
Germplasm Characterization and Trait Discovery

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Abstract

Diverse germplasm is essential to breeding new pigeonpea varieties resilient against both abiotic and biotic challenges. In this chapter, we review the major abiotic and biotic challenges faced by pigeonpea, and trait assessment tools available to the breeding community to address these challenges. We place particular attention on drought tolerance, due to its widespread nature and large effect on yield. We emphasize the utility of wild material to expand the range of available genetic variation; as such material is generally underutilized in breeding programs. A complete genome brings great potential to the pigeonpea breeding community; to fulfill the promise of a genome for breeding for climate resilience, the full range of diversity available must be brought to bear. We hope that conditions in the future allow wild germplasm collections to expand, further capitalizing on the potential to increase pigeonpea yields and resilience against climate change.

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7.1 Introduction

Since the advent of agriculture over 10,000 years ago, the domestication of plants by artificial selection and directed breeding has dramatically increased crop yields and altered plant pheno-

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types such as flowering time, growth form and size, biotic stress responses (e.g., herbivore and disease tolerance), and abiotic stress responses (e.g., heat, drought, salinity tolerance) (Tang et al. 2010; Brown et al. 2009; Purugganan and Fuller 2009; Vaughan et al. 2007). However, the selection of favorable alleles from a population (i.e., artificial selection) can have additional consequences by reducing genetic diversity, reducing adaptive capacity, and maintaining the presence of deleterious alleles (Olsen and Wendel 2013). Crop cultivars, therefore, face genetic constraints that increase vulnerability to variable environments including disease, pests, and abiotic conditions, which impose obstacles to breeding programs tasked to feed a growing population.

To combat the challenges of domestication and substantial reductions of genetic variation within crop lines, the use of diverse germplasm including landraces and wild relatives of crops has proven crucial to crop breeding programs by making additional genetic material available for introgression into crop cultivars and has led to considerable improvement of disease and pest resistance for more than half a century (Vavilov 1922; Hajjar and Hodgkin 2007). While some crop species have substantial germplasm collections of wild relatives, only 2–6% of all international germplasm collections are from crop wild relatives (Maxted and Kell 2009). The lack of sufficient genetic resources poses a significant challenge to breeders and agricultural scientists who are tasked to not only increase crop productivity to feed a growing global population but also accomplish this task in the face of a changing global climate that threatens to exacerbate increasing rates of famine, nutrition related health issues, and threats to conservation of biodiversity. The genetic reservoirs that wild relatives offer are under threat of extinction from habitat fragmentation, and habitat loss (Ford-Lloyd et al. 2011). Climate change further threatens all plant populations and makes abiotic stress tolerance a major target for crop improvement.

Many of the most globally important crops have benefitted from collections of wild relative

germplasm. Yet many legumes, particularly those primarily grown in tropical regions, have received less attention. One of these tropical legumes, pigeonpea, *Cajanus cajan*, is the only cultivated member of the genus (Kassa et al. 2016), while the remaining wild relatives belong to the secondary or tertiary levels based on the gene pool concept of Harlan and De wet (1971). In pigeonpea, domestication is somewhat incomplete, as indehiscent seeds remain a breeding challenge. With a better understanding of evolutionary relationships in *Cajanus* and new wild relative collections, strategies can be developed to systematically sample the variation in wild relatives and utilize that variation in breeding programs (Warschefsky et al. 2014). For example, species in the tertiary pool of *Cajanus* have a wide eco-geographical range in Australia and remain an untapped reserve of adaptive potential (Varshney et al. 2011). Moreover, pigeonpea is locally adapted to a wide range of edaphic and climatic conditions around the world, presenting diverse adaptations that could be incorporated into germplasm collections. In this chapter, we focus specifically on pigeonpea (*Cajanus cajan*) and promote an integrative approach to develop agroecology programs capable of feeding an exploding human population in a changing global climate, by (1) considering evolutionary insight and population genetic theory to guide germplasm collection, (2) using physiological tools to understand plant responses to biotic and abiotic stresses, and (3) employ modern molecular tools for phenotyping and trait selection. We intend to review current literature and promote the integration of cross-disciplinary approaches from physiology, population genetics, ecology, and genomics.

7.1.1 Pigeonpea and Its Wild Relatives

Pigeonpea is a short-lived perennial shrub with relatively high levels of abiotic and biotic stress resistance. Pigeonpea is a short-lived perennial belonging to the Leguminosae genus *Cajanus*

which is composed of 34 species. Pigeonpea is the only cultivated member of the genus, while the remaining wild relatives were placed in the secondary or tertiary gene pools according to the gene pool concept of Harlan and de Wet (1971). Species in the secondary gene pool comprises the putative progenitor of Pigeonpea, *Cajanus cajanifolius* and others such as *Cajanus albicans*, *C. lineatus*, *C. scarabaeoides*, *C. sericeus*, *C. acutifolius*, *C. confertiflorum*, *C. lanceolatus*, *C. reticulatus*, and *C. trinervius* (Mallikarjuna et al. 2011). Species assigned to the tertiary gene pool includes *Cajanus platycarpus*, *C. mollis*, and *C. crassus* (Upadhyaya et al. 2013).

In terms of geographical distribution, most of the species of the genus *Cajanus* are endemic to either southern/southeastern Asia or northern Australia (Fortunato 2000). Among these, 16 *Cajanus* species occur in Asia (8 of which are endemic to India), 15 species in Australia (of which 13 are endemic), one species of *Cajanus* is confined to West Africa, and 2 species (including *Cajanus cajan*) are ubiquitous throughout the old world.

Using morphological and ecological characters such as habit, leaf structure, hairiness, pod size, strophiole characters and other traits, van der Maesen (1986) grouped the genus *Cajanus* into six sections vis-à-vis *Cajanus* (2 species), *Atylosia* (7 species), *Fruticosa* (9 species), *Cantharospermum* (5 species), *Volubilis* (6 species), and *Rhynchosoides* (3 species). Species in sections *Cajanus*, *Atylosia*, and *Fruticosa* have erect growth habit, *Cantharospermum*, *Volubilis*, and *Rhynchosoides* are climbing and creeping species, and *Rhynchosoides* are trailing species. Three *Cajanus* species have been further subdivided into botanical varieties: *C. scarabaeoides* into var. *pedunculatus* and var. *scarabaeoides*, *C. reticulatus* into var. *grandifolius*, var. *reticulatus*, and var. *maritimus*, and *C. volubilis* into var. *burmanicus* and var. *volubilis* (van der Maesen 1986). Molecular phylogeny of the genus *Cajanus* resolved three distinct clades: Indian, Australian, and *Scarabaeoides* (Kassa et al. 2012).

