usually 4-12 cm long. In most of the long duration genotypes the racemes are grouped together at the end of branches, while in early, medium and indeterminate genotypes the racemes are distributed along the branches (Sharma and Green, 1980).

Flower
The flowers are bisexual, zygomorphic and predominantly yellow (Sundaraj and Thulasidas, 1980). More flowers are seen on the top of the peduncle. Small flowers, normally about 2 cm in length are borne on thin, hairy pedicel. The flower size is very small in wild species. Flower size is correlated with seed size (Sharma and Green, 1980). The calyx is gamosepalous with five lobes. The corolla is zygomorphic and petals are imbricate. The largest, auricled and erect petal forms the standard; two lateral, obliquely obovate and incurved clawed petals are known as wings; the two innermost obtuse, incurved and boat shaped petals are fused to form the keel to protect the stigma and style. The standard and the wings are generally of bright yellow colour, whereas keel is greenish yellow. A lot of variation in petal colour can be observed in the germplasm collections. The androecium has 10 stamens bunched into two groups (diadelphous) of 9 and a single free stamen that is attached at the base of androecium. The grouped filaments are fused at the base and cover the gynaecium, while the upper part is free and bear uniform anther of about 1 mm length. Six filaments are long, while the remaining four stamens including the free posterior have short filaments which are supposed to encourage self fertilization (Bahadur and Rao, 1981). The dorsifixed anthers, consisting of two halves, are pale yellow to yellow in colour. The placement of subsessile, dorsoventrally flattened, punctuates and densely hairy ovary is superior. The long, filliform and glabrous style of gynaecium bears a thick, incurved and capitate stigma. The short stalked glandular ovary is unilocular and monocarpellary bearing 2-9 ovules with marginal placenta.

Pod
Pod size is highly variable. The vegetable types have long pods with 4-7 seeds per pod. Depending on the genotype, 2-7 seeds develop in each pod. Seeds are produced in separate locules and the pod may be highly constricted in certain genotypes giving beaded appearance. Pod colour varies from green to dark purple with varying degrees of brownish or purplish streaks. Pod is generally pubescent with varying degrees of simple or glandular hairs. Pod shattering at maturity is uncommon in cultivated varieties as it is an undesirable trait for grain harvest.
Seed

The germplasm of pigeonpea show a variety of seed colour (white, creamy white, silvery, fawn, dark purple which appear as black, pink, red to purple, straw, brown) with or without specks and blotches of different shades. The 100 gram seed weight varies considerably from 5 to 22 g in germplasm materials. The 100 seed weight of short duration cultivated varieties are low (generally 6-8 grams) as compared to long duration varieties (9-13 g). Seed weight of medium duration varieties lie between early and late maturing varieties. The 100 seed weight of vegetable types may reach up to 22 g. Seed do not show dormancy and germination is hypogean.

1.3 Economic Importance and Nutritional Composition

Pigeonpea is one of the most important pulse crops of India. It is an integral part of the subsistence and rainfed farming systems in many parts of India. Being a hardy crop, it is a natural choice for small and marginal farmers particularly, in semi-arid dry-land areas because it can be grown successfully under rainfed or low input condition and provide nutritive food, feed, fodder and fuel wood. In India, it is mainly consumed in the form of split pulse as ‘dal’. Its immature green seeds and pods are also consumed as a green vegetable by the tribal people of many States (provinces) such as Chattisgarh, Jharkhand, Andhra Pradesh, Karnataka, Gujarat and in the entire North-East Hill region, where it is primarily grown in the kitchen garden, backyards, hilly tract and on jhum land. The seed coat together with husk provides a valuable feed for milch animals. The green leaves and tender branches provide nutritive fodder to livestocks. The tall and erect pigeonpea varieties are known to provide not only nutritious food, feed and fodder but also provide fuel wood for the rural people, thus very popular among small and marginal farmers. The dry sticks of pigeonpea plant are also used for making baskets, thatches and storage bins. In addition to atmospheric nitrogen fixation through root nodulation by a wide range of symbiotic Rhizobia strains (Chikowo et al., 2004), the defoliated leaves also add nitrogen and organic matter to the soil (Mafongoya et al., 2006). Being a deep rooted legume, it also improves the physical condition of the soil for the next crop. Krauss (1936) considered pigeonpea for soil binding and advocated its plantation in Hawaii Island for checking soil erosion.

Crude protein ranges from 28–36% in green foliage of pigeonpea (Phatak et al., 1993) and

Table 2: Nutritional composition of mature pigeonpea seeds

<table>
<thead>
<tr>
<th>Nutritional value per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy 343 kcal</td>
</tr>
<tr>
<td>Carbohydrates 62.78 g</td>
</tr>
<tr>
<td>Dietary fiber 15 g</td>
</tr>
<tr>
<td>Fat 1.49 g</td>
</tr>
<tr>
<td>Protein 21.7 g</td>
</tr>
<tr>
<td>Thiamine (vit. B1) 0.643 mg (56%)</td>
</tr>
<tr>
<td>Riboflavin (vit. B2) 0.187 mg (16%)</td>
</tr>
<tr>
<td>Niacin (vit. B3) 2.965 mg (20%)</td>
</tr>
<tr>
<td>Pantothenic acid (B5) 1.266 mg (25%)</td>
</tr>
<tr>
<td>Vitamin B6 0.283 mg (22%)</td>
</tr>
<tr>
<td>Folate (vit. B9) 456 μg (114%)</td>
</tr>
<tr>
<td>Calcium 130 mg (13%)</td>
</tr>
<tr>
<td>Iron 5.23 mg (40%)</td>
</tr>
<tr>
<td>Magnesium 183 mg (52%)</td>
</tr>
<tr>
<td>Manganese 1.791 mg (85%)</td>
</tr>
<tr>
<td>Phosphorus 367 mg (52%)</td>
</tr>
<tr>
<td>Potassium 1392 mg (30%)</td>
</tr>
<tr>
<td>Sodium 17 mg (1%)</td>
</tr>
<tr>
<td>Zinc 2.76 mg (29%)</td>
</tr>
</tbody>
</table>

Source: Mazur et al., 1998
average seed protein content has been reported to be 21.7% (Mazur et al., 1998). Pigeonpea seeds provide essential amino acids like lysine, tyrosine, and arginine, whereas cystine and methionine contents are low (Saxena et al., 2010a). Pigeonpea seeds are also rich in potassium, phosphorus, magnesium, calcium and iodine. However, cystine and methionine contents are low (Nwokolo, 1987). It also contains fair amount of iron and selenium and small amount of zinc, copper and manganese, vitamin A, niacin and small amount of thiamine, riboflavin, vitamin B6, folate and pantothenic acid (Table 2). Pigeonpea leaf extract is used in the traditional treatment of jaundice and diabetes and used a antidote against food poisoning, gingivitis, stomatitis and constipation (Lans, 2007; Ganeshan, 2008; Upadhyay et al., 2010). Several anti-nutritional factors like trypsin inhibitor, protease inhibitors, amylase inhibitors, cyanogenic glycoside, hemagglutinin, alkaloids and tanninphytolectins, polyphenols and oligosaccharides have been reported in pigeonpea seeds. However, simple processing methods like soaking in water, boiling, cooking, germination and fermentation reduce the level of these factors (Singh, 1998; Onwuka, 2006).

2. AREA, PRODUCTION AND PRODUCTIVITY

2.1 Geographic Distribution

Distribution of pigeonpea is asymmetric over the globe. It is grown in different parts of the world covering more than 22 countries including India, Myanmar, Tanzania, Malawi and Kenya (Fig 2) (FAOSTAT, 2013). In South-East Asia, pigeonpea is mainly grown in India, Mayanmar, Nepal, Bangladesh and Phillipines. It is widely cultivated in India where it plays an important role in pulse based cropping systems and occupies second largest area among the pulse crops. Recently this crop has been introduced in China as well where it is planted on the hilly slopes primarily to check soil erosion (Saxena, 2008).

Globally 4.33 million tonnes (mt) of pigeonpea was produced and India alone contributed 2.65 mt followed by Mayanmar (0.9 mt), Tanzania (0.3 mt), Malawi (0.24 mt), Kenya (0.09 mt) and Uganda (0.084 mt) (FAO STAT, 2012). The area and production of pigeonpea for last 6 years in India has been presented in Table 3.