Pigeonpea is a hardy, widely adapted and drought tolerant pulse crop cultivated primarily

by subsistence farmers on 5.32 million hectares of land (FAO 2012) of semiarid tropics and subtropics in south Asia (mainly on the Indian subcontinent), Africa, and Latin America. India accounts 72.5% of the global production area. Pigeonpea is a highly adaptable grain legume that can promote food security in rain-fed agriculture because it tolerates drought, requires very minimal inputs to give a sustainable yield and is resilient to very harsh biotic and abiotic stresses. In fact, a recent study has identified pigeonpea to be more heat and drought tolerant than the majority of grain legume crops (Khoury et al. 2015). As a legume crop, it plays a major role in fixing atmospheric nitrogen through symbiotic nitrogen fixation with soil bacteria as well as through solubilizing of soil-bound phosphorus and thus improves the nutrient status of the soil (Saxena 2008). In addition to its main use as de-hulled split peas (“dhal”) which is the primary source of dietary protein (20–24% per seed) for millions of resource-poor people around the world, pigeonpea also has other uses: its immature seeds and pods are consumed fresh as green vegetables, and stems are used as domestic fuel wood and for making huts and leaves are used as quality fodder (Saxena et al. 2006). In spite of having typical Papilionoideae flowers with a “banner and keel” shape, pigeonpea exhibits a considerable variation (20–70%) in natural insect-aided out-crossing rate and is a partially cross-pollinated species (Saxena et al. 1990). This considerable out-crossing rate may have two major impacts on pigeonpea agronomy and breeding. It creates a problem for maintaining genetic purity in cultivar development, but on the other hand, it has been used effectively in developing elite hybrid varieties through hybridization (Saxena 2008). Some studies have reported that insect species also regularly visited the wild relatives of pigeonpea and noted a few naturally out-crossed plants with distinct traits (Saxena and Kumar 2010). Pigeonpea exhibits high levels of phenotypic and morphological diversity in terms of vegetative, floral, with a wide difference in days to maturity (90–300 days), photoperiod sensitivity, growth habit, and other phenotypic and agronomic traits

(Upadhyaya et al. 2007; Saxena 2008). Pigeonpea germplasm comprises diverse sets of landraces and heterogeneous feral forms with extensive morphological diversity. There are determinate, semi-determinate, and indeterminate genotypes that are adapted to various agroecological settings (Upadhyaya et al. 2007). Regardless of this extensive morphological and phenotypic diversity, molecular genetic analyses in pigeonpea, and wild relatives revealed that there is very low genetic diversity in the domesticated gene pool as compared to the wild groups (Kassa et al. 2012 and references therein; Saxena et al. 2014a, b). This strikingly low polymorphism within the domesticated accessions (including the landraces) signals the severity of the “genetic bottleneck” which happened during pigeonpea domestication. To broaden the genetic base of this highly constrained and narrowed genetic diversity in the domesticated gene pool, there is a need to utilize the high genetic diversity that present in the wild gene pool. The wild relatives are a potential source of novel alleles that can be exploited in breeding and improvement programs in pigeonpea (Saxena et al. 2014b).

7.2 Genetic and Genomic Resources of Pigeonpea and Its Wild Relatives

Pigeonpea accessions have been collected and deposited at various gene banks. The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) holds 13,771 pigeonpea accessions (Gowda et al. 2013). About 11,221 accessions are deposited at the India National Bureau of Plant Genetic Resources (NBPGR) (Singh et al. 2014). Pigeonpea accessions are also deposited elsewhere including 4116 accessions at US Department of Agriculture (USDA) and 1288 accessions at National Genebank of Kenya (Singh et al. 2013). ICRISAT also holds 555 accessions of wild relatives represented by 67 species belonging to the secondary and tertiary gene pool (Upadhyaya et al. 2011). Various cultivated and wild accessions have been

characterized for important agronomic traits including yield, early maturity, high protein content, tolerance to salinity, and drought (Gowda et al. 2013). However, recent studies have shown that the bulk of pigeonpea wild relatives are broadly underrepresented in gene banks and other ex-situ conservation settings and thus are high priority species for further collection to tap important traits to pigeonpea breeders (Khoury et al. 2015).

In the past decade, tremendous progress had been made to enrich genetic and genomic resources of pigeonpea, including the completion of the pigeonpea genome (Varshney et al. 2012). This enables discovery of genes and quantitative trait loci (QTLs) associated with important agronomic traits and development of molecular markers that promote marker assisted breeding. About 30 biparental mapping populations segregating for abiotic and biotic traits have been developed (Varshney et al. 2010). These remain a fantastic resource for trait determination and accelerated molecular breeding. Moreover, efforts are underway to develop multi-parent mapping populations including multi-parent advanced generation intercross (MAGIC) and nested association mapping (NAM) populations (Pazhamala et al. 2015).

Similar effort is needed to expand the range of germplasm resources. With primary through tertiary gene pool species poorly collected in centers of diversity in south Asia and Australia, there is room to greatly expand collections. We advocate for efforts to collect the large aridity and soil type gradients that characterize both south Asia and Western Australia. In both south Asia and Australia, hierarchical sampling across regions has great potential to capture adaptations to drought and other climatic factors that segregate on large spatial scales of degrees of latitude, as well as biotic adaptations such as pest and disease adaptation that likely segregate within populations of wild species. We believe such efforts, if integrated into pre-breeding programs guided by genomic information, have great potential to expand climatic resilience and disease resistance in pigeonpea (e.g., Warschefsky et al. 2014).

7.2.1 Leading Breeding Challenges: Drought, Osmotic Stress, and Other Abiotic Challenges

Of particular importance in a changing climate is understanding plant physiological responses to variable and harsh environments. The relationship between water use, photosynthesis, and plant growth has been studied for over a century by both ecologists and agronomists and offers critical tools for developing agroecology programs. Although the goals of ecologists and agronomists may differ, the underlying physiological theory serves as a common core of principles, equations, and processes that when understood can be applied to a diverse set of fields. It is critical for agronomists to understand the physiological principles that describe how water availability affects plant carbon acquisition, growth, and resource allocation (reviewed in [Chaves et al. 2003](#); [Farooq et al. 2009](#)) because plant physiology offers useful tools to assess the health of crop plants, and manipulate agroecological breeding programs to increase yield and plant productivity. The following section of this chapter will review the core physiological principles at the intersection of plant water relations, gas exchange, and productivity that inform agroecology programs and crop breeders of the utility of variation in physiological tolerance and traits. Specific attention is paid to pigeonpea, and we emphasize that understanding the variation in physiological and morphological phenotypes in crop wild relatives can be used to inform germplasm collection efforts.

Harsh environments can cause significant yield losses and negatively impact biomass, pod number, seed yield, seed weight, and quality in cultivated pigeonpea ([Toker et al. 2007](#); [Khan et al. 2010](#); [Toker and Mutlu 2011](#); [Hasanuzzaman et al. 2013](#); [Pagano 2014](#)). One of the most pressing issues for global food security is water availability because the agriculture industry accounts for some 70% of total freshwater consumption ([FAO 2012](#)), and the irrigation water sources and rain patterns that feed agricultural land are vulnerable to the effects of climate

change. Already, 60% of all crop production suffers from drought conditions ([Grant 2012](#)). In many developing regions of the world, water is a limiting resource and therefore plant water use and traits related to abiotic stress tolerance have been a significant focus of agricultural scientists and agronomists concerned with maintaining productivity in harsh conditions, including water deficit conditions. In response to the numerous climate models that predict changes in global precipitation and increasing drought severity and durations, a firm understanding of plant water relations is critical for improving crop productivity in water-limited environments (see [Ehlers and Goss 2003](#)).

The primary way water leaves a plant is through stomata via transpiration. Transpiration generates a pulling force on the water column in xylem generating negative absolute pressures that can surpass a perfect vacuum. This is possible because water is a polar molecule that forms hydrogen bonds between positive and negative hydrogen atoms that allow molecules to withstand tension. This is known as the cohesion-tension theory and describes the force between water molecules that allow water columns to remain intact when “pulled” through the xylem. When water is limited, plants generate more negative tension within their xylem and are more vulnerable to cavitation and embolism. Cavitation is the breaking of a water column at very negative water potentials to pull gas out of solution and introduces gaseous bubbles into the xylem. An embolism is the formation and spreading of air bubbles and imposes an additional resistance to hydraulic flow and can effectively stop water movement causing wilting and premature senescence. Thus, selective breeding on traits that target efficient water use and drought tolerance are of great importance in all crop systems.

The transpiration of water vapor through stomata is influenced by light environment, heat, and leaf characteristics like hairs and thick epicuticular wax that limit water loss ([Holmes and Keiller 2002](#)) offering leaf level traits for plant physiologists and breeders to get more “crop per drop” ([Yoo et al. 2009](#)). Guided collection efforts

and artificial selection on traits that confer more efficient water use are beneficial in dry or otherwise water-limited conditions, and there are significant tools available to assist in these efforts. Carbon isotope discrimination has long been established as a tool to evaluate plant water-use strategies (Farquhar et al. 1989). In particular, analysis of ^{13}C discrimination is an accurate way to measure water-use efficiency and has been shown to be heritable in several species (Schuster et al. 1992; Donovan and Ehleringer 1994). Carbon isotope discrimination is a superior method to assess water-use efficiency in plants because it is an integrated measure over the life of the sample, and thus ^{13}C discrimination is called often as integrated water-use efficiency (WUE). Only a small amount of plant tissue is needed for this method (on the order of μg) and an accurate mass spectrometer. Other measures of WUE using gas exchange data such as A/E (i.e., assimilation rate/transpiration) are most useful when measured repeatedly over time (e.g., diurnal) because they are vulnerable to changes in environmental conditions such as light, humidity, and soil conditions. Carbon isotopes have been used successfully to assess drought tolerance in other legume crops such as chickpea, but it is not yet a ubiquitous tool among pigeonpea programs (Kashiwagi et al. 2006).

Whole plant water movement is a function of the water potential gradient from the soil, roots, plant leaves, and atmosphere (often referred to SPAC). Water potential in leaf tissue has three main components: gravity potential, osmotic potential, and turgor potential (the turgor potential component is replaced with a pressure potential term when assessing xylem water potentials). Comparative studies have shown pigeonpea maintains a higher leaf water status during times of terminal drought, allowing for prolonged survival in soils with low water potentials (Choudhary et al. 2011). This may be attributed to pigeonpea's ability to osmotically adjust tissues to maintain a more favorable water status (e.g., Flower and Ludlow 1986, 1987). However, it is important to note that osmotic adjustment is an active process

(requiring energy), in contrast to the passive change in solute concentrations that can result from a change in plant water status. Although plants must invest energy to synthesize organic molecules and shuttle ions across cell membranes, a higher capacity for osmotic adjustment enhances tolerance to osmotic stress and can prolong survival (Mao et al. 2009). This can be achieved through the accumulation of a range of small osmolytes, such as proline, malate, and other small metabolites.

Osmotic adjustment is involved in another major abiotic stress, salinity and is very similar to drought in that poses an osmotic challenge to plants (Munns and Tester 2008). Cultivars and wild *Cajanus* have been observed to vary in their salinity tolerance (e.g., Johansen et al. 1988; Subbarao et al. 1990, 1991; Srivastava et al. 2006). Salinity can limit germination, plant vigor, and yield of agricultural crops especially in arid and semiarid regions (Munns and Tester 2008; Latef 2014; Aggarwal et al. 2012; Porcel et al. 2012). Approximately 20% of irrigated land worldwide currently is affected by salinity, particularly in arid, and desert lands, which comprise 25% of the total land area of our planet (Yeo 1999). High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, and genotoxicity (Hasegawa et al. 2000; Munns 2002; Djanaguiraman and Prasad 2013). Together these effects significantly reduce plant growth, development, and survival (Hameed et al. 2014).

Several criteria have been used in screening for salinity tolerance including germination, radicle length, dry weight production, shoot length, cell survival, plant biomass, nodulation, number of pods, grain yield, and K⁺/Na⁺ ratio (Toker and Mutlu 2011). However, there are physiological tools available that can accurately determine how a plant accumulates osmolytes and the relationship between water content and water stress, i.e., the pressure-volume curve. The pressure-volume curve can be generated with a Scholander pressure chamber and a balance and is plotted with the inverse water potential on the

y-axis, and the deficit from saturated water content on the x-axis from repeatedly taking measures as the plant tissue dries. From the resulting curve, several parameters can be extracted such as the osmotic potential, turgor loss point, and bulk elastic modulus (Tyree and Hammel 1972). The pressure-volume curve technique has been used in several other crop systems (e.g., Katerji et al. 1997, 1998) but is not yet integrated into pigeonpea programs despite past interest (i.e., Flower and Ludlow 1986, 1987). This physiological technique is ideal to assess plant water status, capacity for osmotic adjustment, and thus to identify these favorable traits. These osmotic adjustment traits can then be used in QTL analysis to further breeding efforts (e.g., Teulat et al. 1998). Greater integration of physiological and genetic tools is needed in pigeonpea agroecology and breeding programs.