Table 3: Area and Production of Pigeonpea in India

<table>
<thead>
<tr>
<th>Year</th>
<th>Area (Million hectares)</th>
<th>Production (million tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007-08</td>
<td>3.73</td>
<td>3.08</td>
</tr>
<tr>
<td>2008-09</td>
<td>3.38</td>
<td>2.27</td>
</tr>
<tr>
<td>2009-10</td>
<td>3.53</td>
<td>2.46</td>
</tr>
<tr>
<td>2010-11</td>
<td>4.42</td>
<td>2.86</td>
</tr>
<tr>
<td>2011-12</td>
<td>4.04</td>
<td>2.65</td>
</tr>
<tr>
<td>2012-13</td>
<td>3.81</td>
<td>3.07</td>
</tr>
<tr>
<td>2013-14</td>
<td>3.88</td>
<td>3.29</td>
</tr>
</tbody>
</table>

Source: Agricultural Statistics Division, Directorate of Economics and Statistics, Department of Agriculture and Co-operation, Ministry of Agriculture.
2.2 Distribution in India including Regions of Cultivation

India occupies the largest area (3.5 – 4.0 million hectares) of pigeonpea in the world, thereby contributing nearly 80% area globally. Although pigeonpea is grown in 315 districts of India, 26 districts account for about 50% area (Bhatia et al., 2006). In India, it is a widely grown crop covering more than 18 states. About 85% of the pigeonpea is grown in six states namely, Maharashtra, Karnataka, Madhya Pradesh, Andhra Pradesh, Gujarat and Jharkhand (Fig.3). Other states include Uttar Pradesh, Orissa, Tamil Nadu, Bihar and Chattisgarh. To a limited extent, pigeonpea is also cultivated in Rajasthan, Punjab, Haryana, West Bengal and North-Eastern States. Pigeonpea is grown traditionally in the foot hill regions of Dehradun upto 1500 m altitude, where it is locally known as ‘Tur’.

Fig. 3: Major state wise producer of pigeonpea (%)
[Source: GoI, Department of Agriculture & Cooperation (2014-15)]

In India, pigeonpea crop has four distinct maturity groups viz., early (120-140 days), mid-early (141-160 days), medium duration (161-180 days) and long duration (>180 days). Although pigeonpea can grow well on hilly slopes, grass lands, forest lands, degraded lands and ravine areas the cultivated species of pigeonpea does not exist as naturalized population in the wild form in any ecological zone of India. Hence its natural habitat conditions are not known; but it prefers grassy habitats in tropical, cold free zones with optimum 600-1000 mm annual rainfall. Natural population of various wild species of pigeonpea can be found in Eastern and Western Ghats, North-Eastern states and in forests and hilly areas in almost every state (Sardana et al., 2011). Generally, wild species of pigeonpea are spotted on the sunny and drained area of the forest edges, in open places within the forest or on grasslands and hill slopes.

2.3 Zonalization and Varietal Testing System for Release

The Project Directorate on Pulses (PDP), Kanpur Uttar Pradesh was created to look after the All India Co-ordinated Project on Improvement of Pulses (AICPIP) including pigeonpea. The PDP was later upgraded to the level of Directorate of Pulses Research (DPR) in 1984 and subsequently in 1993, it attained the status of Indian Institute of Pulses Research (IIPR). The AICPIP was trifurcated, and a separate network, All India Co-ordinated Research Project (AICRP) on Pigeonpea was created for zone based co-ordinated research catering to the specific needs of pigeonpea.

Pigeonpea is a photo thermo sensitive crop and its phenology differs with the climatic conditions and varieties. Long duration varieties (>180 days) are pre-dominant in North East plains (Eastern Uttar Pradesh, Bihar, Jharkhand, West Bengal, Assam) and parts of Northern Chattisgarh and Bundelkhand region of Uttar Pradesh and Madhya Pradesh. Medium duration (161-180 days) varieties are usually grown in Central (Chhatisgarh, Madhya Pradesh, Gujarat, Maharashtra) and Southern India
(Andhra Pradesh, Karnataka, Tamil Nadu and parts of Odisha), whereas early duration varieties are predominant in North West plains (Punjab, Haryana, parts of Rajasthan and western Uttar Pradesh).

For the purpose of varietal release, elite pigeonpea breeding lines are evaluated to assess their performance with respect to grain yield, disease resistance and their suitability for cultivation in specific zone(s). Testing centres and growing areas have been divided into five zones (Fig 4), namely:

1. North Hill Zone (NHZ) comprising Uttarakhand, Tripura, Nagaland and Assam
2. North East Plain Zone (NEPZ) comprising central and eastern Uttar Pradesh, Bihar, Jharkhand and parts of West Bengal
3. North West Plain Zone (NWPZ) comprising Delhi, Haryana, Rajasthan, Punjab, western Uttar Pradesh and tarai region of Uttarakhand
4. Central Zone (CZ) comprising Madhya Pradesh, central and southern Chattisgarh, Gujrat and Maharashtra
5. South Zone (SZ) comprising Orissa, Telangana, Andhra Pradesh, Karnataka and Tamil Nadu

These zones have manpower and resources to evaluate elite breeding lines and participate in development of matching crop production and protection technologies.

Fig. 4: Zonalization and testing centres of pigeonpea

They are also involved in technology transfer and quality seed production and are catering to specific needs of various agro-climatic zones. There is a three tier varieties testing system for release. Promising elite breeding lines are evaluated as entry in Initial Varietal Trial (IVT) across the zone(s), and the genotype(s) performing better than check variety are promoted for further evaluation in Advanced Varietal Trial 1 (AVT 1) and Advanced Varietal Trial 2 (AVT 2) on the basis of performance in a specific zone or zones. The elite breeding lines are recommended for release on the basis of performance (3 years or more) in multi-locational trials in comparison to the best check variety.

3. GEOGRAPHIC ORIGIN, GENOMIC EVOLUTION AND CHROMOSOME NUMBER

3.1 Centres of Origin and Diversity

India is considered as the centre of origin for pigeonpea (Vavilov, 1951). Many evidences including occurrence of various wild relatives (Table 4) in nature, vast genetic variability in the gene pool, and a few historical as well as archaeological records have been offered to strengthen
the view of Indian origin of pigeonpea (Van der Maesen, 1986, 1990). The alternate hypothesis suggesting Africa as the centre of origin does not seem to be viable as only one wild relative *C. kerstingii* is reported to occur in West Africa. In addition, *C. scarabaeoides* has also been found in Africa, but spread is restricted to the coastal areas only. Consequently, van der Maesen (1980) proposed Africa as the secondary centre of origin (Saxena, 2005). Therefore, the most acceptable route of dispersion describes that the immigrants moved the crop up from India to East Africa, then the route followed to Egypt (via Nile valley), West Africa and finally to the America (Odeny, 2007a; Kassa et al., 2012). Fifteen wild species have been reported in Australia also. Noticeably, majority of these species are endemic, therefore, Australia is considered as the centre of diversity for pigeonpea. However, Kassa et al. (2012) contest this view that considers Australia as the centre of diversity since they observed very low level of genetic diversity among the wild Australian species, which were used for SNP based genetic diversity analysis.

*C. cajan* having a pantropical distribution is the only cultivated species belonging to the genus *Cajanus*. Pigeonpea underwent domestication around 3,500 years ago (Vavilov, 1951; De, 1974; Royes, 1986; 1990).

<table>
<thead>
<tr>
<th>Species</th>
<th>Distribution</th>
<th>Reference(s)/Links</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. scarabaeoides</em></td>
<td>Widely distributed species across Andaman and Nicobar, Andhra Pradesh, Bihar, Chattisgarh, Himachal Pradesh, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, Uttar Pradesh, Uttaranchal, West Bengal</td>
<td>Upadhyaya et al., 2013</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>Peninsular India</td>
<td>van der Maesen, 1990</td>
</tr>
<tr>
<td><em>C. cajanifolius</em></td>
<td>Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chattisgarh, Gujarat, Haryana, Himachal Pradesh, Jammu-Kashmir, Karnataka, Kerala, Mahara- rashtra, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Pondicherry, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal</td>
<td><a href="http://www.iucnredlist.org/details/full/19891613/0">http://www.iucnredlist.org/details/full/19891613/0</a></td>
</tr>
<tr>
<td><em>C. crassus</em> (King) Maesen var crassus</td>
<td>Assam, Central India and NW Himalaya</td>
<td>van der Maesen, 1990</td>
</tr>
<tr>
<td><em>C. elonagatus</em> (Bentham) Maesen</td>
<td>NE India</td>
<td>van der Maesen, 1990</td>
</tr>
<tr>
<td><em>C. grandiflorus</em> (Bentham ex Baker) Maesen</td>
<td>Himalayn region</td>
<td>van der Maesen, 1990</td>
</tr>
<tr>
<td><em>C. mollis</em></td>
<td>Himalayn region</td>
<td>van der Maesen, 1990</td>
</tr>
<tr>
<td><em>C. platycarpus</em></td>
<td>Bihar, Gujarat, Haryana, Himachal Pradesh, Jammu-Kashmir, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan and Uttar Pradesh</td>
<td><a href="http://www.legumes-online.net/ildis/aweb/td114/td_24020.htm">http://www.legumes-online.net/ildis/aweb/td114/td_24020.htm</a></td>
</tr>
</tbody>
</table>

(Source: http://www.theplantlist.org)
Based on the morphological evidences, *C. cajanifolius* is considered as the putative progenitor of present cultivated pigeonpea (De, 1974; van der Maesen, 1986,1990). Morphologically, *C. cajan* and *C. cajanifolius* share similar attributes except for the strophiole characteristics (De, 1974). An elaborated comparison between *C. cajan* and *C. cajanifolius* was established based on various morphological attributes, extent of crossability and cytology of the derived inter-specific hybrids (Mallikarjuna et al., 2012).

### 3.2 Genomic Evolution

Pundir and Singh (1985) reviewed the evolution of *C. cajan* and proposed that an inter-specific hybridization event between *C. scarabeaoides* and *C. cajanifolius* led to the evolution of *C. cajan*. Further, using PCR-RFLP technique different genera belonging to the sub tribe Cajaninae viz., *Cajanus, Rhynchosia, Dunbaria, Flemingia* and *Paracalyx* were studied and involvement of *C. cajanifolius* as the maternal parent was advocated (Lakshmi et al., 2000).

The first report demonstrating the eleven pairs of homologous chromosomes (n=11) in pigeonpea was documented by Roy (1933). Afterward, an investigation using pollen mother cells strengthened the hypothesis of haploid chromosome number to be n=11 (Krishnaswamy and Ayyangar, 1935). Somatic chromosome number of pigeonpea was reported to be 2n=22 (Naithani, 1941). The wild relatives of pigeonpea also contain similar number of chromosomes except in African species *C. kertsingii*, which exhibited a different chromosome count of 2n = 32 (Gill and Hussaini, 1986). Thenceforth, a series of karyotype studies and measurement of the 4C DNA content were done in pigeonpea (Ohri et al., 1994; Greilhuber and Obermayer, 1998; Ohri and Singh, 2002).

The comprehensive karyotype analysis reported by Ohri and Singh (2002) included cultivars of *C. cajan* and other wild species belonging to *Cajanus, Rhynchosia, Dunbaria, Flemingia* and *Paracalyx* and they also reported the 4C DNA content in these species. With regard to chromosome structures, variations were detected in chromosome length and satellite chromosomes (Naithani, 1941; Sinha and Kumar, 1979; Sharma and Gupta, 1982; Pundir and Singh, 1986).

The drafts of pigeonpea whole genome sequence yielded valuable insights about the genome evolution of pigeonpea (Varshney et al., 2011; Singh et al., 2011). Based on K-mer statistics the entire genome size of pigeonpea was estimated to be 833.07 Mb. Furthermore, the whole genome assembly of pigeonpea also supports existence of 11 pairs of homologous chromosomes. The genome shed new light into the evolutionary dynamics of pigeonpea and absence of recent genome wide duplication events was speculated by the authors (Varshney et al., 2011).

Based on the variation in esterase enzyme examined in the seed extracts of *C. cajan* (T21) and six wild species, Krishna and Reddy (1982) postulated that *C. cajanifolius* is the closest wild relative of *C. cajan*. Later, Kollipara et al. (1994) investigated the electrophoretic migration patterns in trypsin and chymo-trypsins inhibitors among 69 strains of *C. cajan* and 17 accessions belonging to seven different wild *Cajanus* species. Similarly, close proximity of *C. cajan* with *C. cajanifolius* was also demonstrated by Jha and Ohri (1996) through developing seed protein profiles of different cultivated and wild accessions. Furthermore, the karyotype analysis and variation in nuclear DNA
content revealed that the karyotype including morphology and number of satellite chromosomes of *C. cajan* was the most similar to that of *C. cajanifolius* (Ohri and Singh, 2002). Several other studies, particularly the molecular marker-based diversity analyses, supported *C. cajanifolius* as the most probable progenitor sharing a similar pattern of DNA variation with *C. cajan*. Nadimplalli et al., (1993) used RFLP markers to examine the relationship between 24 diverse accessions belonging to four different genera, and they established a close relationship between *C. cajan* and *C. cajanifolius*. Higher genetic similarities between *C. cajan* and *C. cajanifolius* were also revealed by other marker systems including mitochondrial DNA-RFLP (Sivaramakrishnan et al., 2002) and genomic SSR markers (Odeny et al., 2007b; Saxena et al., 2010c). More recently, Kassa et al. (2012) performed a comprehensive diversity analysis using over 700 SNP markers across 110 accessions (79 cultivated and 31 wild), and the experimental evidences supported the hypothesis of *C. cajanifolius* being the closest wild progenitor of cultivated pigeonpea. Similarly by analyzing 1,616 SNP markers in a panel of 184 pigeonpea accessions (77 cultivated and 107 wild), a close relationship of *C. cajan* was observed with *C. cajanifolius* as compared to the other wild relatives (Saxena et al., 2014).

### 3.3 Genetic Diversity of Indian Germplasm

Pigeonpea is grown in different agroclimatic regions of India on a variety of soil and diverse physiographic situations. Wide range of variability has been documented for various botanical and agronomic traits viz. plant growth habit, plant height, days to 50% flowering, days to physiological maturity, branching pattern, number of primary branches, branching length, crop duration, photoperiod sensitivity, number of pods per plant, pod length, number of seeds per pod, flower number, flower colour, inflorescence size, pod colour and seed size, seed color, taste, pod dehiscence, seed dormancy, seedling vigour, habitat preferences and biochemical constitution.

Most of the pigeonpea genotypes are photothermo sensitive and a few behave like photoperiod insensitive. Therefore, days to 50% flowering and physiological maturity do not correspond across the North-South region. Grossly, as mentioned earlier Indian pigeonpea genotypes have been classified into distinct maturity groups i.e. early, mid-early, medium and late. Vegetable types (tall, large flower, bigger pods and seed) are distributed in the North East Hill region, Gujarat, Karnataka, Chhattisgarh and Andhra Pradesh. Genotypes with non-branching habit (single stem) have also been identified among collections from Madhya Pradesh and other parts of Central and Northern India. Large amount of variability exists for growth habit (determinate and non-determinate) and branching pattern (erect to spreading). Flower colour is predominantly yellow among the accessions of grain type, while purple flower is predominant among the vegetable types collected from Chhattisgarh and Tripura. Wide scale variation is observed for seed size and seed colour among the germplasm collections. Considerable diversity was observed in pigeonpea germplasm accessions of Bastar region (Nag and Sharma, 2012). Forty nine pigeonpea genotypes originating from different eco-geographical regions exhibited considerable genetic diversity for 12 characters (Rekha et al., 2011). Variability for SSR alleles was shown among 36 pigeonpea lines with varying degrees of resistance against Fusarium wilt (Singh et al., 2013).
3.4 Germplasm Collection at National and International Institutes

There are 13,771 accessions of pigeonpea conserved at ICRISAT (Upadahyay et al., 2007). ICRISAT has developed a composite collection of pigeonpea containing 1000 accessions representative of the diversity of all germplasm collection. Furthermore, a mini core collection comprising 146 accessions has been grouped from the core collection and other materials. In the national gene bank at National Bureau of Plant Genetic Resources (NBPGR), 10,189 accessions of Indian collections are conserved. In addition, NBPGR has also repatriated 5,748 accessions to national gene bank. The extensive use of few parents in pigeonpea improvement programmes has led to the narrowing down of genetic base of the cultivated varieties which defeats the purpose of collecting a large number of germplasm.

4. REPRODUCTIVE BIOLOGY

4.1 Reproduction

The basic floral arrangement in pigeonpea is typical of fabaceae family exhibiting terminal or auxiliary raceme inflorescence. The panicles are either terminal, as in the case of indeterminate types, or corymb-shape bunch in the determinate types. The inflorescence has a long peduncle and flowers are concentrated at the end of branches in late maturing and determinate genotypes, whereas flowers are borne along the branches in most of the early, medium and non-determinate genotypes (Sharma and Green, 1980). In general, flowering starts from the base and proceeds acropetally towards the apex both within the raceme and on the branch. In some cases the first flower appears in the middle of the flowering branch and then move, in either directions. The flowers are papileonaceous (completely bisexual and zygomorphic). Generally the stigma of a mature flower bud is surrounded by anthers which dehisce a day before the opening of flower (Fig. 5). On a bright sunny day, anthesis starts in the early morning, peaks at 9-10 AM and continues till 4 PM. The duration of flower opening varies from 6 to 36 hours depending upon the climate and environmental conditions.