Classic morphological traits associated with drought tolerance can also be selective targets. Due to pigeonpea's generally deep root system, it is considered a drought tolerant crop because having a deeper root system allows access water at greater depths in the soil. Onim (1983) observed differences in root length that was positively correlated with seed yield among genotypes in pigeonpea; therefore, it seems root characteristics and architecture are posed as ideal candidate traits for selection in breeding programs for water-limited environments. This can be aided by root optimization models which can be used to predict and assess plant responses to environmental factors (Dunbabin et al. 2002; Pages et al. 2004). Our understanding of how root architecture contributes to drought tolerance is rapidly growing (e.g., Lynch et al. 2014; Lynch 2015) and can be applied to developing more drought resilient root systems for crops like pigeonpea. Furthermore, some studies classify the variation in pigeonpea life histories into 4 groups of differing life histories regarding time to maturity: 90–120 days, 120–150 days, 150–200 days, and 200–300 days (Choudhary et al. 2011). If these genotypes are made a part of the germplasm collection, one clear strategy to improve yield under water limiting environments is to match genotypes with seasonal patterns,

although some research suggests shorter period genotypes experience greater deficits in seed yield under stress (Lopez et al. 1994). Collection of landraces or wild crop relatives of pigeonpea with locally adapted genotypes in harsh environments can be used to create a comprehensive germplasm collection that affords plant breeders access to desirable traits such as extensive root systems, phenology, water-use efficiency, osmotic adjustment, etc. However, current collections lack sufficient passport data or depth of collection to thoroughly explore local adaptation (Jaggal et al. 2014). Ideally, large collections spanning ecological gradients allow one to find accessions from regions with climates similar to areas that are either now marginal or that are predicted to be marginal with future climate change. Using material from these marginal climates increases the chances it harbors adaptive variation to these conditions. Without large collections, spanning ecological gradients, assessing local adaptation, and utilizing it for breeding in wild relatives are not feasible. Most collections predate GPS technology and lack precise site of collection descriptions. Furthermore, although landrace collections, particularly at ICRISAT, are large (see below), wild relative collections, which harbor higher genetic variation, are limited, as is the case in most other crops (Maxted and Kell 2012).

7.2.2 Biotic Challenges to Pigeonpea Production

Pigeonpea suffers from a range of biotic stresses, from nearly 200 herbivorous insects, nematodes, fungal, bacterial, and viral pathogens. The major diseases affecting pigeonpea production are Fusarium wilt (FW), sterility mosaic virus, Phytophthora blight disease, and major pests causing severe damage are pod borer (*Helicoverpa armigera* and *Maruca vitrata*) and pod fly (*Melanagromyza obtusa*) (Minja et al. 2000). Minor diseases include *Macrophomina* stem canker and yellow mosaic virus (in Asia), leaf spot and powdery mildew (in Africa), and witches broom (in the tropical Americas)

(Kannaiyan et al. 1984). The major diseases and pests can cause substantial harm, particularly for small holder farmers with limited access to pesticides or other inputs. Many of these diseases and pests are badly understudied, particularly outside India. For all these challenges, phenotyping remains a laborious task. Genetics sources of resistance are particularly important to meet these challenges.

Helicoverpa armigera pod boring moths cause massive economic damage in pigeonpea, as well as occasional total crop loss (Minja 2001). The moth can destroy entire seeds, as well as leave partially damaged seeds unsuitable for sale or consumption. Pod sucking Hemipterans of several genera can cause equivalent or greater losses in sub-Saharan and south Asian production zones (Minja 2001). These pests can reduce nutrient accumulation in pods and render seeds unfit for market consumption. Seed feeding dipteran larvae of the species *Melanagromyza chalcosoma* also cause substantial losses, although usually less than the other two types of pod-attacking insects. A variety of management strategies such as altered planting dates or harvest techniques can reduce the severity of these pests. Some pesticides are effective, although the different biology of these classes of pod-attacking insects means that no one weapon is likely to be effective. Some biological agents, such as *Helicoverpa* nuclear polyhedrosis virus (NPV) can be effective but are hard to develop.

Although management can be effective, genetic resistance is very desirable. Cultivated lines and the wild *Cajanus* differ greatly in resistance, with the wild species forming a particularly useful but under-explored reservoir of resistance to insect pest (Shanower et al. 1997; Green et al. 2002; Chougule et al. 2003; Aruna et al. 2005; Mallikarjuna et al. 2007; Sujana et al. 2008; Sharma et al. 2009). Combinations of amylase and protease inhibitors in pigeonpea seeds can reduce feeding by and induce mortality in *Helicoverpa* (Giri and Kachole 1998), and phenolic compounds and morphological structures like trichomes can contribute to deterrence (Green et al. 2003; Sharma et al. 2009). Larger collections of wild material would likely uncover

segregating polymorphisms in resistance to these pests, potentially from distinct loci to those segregating in the cultivated material. Furthermore, the increased examination is needed to determine the extent to which genetic resistance to once pest modulates resistance to the other pod-eating pests.

Wild material is a similarly useful source of resistance to diseases, particularly when coupled with molecular tools facilitated by the pigeonpea genome project to identify resistance genes. Pigeonpea mosaic sterility virus is a vectored disease transmitted by the mite *Aceria cajani* (Jones et al. 2004). Because the disease causes pigeonpea to not flower, it is a green plague. Only found in India, it is perhaps the most damaging of the diseases, although all are problematic. Kulkarni et al. (2003) described broad based resistance to *Fusarium* in *Cajanus scarabaeoides*, a species in the secondary gene pool. Jones et al. (2004) described similar mosaic sterility virus resistance in *C. scarabaeoides*. Gangwar and Bajpai (2008) also found mosaic sterility virus resistance from *C. scarabaeoides* and *C. acutifolius* to segregate in interspecific crosses with cultivated pigeonpea. Since the publication of the pigeonpea genome, some resistance loci have been identified (Singh et al. 2015).

7.2.3 Economic Traits in Wild Relatives of Pigeonpea

Abiotic (e.g., drought and salinity) and biotic (e.g., diseases and pests) stresses constrain and adversely affect pigeonpea production and cause huge economic damage. Breeding strategies to tackle these problems in pigeonpea have been attempted by various researchers (reviewed by Saxena 2008). The breeding programs for developing disease resistant cultivars using resistance gene sources from cultivated pigeonpea germplasm did not succeed in controlling devastating pests (e.g., pod borer). The cultivated gene pool has low genetic polymorphism and lacks resistance alleles to a number of diseases (e.g., Kassa et al. 2012; Saxena et al. 2014)

Alternative approaches of utilizing wild species as a source of resistance have shown promising results, as there are wider genetic diversity and the presence of resistance genes in the wild gene pool.