4.2 Methods of Pollination, Known Pollinators and Pollen Viability

Pigeonpea possesses cleistogamous flowers which favour self pollination. However, 14-20 % natural outcrossing was observed in pigeonpea (Sharma and Green, 1980; Howard et al., 1919). Pigeonpea is often-cross pollinated through entomophily. Self-pollination occurs in the bud before the flowers open, while cross pollination is effected with the help of insects. Reddy and Mishra (1981) reported low frequency of self fertilization when flower buds were pollinated with foreign pollen without emasculation. Onim (1981a) observed that anthers dehisce during the bud stage but they start germinating 24-28 hours after dehiscence when...
flowers start to wither. Pigeonpea is protogynous and the stigma becomes receptive 68 hours before anthesis and stigma receptivity is maintained even 20 hours post anthesis (Prasad et al., 1977). Fertilization occurs within 48-54 hours after pollination (Dutta and Deb, 1970). Several factors like flowering habit of a genotype, presence of insect population, temperature, humidity, wind velocity and wind direction affect natural outcrossing at a given time and space (Bhatia et al., 1981). Most important pollinators are *Apis* spp. (*A. dorsata*, *A. laboriosa*, *A. florea* and *A. cerana*), *Megachile* spp. (*M. lanata* and *M. flavipes*) and *Xylocopa* spp. (Pathak, 1970; Williams, 1977). The rate of outcrossing varies from place to place depending on the extent of pollinator bees and climatic conditions. Outcrossing ranged from 20 to 70% at various locations (Saxena et al., 1990, 2010b). High rate of outcrossing in pigeonpea creates problems in the maintenance of varietal purity.

### 4.3 Seed Production and Dispersal

Majority of flowers drop before developing into a pod and only a small portion gives rise to mature pods (Datta and Deb, 1970; Howard et al., 1919). There is rapid development of endosperm during the first week after fertilization. Seed development is visible 7 days after pollination. Though the seed of 30 days old pod attains physiological maturity, it requires 10-15 additional days for obtaining seed with desirable moisture content. Pod shattering is the natural means for seed dissemination in wild relatives of pigeonpea and these seeds germinate in next crop season. Therefore, restriction of two years has to be made to grow pigeonpea in the same fields/plots. The pods of cultivated pigeonpea are by and large indehiscent and do not shatter at maturity. However, if the mature plants are left in the field for a long time, pods show tendency of shattering. Pigeonpea seeds do not show dormancy and can be grown immediately after harvest (Andersson and de Vicente, 2010).

### 4.4 Mating Systems

Large and bright coloured flowers coupled with the presence of nectar attract a variety of pollinators. On an average 20% out crossing has been observed due to insects in pigeonpea (Saxena et al., 1990). To harness the potential of outcrossing nature for heterosis breeding, instances of genetic male sterility (GMS) systems were reported in pigeonpea which was achieved by spontaneous mutations (Saxena et al., 2010b). GMS plants are eliminated from the population unless they outcross with male fertile genotypes. Male sterile plants induced through mutagen could not be maintained further (Dundas, 1990). Recently, cytoplasmic-genetic male sterility (CGMS) system has been established in pigeonpea based on a range of sterilizing cytoplasmic *viz.*, A1 cytoplasm derived from (*C. sericeus*), A2 cytoplasm from *C. scarabaeoides*, A3 cytoplasm from *C. volubilis*, A4 cytoplasm from *C. cajanifolius*, A5 cytoplasm from *C. cajan*, A6 cytoplasm from *C. lineatus*, A7 cytoplasm from *C. platycarpus* (Saxena et al., 2010b). Figure No. 6a shows the visible difference between the anthers of sterile and fertile flowers.

![Fig. 6a: Difference in anthers and pollen load in fertile and sterile flowers](image-url)
CGMS approach involves three lines i) A-line ii) B-line which is isogenic to A-line except for the cytoplasm and iii) R-line that should restore fertility in the F1 hybrids. Based on their specific utility in the CGMS programme, A, B and R lines are also referred to as sterile-line, maintainer-line and restorer-line, respectively. Owing to the involvement of three different lines, CGMS approach is also known as ABR system. Technically, the CGMS breeding scheme comprises of two components that is production of F1 hybrids and maintenance of parental lines. As illustrated in Figure 6b, the fertile hybrids are produced by crossing A-line with R-line i.e. ‘A × R’ progeny. Concerning maintenance of A-line, it is crossed with its isogenic line i.e. B-line. On the other hand, R and B-lines are maintained simply by selfing the individual plant.

4.5 Methods of Reproductive Isolation

Reproductive isolation is essential for the production of genetically pure seeds as pigeonpea is often cross pollinated crop and therefore. Depending on the purity standards, a minimum of 200 m (for certified seed) to 500 m (for breeder seed production) isolation distance is required for producing true to type seed of a genotype. Alternatively, entire seed production plot may be isolated using appropriate nets which is rarely feasible under normal circumstances unless it is necessary for certain specific programmes like maintenance of CMS lines in hybrid breeding. The reproductive isolation can be maintained by means of bagging the individual plant with nylon nets before flowers initiation i.e. at flower bud initiation (Saxena, 2006; Saxena and Nadarajan, 2010).

4.6 Potential of Vegetative Propagation

Pigeonpea plants can be regenerated from the vegetative tissue in the artificial medium (Chandra and Pental, 2003; Thu et al., 2003; Eapen, 2008). Like other grain legumes, seed is the key source of propagation. However, regrowth has been noticed in pigeonpea after cutting or ratooning the plants (Andersson and de Vicente, 2010).

5. HYBRIDIZATION AND INTROGRESSION

5.1 Intraspecific Crosses

Pigeonpea is an often cross pollinated crop in nature, the extent of natural open pollination goes upto 24% (Bhatia et al., 1981). The natural pollination is usually mediated through a variety of insects particularly Megachile lanata, Apis florea (Pathak, 1970), M. conjuncta and M. bicolor (Saxena and Kumar, 2010), A. dorsata (Williams, 1977) and M. velutina and Xylocopa sp. (Li et al., 2011).

5.2 Naturally Occurring Interspecific Crosses

Based on the extent of crossability or gene flow, the concept of gene pool was proposed by Harlan...
and de Wet (1971). A total of 31 species of pigeonpea are distributed across primary (only one, \textit{C. cajan}), secondary (10 species) and tertiary (20 species) gene pools (Ramanadam, 1990). Figure 7 depicts distribution of these species across different gene pools.

![Diagram illustrating the pigeonpea gene pool](https://ksiconnect.icrisat.org/wp-content/uploads/2013/03/conquering-gene-pools-pigeonpea.revised.pdf)

In regard to the natural inter-specific hybridization, several instances of out crossing leading to the development of viable inter-specific hybrids have been documented in pigeonpea (Saxena and Kumar, 2010). Saxena and Kumar (2010) examined the degree of natural out crossing in the four wild relatives from the secondary gene pool viz. \textit{C. scarabaeoides} (ICPW 89), \textit{C. albicans} (ICPW 13), \textit{C. sericeus} (ICPW 162) and \textit{C. lineatus} (ICPW 42). The percentage of natural out-crossing was calculated on the basis of the number of hybrid individuals observed in the succeeding generations. Consequently, variable levels of out-crossing were reported like \textit{C. scarabaeoides} (8.3%), \textit{C. albicans} (10%), \textit{C. sericeus} (2.3%) and \textit{C. lineatus} (17%) compared to the pigeonpea line (ICP 5529), which exhibited 22% out crossing. Notably, the low levels of out crossing observed in the wild species may be attributable to the non-preference of pollinating insects discarding the possibilities of existence of potent compatibility barriers. Several species belonging to secondary gene pool exhibit sexually compatible reactions with \textit{C. cajan} include \textit{C. cajanifolius} (Reddy et al., 1981; Pundir and Singh, 1987; Yadav and Padmaja, 2002), \textit{C. scarabaeoides} (Roy and De, 1965; Pundir and Singh, 1987), \textit{C. albicans} (Pundir and Singh, 1987), \textit{C. sericeus} (Kumar et al., 1985; Pundir and Singh, 1987; Yadav and Padmaja, 2002), \textit{C. lanceolatus} and \textit{C. latisepalus} (Kumar, 1985), \textit{C. reticulatus} and \textit{C. acutifolius} (Dundas, 1984; Reddy et al., 2001; Mallikarjuna and Saxena, 2005).