Wild species have coexisted with pests and pathogens on an evolutionary time scale, and they have developed alleles conferring pest and pathogen resistance (Acosta-Gallegos et al. 1998). These natural defense mechanisms for diseases and pests have been lost during domestication, and intense selection for agriculturally desirable traits such as high yield improved nutritional quality and other desirable agronomic traits. Most wild species have unique traits (e.g., the presence of trichomes) that confer resistance to these diseases and pests (Aruna et al. 2005). Wild species in the secondary and tertiary gene pools also possess useful genes for extra-early flowering and maturity, photoperiod insensitivity, good flowering and pod setting, true annuality, rapid seedling growth (Mallikarjuna and Moss 1995). Wild relatives of pigeonpea are also genetic sources for salinity tolerance (Subbarao 1988; Srivastava et al. 2006), drought tolerance, resistance to sterility mosaic virus, Phytophthora blight disease (Reddy et al. 1996; Mallikarjuna et al. 2005, 2006), tolerance to pod borers (*Helicoverpa armigera* and *Maruca testulalis*), and pod fly (*Melanagromyza obtusa*) (Saxena 2008). Species in the secondary gene pool such as *Cajanus albicans*, *C. lineatus*, *C. scarabaeoides*, *C. sericeus* have genes for high seed protein, and *Cajanus sericeus* has genes for resistance to sterility mosaic virus and P2 race of Phytophthora blight disease (Saxena 2008). Wild relatives of pigeonpea such as *Cajanus scarabaeoides*, *C. sericeus*, *C. acutifolius*, *C. albicans*, *Rhynchosia aurea*, *R. bracteata*, and *Flemingia bracteata* have shown high resistance to pod borer. *Cajanus platycarpus* has shown resistance to the most virulent race of phytophthora blight disease and the only source of resistance to the P3 race of Phytophthora blight disease (Saxena 2008). Some of the wild relatives of pigeonpea have shown a high level of resistance to pod fly (*Melanagromyza obtusa*) and pod wasp (*Tanaostigmodes cajaninae*) (Sharma et al. 2003).

7.3 Cytoplasmic Male Sterility System in Pigeonpea Breeding

All wild species of *Cajanus* have the same number of chromosomes ($2n = 22$) and similar karyotype and their interspecific hybrids showed chromosomal homology and complete chromosomal pairing. Efforts have been made to utilize desirable genes of the wild relatives in pigeonpea breeding and improvement programs (Ariyanayagam et al. 1995; Saxena et al. 1996; Tikka et al. 1997; Wanjari et al. 2000; Saxena and Kumar 2003, 2010; Saxena et al. 2005a). Wild species of pigeonpea have been utilized in breeding programs to develop cleistogamous lines (Saxena et al. 1992a, 2010), genetic dwarfs (Saxena and Sharma 1995), and cytoplasmic male sterile (CMS) lines (Saxena 2006). Breeders at ICRISAT have been utilizing the partial out-crossing nature of pigeonpea to develop a high yielding hybrid cultivar (Reddy et al. 1978). Saxena et al. (1992b) developed a pigeonpea hybrid ICPH 8, which showed increased yield gain (30.5%) over the best performing pure line control. Despite the success of releasing high yielding ICPH8 to farmers, it was not adopted effectively due to the high cost of seed production of the hybrid. This led to the development of the CMS breeding system, which was more efficient in large-scale hybrid seed production. Cytoplasmic male sterility (CMS) is a phenotypic expression of incompatibility between nuclear and cytoplasmic genomes and is a maternally inherited trait that has been successfully used as an efficient pollination control system in developing hybrid seed production (Havey 2004). In most cases, CMS is caused by the interaction between the recessive nuclear genes and specific genetic factors housed in the mitochondrial genome which cause dysfunctionality of the anthers and result in male sterility. Fertility can be restored if dominant genes substitute the recessive nuclear genes or fertility-inducing factors arise in the mitochondrial genome. Three parents are required to maintain the CMS-based breeding system: a male sterile line (A-line), maintainer line (B-line), and fertility restorer line (R-line). CMS systems can be caused by

spontaneous mutation, intraspecific, interspecific, or intergeneric crosses. About 75% of the CMS systems are a result of interspecific and intergeneric crosses (Kaul 1988). The absence of CMS lines within pigeonpea germplasm led to the synthesis of CMS lines through interspecific crosses between cultivated pigeonpea and wild species using the cytoplasm genome of the wild parent and nuclear genome of the cultivated parent (Saxena 2008). To date, seven CMS breeding lines (A₁–A₇) have been developed by crossing wild parental lines with the cultivated pigeonpea parent. However, all except one failed to use in a commercial seed production system. The exception is the CMS system (A₄) that uses the wild progenitor species *Cajanus cajanifolius* cytoplasm (Saxena et al. 2005b). High yielding and stable seeds are produced and are being used extensively by breeders to develop commercial pigeonpea hybrids.

7.4 Hybrid Vigor in Pigeonpea

For the past five decades, breeders, particularly in India, have actively pursuing pigeonpea improvement with the objective of developing high yielding varieties. Though over 100 new cultivars were released, crop productivity remains low and stagnant at around 750 kg/ha (Saxena and Sawargaonkar 2014). However, recent progress made on hybrid technology in pigeonpea resulted in high yielding hybrid cultivars. Currently, efforts are underway to identify diverse heterotic groups in pigeonpea with the objective of developing exceptionally high yielding hybrids using a diverse set of elite and hybrid parents (Saxena and Sawargaonkar 2014).

After decades of intense research, the world's first commercial CMS-based legume (pigeonpea) ("magic pea hybrid") was developed by ICRI-SAT. This breakthrough was achieved through a CMS system of using the wild progenitor of pigeonpea, *Cajanus cajanifolius*, as a parental line (Saxena 2009). Unlike previous attempts of hybrid development (ICPH 8) in pigeonpea, three CMS hybrids were developed and released. These are ICPH 2671 (Saxena et al. 2013a),

ICPH 2740 (Saxena and Sawargaonkar 2014), and ICPH 3762 (Pazhamala et al. 2015), which are stable across diverse environments and had an excellent male fertility restorer system. The hybrids give about 40% yield increase under farmers' field over the best controls and showed resistance to wilt and sterility mosaic diseases (Saxena 2009; Saxena and Sawargaonkar 2014). To augment the CMS technology, genomic analysis of the mitochondrial genome of the three lines (CMS, maintainer, and restorer lines) is being pursued (Pazhamala et al. 2015). Additionally, cross-applicable markers for testing pigeonpea hybrid purity are already developed (Bohra et al. 2014).

7.4.1 Molecular Tools and Trait Discovery

Development of new genomic tools and resources has brought a revolution in plant breeding. It enables deciphering and genetic dissection of traits of importance. Next generation sequencing (NGS) technologies coupled with bioinformatics advances are availing abundant genomic information of many crops. Discovering of new genes, QTLs, and molecular markers associated with important agronomic traits is now becoming routine (Varshney et al. 2009, 2015; Varshney 2016).

In pigeonpea, rapid progress has been made recently, and abundant genomic resources are currently available. This will speed up trait discovery and promote molecular plant breeding. Molecular markers starting from the classical gel and hybridized-based technologies (e.g., RAPD, RFLP, AFLP, DArT) to high throughput SNP markers have been developed for pigeonpea over the years (see review, Pazhamala et al. 2015). Moreover, a draft genome sequence of pigeonpea has been developed (Varshney et al. 2012a) and is the basis for future advances. Recently, efforts have been made to identify genes and QTLs associated with important agronomic traits such as drought tolerance (Varshney et al. 2012a), plant height and earliness (Kumawat et al. 2012), and determinacy (Mir et al. 2014).