### 5.3 Experimental Interspecific Crosses

In general, the species belonging to secondary gene pool are easily crossable with the cultivated pigeonpea using the traditional hybridization methods. This in turn avoids the need for employing additional technical interventions including hormone-aided pollination and embryo rescue. However, these interventions may be required to enable recovery of greater quantities of the hybrid seeds from the interspecific cross (Mallikarjuna et al., 2011). However, reciprocal crosses involving wild species as female parent did not produce healthy embryos (Mallikarjuna and Saxena, 2002). Thiruvengadam and Muthiah (2007) generated viable hybrids from the cross \textit{C. cajan} × \textit{C. cajanifolius} only when they used \textit{C. cajanifolius} as male parents, while reciprocal crosses yielded hybrids that could not set
pods. Similarly, crosses between *C. cajan* and *C. acutifolius* provided aborted embryos when *C. acutifolius* was chosen as female parent (Mallikarjuna and Saxena, 2002). Concerning cytology, while characterizing the hybrids (*Cajanus cajan* × *C. reticulatus var. grandifolius*), it was observed that during diakinesis and metaphase-I, only 41.9% of the cell had bivalents. On the other hand, several abnormal forms including quadrivalents, trivalents, and univalents were detected to considerable extent i.e. upto 45.4% (Reddy et al., 2001).

In contrast to compatibility with secondary gene pool, regular abortion of hybrid embryos has been reported in case of experimental crosses involving parents from tertiary gene pool in particular *C. platycarpus* (Pundir and Singh, 1987). However, to assist inter-specific hybridization with *C. platycarpus*, effective techniques involving colchicine treatment and embryo rescue using in vitro culture practices have successfully been employed in pigeonpea (Mallikarjuna and Moss, 1995; Mallikarjuna, 1998; Mallikarjuna et al., 2006). By using embryo rescue technique, Mallikarjuna (2007) successfully generated several BC4F1 plants from the cross (*C. platycarpus* × *C. cajan*) × *C. cajan*. Alternatively, to escape the cumbersome embryo rescue practises, colchicine treatment of the derived F1 (*C. platycarpus* × *C. cajan*) plants was suggested by Mallikarjuna et al. (2011), who developed tetraploid plants showing enormous vegetative growth. However, variations in ploidy levels did not permit further back crossing of these tetraploid individuals (4x) with the cultivated *C. cajan* (2x). The occurrence of post-zygotic barriers has been proposed as the underlying reason for the incompatible response mentioned above (Mallikarjuna and Moss, 1995). However, while investigating inter-specific hybridization using another species from tertiary gene pool (*C. volubilis* × *C. cajan*) existence of pre-fertilization barriers was also reported (Jayaprakash and Sarla, 2001) and application of ε-amino caproic acid (EACA - an amino acid) to the pollen germination medium was suggested as it resulted in better pollen germination. To address the issue of early embryo abortion, application of gibberellic acid (GA$_3$) to the pollinated pistils has been advocated to allow recovery of mature embryos that are usually more responsive to embryo rescue technique (Mallikarjuna et al., 2011). In addition, application of GA$_3$ or kinetin is also known to enhance the rate of pod set and number of seeds per pod during inter-specific hybridization (Kumar et al., 1985; Dhanju and Gill, 1985).

### 5.4 Genetic Introgression

Wild relatives of pigeonpea are known to contain several agriculturally important genes that impart tolerance to a wide range of biotic and abiotic stresses such as sterility mosaic disease (SMD), phytophthora blight, root-knot nematode, pod borer, pod fly, salinity and drought etc. (Rao et al., 2003; Bohra et al., 2010). In this context, distant hybridization emerges as a viable option not only to incorporate beneficial exotic alleles of desired traits into cultivated-types, but also for broadening the extremely narrow genetic base of pigeonpea. In terms of trait-introgression, inter-specific hybridization led to the recovery of remarkably distinct phenotypes in pigeonpea. For example, some of the derivatives of the wild cross (*C. cajan* × *C. scarabaeoides*) exhibited enhanced level of protein content i.e. more than 27% (Reddy et al., 1997). Besides higher protein content, Reddy (1990) obtained a valuable segregant with different plant architecture (dwarf
stature, designated as D0) from an inter-specific cross involving C. cajan and C. scarabaeoides as parents. Similarly, progenies with partially cleistogamous flowers showing very low level of cross-pollination were also recovered from another inter-specific cross i.e. C. cajan × C. lineatus (Saxena et al., 1998).

6. HUMAN HEALTH IMPLICATIONS

Pigeonpea seeds are known to contain 18-26 % protein and in case of wild Cajanus species up to 30% protein content has been observed (Odeny, 2007a). The nutritional value of pigeonpea is evident from the information provided in Table 1. Additionally, Saxena et al. (2010a) have enlisted a range of major anti-nutritional factors which are reported in pigeonpea. These anti-nutritional factors include phenolic compounds, tannins, phyt lectins, oligosaccarides like raffinose and stachyose and a variety of inhibitors that negatively affect the digestive enzymes including trypsin, chymotrypsin and amylase (Singh 1988). Compounds like phyt lectins are heat-labile hence get destroyed easily while cooking (Saxena et al., 2010a). Concerning its therapeutic uses, consumption of immature pigeonpea seeds has been considered to have ameliorating effects in case of kidney related disorders. Similarly, leaf extracts from pigeonpea are reported to have noticeable impact in curing diverse diseases such as malaria, diabetes, dysentery and hepatitis (Oke, 2014). The flavonoids found in pigeonpea leaves have important pharmacological properties such as anti-cancer and anti-inflammatory (Saxena et al., 2010a). Similarly, the anti-cancer properties of cajanol (an isoflavanone isolated from pigeonpea roots) were also demonstrated in vitro against human breast cancer cells by Luo et al. (2010). Experimental evidences showing the hypocholesterolemic effect of stilbenes-containing extracts from pigeonpea have also been furnished by Luo et al. (2008). The wide ranging biological activities and medicinal properties of diverse chemical compounds obtained from pigeonpea were reviewed recently by Pal et al. (2011).

7. KNOWN INTERACTIONS WITH OTHER ORGANISMS IN MANAGED AND UNMANAGED ECOSYSTEMS

7.1 Interactions in Unmanaged and Managed Ecosystems

Pigeonpea prefers grassy habitats in tropical and sub-tropical cold free zones with an optimum annual rain fall of 600-1000 mm. It can also grow in open areas in the forests, hilly slopes and degraded lands under natural habitat. Wild pigeonpea colonizes the drained, sunny and open spaces in forests and creeping types climb on the trees to get light. However, pigeonpea has not been known to grow as naturalized population, hence its interaction in the unmanaged ecosystem is uncertain. Stabilizing yield in pigeonpea is a major concern as its production is very much affected by several biotic and abiotic factors (Varshney et al., 2013). Pigeonpea is grown as a rainfed crop
and precipitation affects this crop differentially in different regions depending on the soil type, slope of land and natural drainage. In the absence of proper rains the crop may fail or its yield will be severely affected; excess rain is also very harmful to this crop in the early growth stages (up to 60 days). In the poorly drained fields heavy rain immediately after sowing may lead to heavy loss of plant stand which subsequently will affect the yield. Waterlogging during initial growth stage, low temperature during flowering stage, high temperature during pod formation and drought are impediments in realizing the productivity potential of pigeonpea cultivars. Although pigeonpea has deep roots, yield losses due to these stresses are large and widespread, especially when they occur during critical seedling and reproductive stages.

7.2 Important Insect Pests, Nature of Damage and their control in Managed Ecosystems

7.2.1 Major Insect Pests

A variety of insect species (over 200) affects pigeonpea plant and seeds, and feeds on roots, shoots, flowers, and seeds. However, majority of these insects are sporadic in their distribution and do not cause economic damage and therefore, may not be regarded as pests. In general, pigeonpea can tolerate foliage damage during vegetative growth and need no chemical control. The damage caused to reproductive parts is difficult to compensate in short duration varieties, while in long duration cultivars recovery from damage is slow and dependent on the plant type, soil moisture and climatic conditions.

The devastating insect pests which attack pigeonpea at the reproductive phase (flower buds, flowers, pods, and seeds) are pod borer and pod fly. During storage, bruchids are the most dangerous. The insect pests of pigeonpea are briefly described here:

(i) *Helicoverpa pod borer* (*Helicoverpa armigera*): This insect pest is also known as gram pod borer (Fig 8). The larvae damage the pods or flower buds and feed on leaves as well. The typical circular hole on pods can be observed through which the seeds are damaged by the pod borer.

(ii) *Maruca pod borer* (*Maruca vitrata*): It is an insect pest causing huge damage especially to short duration pigeonpea in different parts of the country. Usually the larvae of this insect web tender leaves along with buds, flowers and immature pods and feed on them, thus causing substantial economic damage (Fig 9).

(iii) *Pod Fly* (*Melanagromyza obtusa*): It is considered to be one of the dreaded pests of pigeonpea. The maggot of the insect feeds on the developing grain (Fig 10). The infested pods do not show any external evidence of damage until these maggots are fully grow and larvae make holes in the pod walls. The maggots bore the grains and make tunnels in them. This hole provides an emergence “window” through which the adults exit the pod.
Damaged grains or seeds do not mature and fungus can be seen on excreta inside pods. The infested seeds or grains lose their viability and do no germinate.

(iii) **Blister beetle** (*Zonabris pustulata*): This pest feeds voraciously on the flowers of pigeonpea and greatly reduces the pod setting (Fig 11). This insect can cause huge damage in case of favourable conditions i.e. high humidity and mild temperature. It is a serious pest in short duration varieties.

(v) **Pigeonpea plume moth** (*Exelastis atomosa* Wals.): The larvae damage seeds as well as cause flowers, buds and pods to drop. Small spiny greenish brown caterpillar and pupae can be seen on the pods. It also enters into pod and feeds on developing grains resulting in reduced yields.

(vi) **Pigeonpea blue butterfly** (*Euchrysops cneius, Lampides boeticus and Catochrysops strabo*): The larvae feed on flowers, seeds and pods of pigeonpea. Specific control for these insects is rarely required but the general management recommendations for *H. armigera* may be used here.

(vii) **Pigeonpea pod sucking bug** (*Clavigralla gibbosa*): The adults and nymphs insect, of this use their piercing mouthparts to penetrate the pod wall and suck the liquid from developing seeds. Damaged seeds become shriveled, and develop dark patches. Seeds spoiled by pod sucking bugs neither germinate nor are acceptable as human food.

(viii) **Red spider mite** (*Tetranychus spp.*): Spider mites cause yellow or white spots on the upper surface of the infested leaflets. Heavy infestation results in bronzing of the leaves followed by defoliation.

(ix) **Eriophyid mite** (*Aceria cajani*): The eriphyid mite, *A. cajani* is the vector of the pigeonpea sterility mosaic disease (SMD), the most serious viral disease of this crop. Plants infected with sterility mosaic disease develop light green, chlorotic foliage. The early infection results in reproductively sterile plants.

7.2.2 Major Nematodes

Nematodes are widely distributed in most of the pigeonpea growing regions in India. Pigeonpea is vulnerable to many plant parasitic nematodes *viz.*, *Meloidogyne* spp., *Heterodera cajani*, *Helicotylenchus* spp., *Hoplolaimus* spp., *Rotylenchus* spp. and *Tylenchorhynchus* spp. Root Knot Nematode (*M. javanica*) and pigeonpea cyst nematode (*Heterodera cajani*) are the most important parasites.

The lower larval population of *H. armigera*, *E. atomosa* and *L. boeticus* as well as their damage were recorded, when crop was treated with indoxacarb (0.007%), which was at par with spinosad (0.009%), fenvalerate (0.01%) and monocrotophos (0.36%) (Ghetiya and Mehta, 2011).

7.2.3 Integrated Pest Management Practices in Pigeonpea

1. Deep ploughing during summer to expose the
hibernating pupae of pod borers to adverse weather conditions and natural enemies.

2. Mixing of sorghum/maize seeds (250-500 g/ha) to function as live bird perches. These plants also help in conserving natural enemies.

3. Installation of pheromone traps @ 40/ha and replace helilure at an interval of 21 days as per need.

4. Application of HaNPV @ 250 LE/ha protects the crop from H. armigera.

5. Spraying of monocrotophos 36 SL @ 500 ml/ha is effective against pod fly and pod borers.

Since pigeonpea is ravaged by an array of pest species, the use of specific bio-pesticides that reduce the population of one or two key insects will not control the pest problem completely. Therefore, the role of synthetic insecticides in suppressing pest complex infesting pigeonpea will continue to play an important role. However, integrated pest management involving tolerant varieties, monitoring through pheromone traps, need based use of insecticides and crop rotations and other agronomic manipulations need to be adopted to control damages of pests.

7.3 Important Diseases, Causal agents and their Control in Managed Ecosystems

(i) **Fusarium wilt** *(Fusarium udum Butler)*

*Fusarium* wilt is the most important and widespread fungal disease in all pigeonpea growing areas (Pande et al., 2013). The primary source of inoculum is soil. Infected seeds can also transmit the disease. Symptoms like drooping and subsequent drying of the plants can be observed in field conditions (Fig 12). The stem of infected plant when cut vertically, show black lines which indicate infection of *Fusarium* wilt.

(ii) **Sterility mosaic disease** *(Pigeonpea sterility mosaic virus)*

SMD causes stunting, small yellowish green leaves, bushy habit and complete cessation of growth of reproductive structures. The degree of sterility due to the suppression of flowers and fruits may vary in different pigeonpea cultivars (Sharma et al., 2015). The disease has now spread to different pigeonpea growing areas particularly in Bihar, Karnataka, Tamil Nadu, Gujarat and Maharashtra. Management of the disease through cultural practices is not well studied.

(iii) **Phytophthora blight** *(Phytophthora drechsleri Tucker f. sp. Cajani)*

In addition to *fusarium* wilt and SMD, *phytophthora* blight is another important disease of pigeonpea (Pande et al. 2011). It is a foliar disease affecting both leaves and stems (Fig 13). The disease can appear as soon as the pigeonpea seedlings emerge and can go unnoticed as the small germinated seedlings collapse on the ground with “damping-off” type of symptoms. The disease in the field is usually recognised when the seedlings are about two weeks old. Water soaked lesions appear on the leaves or breaking of stem with broken upper part of the plant still attached to the basal portion of the stem. With close observation, brown, shrunked lesions can be seen on the above ground part of the
stem even before the breaking of the stem occurs. In the grown-up plants, development of cankerous out growths (gall) on the edges of the stem lesions is common. The disease usually appears in the lowlying areas of the field and in waterpaths as it is promoted by higher humidity. The mortality can be reduced by sowing pigeonpea on ridges permitting less splashes of rains as well as better drainage.

8. AGRONOMIC PRACTICES

8.1 Climate

Various cultivars of pigeonpea are grown from sea level up to 3,000 m altitude (Van Den Beldt, 1988). Reddy and Virmani (1981) reported that pigeonpea can be grown between 14°N and 28°N latitude, with a temperature ranging from 26° to 30°C in the rainy season (June - October) and 17° - 22°C in the post rainy (November - March) season. Pigeonpea is very sensitive to low radiation at flowering and pod development. Therefore, flowering during the monsoon and cloudy weather lead to flower/bud drop and poor pod formation. Pigeonpea is comparatively tolerant to drought and high temperatures as compared to other pulse crops. Although the plant can survive in very dry conditions, it has been observed that seed yield is minimal under these conditions. Pigeonpea is not suitable for heavy rainfall areas unless there is proper drainage system. Pigeonpea can survive at low temperature to a certain degree but it is highly sensitive to frost damage which causes burn injury and leads to heavy defoliation followed by drastic delay in reproductive phase resulting in negligible pod set.

8.2 Sowing Season

Pigeonpea varieties are mainly categorised in three maturity groups (short, medium and long duration), and planting is usually done with the onset of the monsoon. Early sowing of short duration varieties in the first fortnight of June with irrigation or pre-monsoon rains gives higher seed yield. Delayed sowings result in progressive reductions in yield due to early flowering and slow growth. Late sowings of short duration varieties may result in poor pod and grain development, due to onset of cold season at the pod-filling stage. Sowing in first fortnight of June also ensures availability of field for post rainy season crops by the end of November. Therefore, sowing should not be delayed beyond June for early maturing varieties. For medium duration varieties of central and southern zones, sowing at the onset of rains (15th June - 15th July) ensures sufficient vegetative growth before flowering. The long duration varieties should be planted between second and last week of July in the North East plains. Late sowings produce less vegetative growth and may expose the crop to terminal drought and heat stress. September is the optimum time for post-rainy season sowing in peninsular India. Delay in sowing affects the vegetative growth and exposes the crop to high temperature during the reproductive phase. The recommended crop management practices may be followed to raise a good crop.