Recently, increasing the effort has been dedicated to building the types of complex populations that are needed to take advantage of the depth of data provided by next generation sequencing approaches. One approach is to establishing genome-wide association study panels for gene discovery across diverse germplasm. Some early success with GWAS has already occurred (e.g., Huang and Han 2013; Korte and Farlow 2013). In addition to GWAS, complex crossing designs can combine the benefits of GWAS with QTL. A nested association mapping (NAM) population for pigeonpea has been built (Yu et al. 2008; McMullen et al. 2009). These populations are an excellent tool for uncovering the genetic basis of traits because they use widely divergent parents and bring to bear the combined benefits of both QTL mapping and genome-wide association. A related tool, a multi-parent advanced generation intercross (MAGIC) population, has also been developed for pigeonpea (Cavanagh et al. 2008). Although MAGIC populations generally have fewer parents than NAM populations and reduced capacity for gene discovery, if they are set up with elite parents they will more quickly yield progeny suitable for breeding programs. Both sets of tools are ultimately useful and important. Full genome sequencing of parents of many of the lines used in these populations will further facilitate their use (Kumar et al. 2016). In addition to these critical population tools, we encourage the more widespread use of advance backcross introgression lines to effectively capture variation in wild germplasm (e.g., Tanksley and McCouch 1997; Warschefsky et al. 2014). Although we are not aware of such efforts, we see great potential in these crossing designs for the effective use of wild germplasm.

7.4.2 Conclusion

Germplasm resources remain a critical component of all breeding programs. At a time of rapid habitat loss in all ecosystems, and a time of declining funding for many gene banks, and germplasm centers, germplasm conservation is of

increasing importance. The risk of loss of many collections, such as that at the Vavilov Institute for Research in St. Petersburg, Russia, remains high. Many other collections, such as all those in the Consultative Group on International Agricultural Research and the USDA have unstable funding sources. And wild populations of all species, such as *Cajanus* species in South Asia, have declining habitat areas and new threats from invasive species and land conversion. In the face of these risks, steps are required to better protect germplasm.

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References

- Acosta-Gallegos JA, Quintero C, Vargas J, Toro O, Tohme J, Cardona C (1998) A new variant of arcelin in wild common bean, *Phaseolus vulgaris* L. from southern Mexico. *Genet Resour Crop Evol* 45:235–242
- Aggarwal A, Kadian N, Neetu K, Tanwar A, Gupta KK (2012) Arbuscular mycorrhizal symbiosis and alleviation of salinity stress. *J Appl Nat Sci* 4(1):144–155
- Ariyanayagam RP, Rao AN, Zaveri PP (1995) Cytoplasmic-genic male-sterility in interspecific matings of *Cajanus*. *Crop Sci* 35:981–985
- Aruna R, Manohar RD, Reddy LJ, Upadhaya HD, Sharma HC (2005) Inheritance of trichomes and resistance to pod borer (*Helicoverpa armigera*) and their association in interspecific crosses between cultivated pigeonpea (*Cajanus cajan*) and its wild relative *C. scarabaeoides*. *Euphytica* 145:247–257

- Bohra A, Singh IP, Yadav AK, Pathak A, Soren KR, Chaturvedi SK et al (2014) Utility of informative SSR markers in the molecular characterization of cytoplasmic genetic male sterility-based hybrid and its parents in pigeonpea. *Natl Acad Sci Lett* 38:13–19
- Brown TA, Jones MK, Powell W, Allaby RG (2009) The complex origins of domesticated crops in the Fertile Crescent. *Trends Ecol Evol* 24:103–109
- Cavanagh C, Morell M, Mackay I, Powell W (2008) From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. *Curr Opin Plant Biol* 11(2):215–221
- Chaves M, João PM, João SP (2003) Understanding plant responses to drought—from genes to the whole plant. *Funct Plant Biol* 30(3):239–264
- Choudhary AK, Sultana R, Pratap A, Nadarajan N, Jha UC (2011) Breeding for abiotic stresses in pigeonpea. *J Food Legumes* 24(3):165–174
- Chougule NP, Hivrare VK, Chhabda PJ, Giri AP, Kachhole MS (2003) Differential inhibition of *Helicoverpa armigera* gut proteinases by proteinase inhibitors of pigeonpea (*Cajanus cajan*) and its wild relatives. *Phytochemistry* 64:681–687
- Djanaguiraman M, Prasad PV (2013) Effects of salinity on ion transport, water relations and oxidative damage. In: *Ecophysiology and responses of plants under salt stress*, Springer, New York, pp 89–114
- Donovan LA, Ehleringer JR (1994) Potential for selection on plants for water-use efficiency as estimated by carbon isotope discrimination. *Am J Bot* 81:927–935
- Dunbabin VM et al (2002) Modelling the interactions between water and nutrient uptake and root growth. *Plant Soil* 239:19–38
- Ehlers W, Goss MJ (2003) *Water dynamics in plant production*. CABI, Wallingford, Oxfordshire, UK
- FAO (2012) Online agriculture statistics. <http://www.faostat.org>
- Farooq M et al (2009) Plant drought stress: effects, mechanisms and management. *Sustainable Agriculture*. Springer, Netherlands, pp 153–188
- Farquhar GD, Hubick KT, Condon AG, Richards RA (1989) Carbon isotope fractionation and plant water-use efficiency. *Stable isotopes in ecological research*. Springer, New York, pp 21–40
- Flower DJ, Ludlow MM (1986) Contribution of osmotic adjustment to the dehydration tolerance of water-stressed pigeon pea (*Cajanus cajan* (L.) millsp.) leaves. *Plant, Cell Environ* 9:33–40
- Flower DJ, Ludlow MM (1987) Variation among accessions of pigeonpea (*Cajanus cajan*) in osmotic adjustment and dehydration tolerance of leaves. *Field Crops Res* 17:229–243
- Ford-Lloyd BV, Schmidt M, Armstrong SJ, Barazani O, Engels J, Hadas R, Hammer K, et al. (2011) Crop wild relatives—undervalued, underutilized and under threat? *BioScience* 61(7):559–565
- Fortunato RH (2000) Systematic relationships in *Rhynchosia* (Cajaniinae Phaseoleae—Papilionoideae—Fabaceae) from the neotropics. In: Bruneau A (ed) Herendeen PS. Kew, UK, The Royal Botanic Gardens, pp 339–354
- Gangwar LK, Bajpai GC (2008) Screening of interspecific hybrids against sterility mosaic disease in pigeonpea. *Legume Res-An Int J* 31:306–307
- Giri AP, Kachole MS (1998) Amylase inhibitors of pigeonpea (*Cajanus cajan*) seeds. *Phytochemistry* 47:197–202
- Gowda CLL, Upadhyaya HD, Sharma S, Varshney RK, Dwivedi SL (2013) Exploiting genomic resources for efficient conservation and use of chickpea, groundnut, and pigeonpea collections for crop improvement. *Plant Genome* 6:1–11. doi:10.3835/plantgenome2013.05.0016
- Grant OM (2012) Understanding and exploiting the impact of drought stress on plant physiology. In: *Abiotic stress responses in plants*. Springer, New York, pp 89–104
- Green PWC, Stevenson PC, Simmonds MSJ, Sharma HC (2002) Can larvae of the pod-borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae), select between wild and cultivated pigeonpea *Cajanus* sp. (Fabaceae)? *Bull Entomol Res* 92:45–51
- Green PW, Stevenson PC, Simmonds MS, Sharma HC (2003) Phenolic compounds on the pod-surface of pigeonpea, *Cajanus cajan*, mediate feeding behavior of *Helicoverpa armigera* larvae. *J Chem Ecol* 29:811–821
- Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica* 156(1–2):1–13
- Harlan JR, de Wet JMJ (1971) Toward a rational classification of cultivated plants. *Taxon* 20:509–517
- Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int J Mol Sci* 14(5):9643–9684
- Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Ann Rev Plant Biol* 51(1):463–499
- Havey MJ (2004) The use of cytoplasmic male sterility for hybrid seed production. In: Daniell H, Chase C (eds) *Molecular biology and biotechnology of plant organelles*. Springer, Dordrecht, The Netherlands, pp 617–628
- Holmes MG, Keiller DR (2002) Effects of pubescence and waxes on the reflectance of leaves in the ultraviolet and photosynthetic wavebands: a comparison of a range of species. *Plant, Cell & Environ* 25(1):85–93
- Huang X, Han B (2014) Natural variations and genome-wide association studies in crop plants. *Ann Rev Plant Biol* 65:531–551
- Jaggal LG, Patil BR, Salimath PM (2014) Evaluation of mini core accessions of pigeonpea (*Cajanus cajan* (L.) Millsp.) against sterility mosaic disease and fusarium wilt. *Karnataka J Agric Sci* 27:337–339
- Johansen C, Saxena NP, Chauhan YS, Rao GS, Pundir RPS, Rao JVDKK, Jana MK (1988) Genotypic variation in salinity response of chickpea and