8.3 Seed Production

Seed is the most important input of the crop
production. Seed may be defined as the propagating material comprising any part of the plant that grows into a new plant and makes a link between previous generation and present generation. Seed production is the art of producing genetically pure plant propagules in large quantity with the aid of science and technology. It is important that seed of a new and superior variety should be multiplied and made available in adequate quantities as soon as possible so as to benefit the farming community. Seed of released varieties must be maintained and kept ready for pushing them into the commercial seed chain. Seed production is carried out under standardized and well organized conditions. During seed production strict attention is given to maintain the genetic purity and other qualities of the seed. Genetic purity of seed is maintained by regular rouging of off-type plants. For the purpose of identifying off-types, the nodal person involved in seed production must possess the list of diagnostic traits of the variety. Availability of quality seed of improved varieties has been a major constraint. Package of practices for raising a seed crop remains same for pure line or hybrid seed production. The crop management practices for raising a good crop are given in Table 5.

Table 5. Crop management practices for raising a good crop

<table>
<thead>
<tr>
<th>Crop management</th>
<th>Seed treatment</th>
<th>Soil treatment</th>
<th>Fertilizers</th>
<th>Weeds</th>
<th>Disease management</th>
<th>Sterility mosaic disease</th>
<th>Fusarium wilt</th>
<th>Alternaria blight</th>
<th>Phytophthora blight</th>
<th>Pod borer and pod fly control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed treatment</td>
<td>Seed treatment with carbendazim 50 WP) 2g + thiride 75 WP) 2g + Metalaxyl-M 45.3%) 2g per Kg seed</td>
<td>Trichoderma viride @ 2.5 kg/ha+ Farm Yard Mannure + neem cake @ 5 q/ha Carbofuran @ 1Kg ai/ha</td>
<td>Basal application 20 kg N + 40-60 kg P2O5+ 20Kg K2O + 10 Kg ZnSO4 in 1 ha area</td>
<td>Pre-emergence application of pendimethalin @ 1.5 kg/ha Two hand weedings at 30 and 60 DAS</td>
<td>1. Deep ploughing and exposing field to sun during hot summer months 2. Field sanitation, removal of all stubbles and plant remains 3. Ridge sowing 4. Destruction of off-season volunteer plants from the vicinity of seed plot</td>
<td>Preventive measure of acaricide Dicofol 18.5% EC @ 2.5 ml per liter water</td>
<td>See treatment using fungicide Carbendazim @2.5 g/kg of seed/Trichoderma harzianum @6-10g/kg</td>
<td>In the event of disease, foliar spray of mancozeb @ 0.2%</td>
<td>As a preventive measure: foliar spraying of Metalaxyl-M 45.3% @ 3g/l at 30 and 45 days after sowing.</td>
<td>At podding stage, 2-3 foliar sprays of Spinosad 45SC @ 0.4-0.5 ml/liter at an interval of 15 days</td>
</tr>
</tbody>
</table>
9. BREEDING OBJECTIVES

9.1 Milestones in Breeding

In the pre independence time all cultivated pigeonpea genotypes were landraces or local types which were either long duration type (>180 days) in the Northern plains or medium late in the central and southern zone. With the creation of AICPIP, pigeonpea breeding efforts were initiated simultaneously at 31 locations in different agroclimatic zones of the country for developing early, medium and long duration varieties suitable for different zones (Ramanujam and Singh, 1981). Multilocation testing of genotypes for yield and biotic stresses paved the way for the identification of more stable varieties. Varieties were bred either through hybridization followed by pure line selection or by selecting desirable plants from the heterogeneous germplasm followed by pure line selection. During this time a need for reducing the crop duration was felt. UPAS120 was the first high yielding short duration (120-140 days) crop released in 1976. ICRISAT has played an important role in the introduction of medium and short duration varieties for the Central and Southern zone. About 40% of the varieties bred so far have evolved through selections made from landraces or heterogeneous population or spontaneous mutations. Vishakha 1 (TT 6) was the first variety developed through mutation breeding by BARC. Subsequently, three more varieties were developed through mutation breeding. Inspite of long and continued breeding efforts, the average productivity of pigeonpea has not increased significantly in the last five decades.

9.2 Heterosis Breeding in Pigeonpea

Existence of high natural out crossing and identification of genetic male sterility offered new avenues for exploiting heterosis leading to the initiation of ICAR sponsored programme on “Promotion of Hybrids in Selected Crops” in 1989. Heterosis breeding was tried for breaking the yield barrier through exploitation of hybrid vigour. Initially genetic male sterility (GMS) was utilised for the development of hybrids and, as a result an early maturing hybrid ICPH 8, which showed more than 40 % superiority over the best check UPAS 120, was released for commercial cultivation (Saxena et al., 1992). Subsequently, 5 more GMS based hybrids were released for general cultivation. Though ICPH 8 and other hybrids had substantial yield advantage, they could not survive due to difficulties in seed production associated with GMS system that led to very high cost of hybrid seed (Saxena et al., 2010b). In order to overcome the lacuna of GMS, cytoplasmic genetic male sterility (CGMS) system was adopted for the production of hybrid seed. The hybrid programme was further strengthened through the National Agricultural Technology Project (NATP) in 1998 and the Integrated Scheme on Oilseeds, Pulses, Oilpalm and Maize (ISOPOM) in 2005. In 2004, the first CMS based medium duration hybrid GTH 1 was released in India (Varshney et al., 2010). Subsequently, using A4 cytoplasm a medium duration hybrid RVICPH 2671 was released in Madhya Pradesh.

9.3 Major Traits of Interest and Priorities in Breeding

The important agronomic and yield attributing traits of pigeonpea for enhancing productivity are: days to 50% flowering, days to 75% maturity, plant height, plant type, branching pattern, number of
pods per plant, number of seeds per pod, pod length, number of primary branches, number of secondary branches, branching length, 100 seed weight, seed yield per plant and seed yield per plot (Saxena, 2008). Tolerance against biotic and abiotic stresses is an important parameter for selection. The objectives of the breeding programme depend on the local needs of the cultivators, prevailing cropping system, climatic condition and constraints of production. Though breeding objectives vary in different agroclimatic zones as per the local needs, varieties are generally bred keeping in view appropriate maturity and enhanced harvest index through manipulating plant architecture for different cropping situations. Attention is also given to induce photo insensitivity, enhancing yield and ensure stability of the released varieties by incorporating resistance against *Fusarium* wilt, SMD and *Phytophthora* blight and tolerance against abiotic stresses like drought, heat and cold.

Identification of potential donors and incorporation of disease resistance into newly developed cultivars remains the most sustainable, economically attractive and ecofriendly strategy to reduce yield losses in crop plants. With the aim of identifying the *Fusarium* wilt resistant sources, multi-location and multi-year screening of genotypes led to the identification of promising wilt resistance genotypes in pigeonpea like IPA 16 F, IPA 8 F, IPA 9 F and IPA 12 F (Singh et al., 2011). Several varieties have been developed in pigeonpea like ICP 8863, ICPL 87119, BDN 1, BDN 2, BSMR 736, IPA 203 which are known to exhibit resistance against *Fusarium* wilt (Choudhary et al., 2013). Similarly, Kulkarni et al. (2003) reported some *C. scarabaeoides* accessions, which were found to be resistant to pigeonpea sterility mosaic virus. Among the pigeonpea lines commercially available for cultivation, popular varieties like ICPL 87119, BSMR 736, BSMR 853, ICP 7035 also show notable levels of resistance against SMD. Recently, screening of pigeonpea core collection comprising 146 accessions have revealed a set of 24 accessions that have promising levels of resistance to SMD. More important, five accessions exhibited marked resistance not only to SMD but also against *Fusarium* wilt (Sharma et al., 2012). In case of *Phytophthora* blight, only a limited number of promising lines like KPBR 80 and ICP 9252 have been reported so far, which show resistance against specific races (Saxena, 2005).

### 9.4 Advancements and Challenges

Cleistogamy favours self pollination in pigeonpea but it exhibits a range of natural out crossing rate due to insect aided pollination (Saxena and Kumar, 2010). There are no reports of inbreeding depression and this crop exhibits commercially exploitable level of heterosis which is why major breeding methodologies so far adopted are based on exploiting additive genetic variance. Considerable advancement has been made in extracting F1 heterosis through the development of hybrids in short and medium duration group. Pure line breeding has been the main strategy for genetic enhancement, but population improvement has also been tried in pigeonpea (Khan, 1973; Onim, 1981b). It was suggested that out-crossing potential of pigeonpea should be utilized in the formation of random mating composites which will serve as the dynamic reservoir of variability and can also be used as base populations for studies in natural selection, mass selection and recurrent selection. Stratified mass selection and mass selection with progeny testing were used for yield improvement but very little response was observed (Onim, 1981b). Pigeonpea has shown substantial amount of non-additive genetic variance and
hybrid vigour for yield. The discovery of stable genetic male sterility coupled with its out crossing nature, has opened the possibility of commercial utilization of the heterosis in pigeonpea (Saxena et al., 2010b). The hybrid technology, based on cytoplasmic nuclear male sterility system, has given an opportunity for achieving the long-cherished goal of breaking yield barrier in pigeonpea (Saxena and Nadarajan, 2010). The short and medium-duration types and disease resistant cultivars have made a significant impact on increasing area under pigeonpea cultivation.