- pigeonpea. In: Proceedings of the International Congress of Plant Physiology, New Delhi, India, 15–20 February 1988
- Jones AT, Kumar PL, Saxena KB, Kulkarni NK, Muniyappa V, Waliyar F (2004) Sterility mosaic disease—the “green plague” of pigeonpea: advances in understanding the etiology, transmission and control of a major virus disease. *Plant Disease* 88(5):436–445
- Kannaiyan J, Nene YL, Reddy MV, Ryan JG, Raju TN (1984) Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa and the Americas. *Int J Pest Manage* 30:62–72
- Kashiwagi J, Krishnamurthy L, Singh S, Gaur PM, Upadhyaya HD, Panwar JDS, Basu PS, Ito O, Tobita S (2006) Relationship between transpiration efficiency and carbon isotope discrimination in chickpea (*Cicer arietinum* L.). *J SAT Agric Res* 2:1–3
- Kassa MT, Varma Penmetsa R, Carrasquilla-Garcia N, Sarma BK, Datta S et al (2012) Genetic patterns of domestication in pigeonpea (*Cajanus cajan* (L.) Millsp.) and wild *Cajanus* relatives. *PLoS ONE* 7: e39563
- Kassa M, Van der Maesen JG, Krieg C, von Wettberg EJB (2016) Historical and phylogenetic perspectives of pigeonpea. *Legume Perspect* 11:7–9
- Katerji N, Van Hoorn JW, Hamdy A, Mastrorilli M, Karzel EM (1997) Osmotic adjustment of sugar beets in response to soil salinity and its influence on stomatal conductance, growth and yield. *Agric Water Manage* 34:57–69
- Katerji N, Van Hoorn JW, Hamdy A, Mastrorilli M (1998) Response of tomatoes, a crop of indeterminate growth, to soil salinity. *Agric Water Manage* 38:59–68
- Kaul M (1988) Male sterility in higher plants. In: Frankel R, Grossman M, Maliga P (eds) *Monographs on theoretical and applied genetics* 10. Springer-Verlag, Berlin, pp 775–795
- Khoury CK, Castañeda-Alvarez NP, Achicanoy HA, Sosa CC, Bernau V, Kassa MT, Norton SL, van der Maesen LJG, Upadhyaya HD, Ramirez-Vallegas R, Jarvis A, Struik PC (2015) Crop wild relatives of pigeonpea [*Cajanus cajan* (L.) Millsp.]: Distributions, ex situ conservation status, and potential genetic resources for abiotic stress tolerance. *BiolConserv* 184:259–270
- Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods* 9(1):29
- Kulkarni NK, Reddy AS, Kumar PL, Vijaynarasimha J, Rangaswamy KT, Muniyappa V, Reddy DVR et al (2003) Broad-based resistance to pigeonpea sterility mosaic disease in accessions of *Cajanus scarabaeoides* (L.) Benth. *Indian J Plant Prot* 31:6–11
- Kumar V, Khan AW, Saxena RK, Garg V, Varshney RK (2016) First-generation HapMap in *Cajanus* spp. reveals untapped variations in parental lines of mapping populations. *Plant Biotechnol J*
- Kumawat G, Raje RS, Bhutani S, Pal JK, Mithra SVCR, Gaikwad K et al (2012) Molecular mapping of QTLs for plant type and earliness traits in pigeonpea (*Cajanus cajan* L. Millsp.). *BMC Genet* 13:84
- Latef AAHA, Miransari M (2014) The role of arbuscular mycorrhizal fungi in alleviation of salt stress. In: *Use of Microbes for the Alleviation of Soil Stresses*, Springer, New York, pp 23–38
- Lopez FB, Johansen C, Chauhan YS (1994) Limitations to seed yield in short-duration pigeonpea under water stress. *Field Crops Res* 36:95–102. doi:10.1016/0378-4290(94)90058-2
- Lynch JP (2015) Root phenes that reduce the metabolic costs of soil exploration: opportunities for 21st century agriculture. *Plant, Cell Environ* 38:1775–1784
- Lynch JP, Chimungu JG, Brown KM (2014) Root anatomical phenes associated with water acquisition from drying soil: targets for crop improvement. *J Exp Bot*, eru162
- Mallikarjuna N, Moss JP (1995) Production of hybrids between *Cajanus platycarpus* and *Cajanus cajan*. *Euphytica* 83:43–46
- Mallikarjuna N, Deepak J, Reddy MV, Usharani DT (2005) Introgression of Phytophthora blight disease resistance from *Cajanus platycarpus* into short duration pigeonpea [*Cajanus cajan* (L.) Millsp.]. *Indian J Genet Plant Breed* 65:261–263
- Mallikarjuna N, Jadhav D, Reddy P (2006) Introgression of *Cajanus platycarpus* genome into cultivated pigeonpea. *C. cajan*. *Euphytica* 149:161–167
- Mallikarjuna N, Sharma HC, Upadhyaya HD (2007) Exploitation of wild relatives of pigeonpea and chickpea for resistance to *Helicoverpa armigera*. *J SAT Agric Res* 3:4
- Mallikarjuna N, Saxena KB, Jadhav DR (2011) *Cajanus*. In: Kole C (ed) *Wild crop relatives: genomic and breeding resources, legume crops and forages*. Springer-Verlag, Berlin, Heidelberg, pp 21–33
- Mao X, Zhang H, Tian S, Chang X, Jing R (2009) TaSnRK2.4, a SNF1-type serine-threonine protein kinase of wheat (*Triticum aestivum* L.) confers enhanced multi-stress tolerance in *Arabidopsis*. *J Exp Bot* 61:683–696
- Maxted N, Kell SP (2009) Establishment of a global network for the in situ conservation of crop wild relatives: status and needs. *FAO Commission on Genetic Resources for Food and Agriculture*, Rome, Italy, p 266
- McMullen MD, Kresovich S, Villeda HS, Bradbury P, Li H, Sun Q, Flint-Garcia S, Thornsberry J, Acharya C, Bottoms C, Brown P (2009) Genetic properties of the maize nested association mapping population. *Science* 325(5941):737–740
- Minja EM (2001) Yield losses due to field pests and integrated pest management strategies for pigeonpea—A synthesis. In *Status and potential of pigeonpea in Eastern and Southern Africa: proceedings of a regional workshop*, 12–15 Sep 2000, Nairobi, Kenya. B-5030 Gembloux, Belgium: Gembloux Agricultural University; and Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 232 pp (p. 48). ISBN 92-9066-432-0