In the national agricultural research system (NARS), genetic improvement in pigeonpea has been brought primarily through pure line breeding. Population improvement programmes, tried elsewhere, have not been successful. After long continued efforts of heterosis breeding, hybrids are now a reality in pigeonpea. Recently, CMS based (three line system; ‘A’, ‘B’ and ‘R’) hybrids have been released for cultivation. The ‘B’, ‘R’ and the released varieties of pigeonpea are purelines and, hence, their maintenance and seed production techniques are same. However, specialized techniques are employed to maintain ‘A’ line (CMS line) and to produce F1 hybrid seed. Several biotic factors severely constrain the productivity of pigeonpea (Varshney et al., 2010, 2013). The losses caused by diseases can be effectively controlled through host-plant resistance breeding (Sharma et al., 2012). The major challenges ahead are to enhance genetic yield potential and manage damages are being caused by the gram pod borer and other insects and some of the diseases where high level of genetic resistance is not available within the pigeonpea germplasm (cultivated and wild ones).

10. BIOTECHNOLOGICAL INTERVENTIONS IN PIGEONPEA

10.1 Transgenic Approaches for Genetic Improvement

Transgenic technology offers a means to introgressing traits that are not amenable to transfer using conventional breeding techniques or adequate variability is not available in the exploitable gene pool. However, poor transformation and regeneration capacities along with differential response of genotypes to regeneration protocols offer the major impediments to development of transgenic pigeonpea (Geetha et al. 1999; Lawrence and Koundal, 2001; Rao et al., 2008; Eapen, 2008). Differential reactions to various A. tumefaciens strains were also elucidated (Surekha et al., 2007; Chandra and Pental, 2003). Concerning gene transfer, various methods like Agrobacterium-mediated, biolistic and electroporation have been developed to facilitate foreign DNA transfer in plants (Eapen, 2008).

Among the methods mentioned above, Agrobacterium mediated particularly using A. tumefaciens strain ‘LBA4404’ has been widely employed in pigeonpea (Geetha et al., 1999, Surekha et al., 2007). In contrast, Dayal et al. (2003) developed a reliable regeneration protocol using leaves as explants and micro-particle bombardment method for DNA transfer. Thu et al. (2003) achieved transformation with both A. tumefaciens mediated and micro-particle bombardment-driven gene transfer. Lawrence and
Koundal (2001) transformed pigeon pea plant with CaMV 35S promoter-led cowpea protease inhibitor gene. Similarly, Kumar et al. (2004) used hpt and rice chitinase genes in pigeon pea transformation. To generate pest-resistant pigeon pea. Sharma et al. (2006) demonstrated the successful integration of the Bt cry1Ab gene. Successful regeneration has been obtained in pigeon pea using both ways viz. organogenesis and somatic embryogenesis (Patel et al., 1994; George and Eapen, 1994). In addition, direct regeneration as well as callus induction have also been reported in pigeon pea (Lawrence and Koundal, 2001; Kumar et al., 1983; Chandra and Pental, 2003). Broad range of explants including cotyledo- nodary nodes, shoot apices, decapitated embryonic axis, leaf etc. has been used for in vitro regeneration of transformed plants in pigeon pea (Eapen, 2008; Chandra and Pental, 2003). As an alternative to escape the labour intensive and low throughput in vitro regeneration, Rao et al. (2008) developed in planta transformation system for pigeon pea. In this investigation, a total of 48 plants generated PCR products for both gus (uidA) and npt II genes, and the integration and transmission were confirmed by Southern blot analysis. However, even after using all above mentioned techniques, there is no transgenic cultivar available as of now for cultivation possessing abiotic or biotic stress resistance in pigeon pea.

10.2 Genomics and Molecular Breeding in Pigeonpea

Availability of genomic resources like robust markers/quantitative trait loci (QTLs) sets a prerequisite for undertaking molecular breeding. In pigeon pea, handful of SSRs was available in public domain till 2010. However, significant research investments have been made during the last five years and due to which tremendous genomic resources have been generated. As a result, several mapping populations segregating for agronomic traits including SMD and FW resistance and fertility restoration were developed (Varshney et al., 2010). To this end, Bohra et al. (2011) reported high throughput development of SSR markers via mining bacterial artificial chromosome (BAC)-end sequences (Bohra et al., 2011). The 3,072 BES-SSR markers were obtained, which were subsequently used in various genomics application like genetic linkage mapping and QTL analysis.

The first SSR based genetic map was reported for an inter-specific mapping population (Bohra et al., 2011). Later, a series of SSR based genetic maps were constructed for different intra-specific F2 populations. A number of SSR markers were successfully mapped ranging from 59 (ICPB 2049 × ICPL 99050) to 140 (ICPA 2043 × ICPR 3467) spanning 586 and 881.6 cM, respectively. In parallel, these mapping populations were used for discovery of the gene(s)/QTL(s) governing the traits of interest (Bohra et al., 2012). These QTLs explained substantial amount of the phenotypic variation for the trait under investigation. QTLs restoring fertility accounted to 24% of the variation from an F2 population ICPA 2043 × ICPR 3467. Similarly, six QTLs linked with the SMD resistance were also discovered from two different populations viz. ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035 (Gnanesh et al., 2011). In addition to SSRs, other markers like random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) were also found to be associated with Fusarium wilt, Plant type and SMD resistance using bulked segregants analysis (BSA) (Dhanasekar et al., 2010; Ganapathy et al., 2009; Kotresh et al., 2006). Recently, Kumawat et al. (2012) detected QTLs for plant type and earliness in an F2 population viz Pusa Dwarf × HDM04-1.
A popular pigeonpea cultivar ‘Asha (ICPL 87119)’ was chosen for sequencing the whole genome by using NGS platforms including 454 GS-FLX (Singh et al., 2011) and Hiseq 2000 Sequencing Systems (Varshney et al., 2012). Approximately, 72% (606 Mb) and 61% (~511 Mb) of the pigeonpea genome was successfully assembled in the two separate sequencing attempts by Varshney et al. (2012) and Singh et al., (2011), respectively. A total of 48,680 protein coding genes were predicted in the genome and 51.67% of the genome was represented by repetitive elements (Varshney et al., 2012). Similar number of protein coding genes (47,004) was reported by Singh et al. (2011). The number of protein coding genes was similar to those observed in the genomes of other related legumes species as well as rice and Arabidopsis. Furthermore, in silico mining of the whole genome sequence permitted access to large-scale DNA markers especially SSRs and SNPs (Varshney et al., 2012). A total of 309,052 SSRs were detected across the genome, of which primer pairs were successfully designed for 23,410 SSRs. In parallel, analysis of the transcript reads from 12 different pigeonpea genotypes resulted in identification of functionally-relevant SNP markers. As a result, 28,104 novel SNPs were added to the marker repertoire of pigeonpea. The genotypes involved in SNP identification represented parents of different mapping population that segregate for various important traits (Varshney et al., 2012). Cost effective 116 KASPar assays for pigeonpea were also developed recently to study the level of genetic variability and for other applications (Saxena et al., 2014). Likewise, after experimental validation, Singh et al. (2011) provided a set of SSR markers comprising 437 markers and designated these markers as hypervariable ‘Arhar’ simple sequence repeat (HASSR) markers. Certainly, all these predictive molecular markers will be of great help to pigeonpea breeders for accelerating the progress of crop improvement. In addition to pigeonpea genome sequencing, mitochondrial genomes of four Cajanus genotypes : the CMS line ICPA 2039, its cognate maintainer line ICPB 2039, the hybrid line ICPH 2433 and the wild relative ICPW 29 (accession from C. cajanifolius), source of A4 cytoplasm were also sequenced (Tuteja et al., 2013).
11. REFERENCES


Ohri D, Singh SP (2002) Karyotypic and genome size variation in Cajanus cajan (L.) Millsp. (Pigeonpea) and some wild relatives. Genetic Resources and Crop Evolution 49:1–10


Pundir RPS and Singh RB (1985) Cytogenetics of F1 hybrids between \textit{Cajanus} and \textit{Atylosia} species and its phylogenetic implications. Theoretical and Applied Genetics 71:216–220


Roy A and De DN (1965) Inter-generic hybridization of *Cajanus* and *Atylosia*. Science and Culture 31: 93.


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