- Minja EM, Shanower TG, Silim SN, Karuru O (2000) Efficacy of different insecticides for pigeonpea pest management in Kenya. *ICPN* 7:30–43
- Mir RR, Kudapa H, Srikanth S, Saxena RK, Sharma A, Azam S et al (2014) Candidate gene analysis for determinacy in pigeonpea (*Cajanus* spp.). *Theor Appl Genet* 127:2663–2678
- Munns R (2002) Salinity, growth and phytohormones. Salinity: environment—plants—molecules. Kluwer Academic Publishers, Dordrecht, pp 271–290
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651–681
- Olsen KM, Wendel JF (2013) A bountiful harvest: genomic insights into crop domestication phenotypes. *Annu Rev Plant Biol* 64:47–70
- Onim JFM (1983) Association between grain yield and drought resistance in marginal rainfall areas in Kenya. In: Holmes JC, Tahor WM (eds) More food from better technology, pp 864–872
- Pages L et al (2004) Root Typ: a generic model to depict and analyse the root system architecture. *Plant Soil* 258:103–119
- Pazhamala L, Saxena RK, Singh VK, Sameerkumar CV, Kumar V, Sinha P et al (2015) Genomics-assisted breeding for boosting crop improvement in pigeonpea (*Cajanus cajan* L.). *Front. Plant Sci* 6:50
- Porcel R, Aroca R, Ruiz-Lozano JM (2012) Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. *Agron Sustain Dev* 32(1):181–200
- Purugganan MD, Fuller DQ (2009) The nature of selection during plant domestication. *Nature* 457:843–848
- Reddy BVS, Green JM, Bisen SS (1978) Genetic male-sterility in pigeonpea. *Crop Sci* 18:362–364
- Reddy MV, Raju TN, Sheila VK (1996) Phytophthora blight disease in wild pigeonpea. *ICPN* 3:52–53
- Saxena KB (2006) Hybrid pigeonpea seed production manual. International Crops research Institute for the Semi Arid Tropics. Patancheru, India. Bulletin 74: 27
- Saxena KB (2008) Genetic improvement of pigeonpea—a review. *Trop Plant Biol* 1:159–178
- Saxena KB (2009) Evolution of hybrid breeding technology in pigeonpea. In: Ali M, Kumar S, (eds.) Milestones in food legume research. Indian Institute of Pulses Research, Kanpur, India. 82–114
- Saxena KB, Kumar RV (2003) Development of a cytoplasmic- nuclear male-sterility system in pigeonpea using *C. scarabaeoides* (L.) Thouars. *Indian J Genet* 63:225–229
- Saxena KB, Kumar RV (2010) Insect-aided natural out-crossing in four wild relatives of pigeonpea. *Euphytica* 173:329–335
- Saxena KB, Sawargaonkar SL (2014) First information on heterotic groups in pigeonpea [*Cajanus cajan* (L.) Millsp.]. *Euphytica* 200:187–196
- Saxena KB, Sharma D (1995) Sources of dwarfism in pigeonpea. *Indian J Pulses Res* 8:1–6
- Saxena KB, Singh L, Gupta MD (1990) Variation for natural outcrossing in pigeonpea. *Euphytica* 46:143–148
- Saxena KB, Singh L, Ariyanayagam RP (1992a) Role of partial cleistogamy in maintaining genetic purity of pigeonpea. *Euphytica* 66:225–229
- Saxena KB, Chauhan YS, Johansen C, Singh L (1992b) Recent developments in hybrid pigeonpea research. *New Frontiers in Pulses Research and Development*. Indian Institute of Pulses Research, Kanpur, India, pp 58–69
- Saxena KB, Rao AN, Singh U, Remnanadan P (1996) Interspecies variation in *Cajanus platicarpus* for some agronomic traits and crossability. *ICPN* 3:49–51
- Saxena KB, Srivastava DP, Chauhan YS, Ali M (2005a) Hybrid pigeonpea. In: Ali M, Kumar S (eds) *Advances in pigeonpea research*. IIPR Kanpur, India, pp 96–133
- Saxena KB, Kumar RV, Srivastava N, Bao S (2005b) A cytoplasmic-nuclear male-sterility system derived from a cross between *Cajanus cajanifolius* and *Cajanus cajan*. *Euphytica* 145:289–294
- Saxena KB, Sultana R, Mallikarjuna N, Saxena RK, Kumar RV, Sawargaonkar SL, Varshney RK (2010) Male-sterility systems in pigeonpea and their role in enhancing yield. *Plant Breed* 129:125–134
- Saxena RK, Von Wettberg E, Upadhyaya HD, Sanchez V, Songok S, Saxena K, Varshney RK (2014) Genetic diversity and demographic history of *Cajanus* spp. illustrated from genome-wide SNPs. *PLoS ONE* 9:e88568
- Schuster WS, Sandquist DR, Phillips SL, Ehleringer JR (1992) Comparisons of carbon isotope discrimination in populations of aridland plant species differing in lifespan. *Oecologia* 91:332–337
- Shanower TG, Yoshida M, Peter JA (1997) Survival, growth, fecundity, and behavior of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on pigeonpea and two wild *Cajanus* species. *J Econ Entomol* 90:837–841
- Sharma HC, Pampapathy G, Reddy LJ (2003) Wild relatives of pigeonpea as a source of resistance to the pod fly (*Melanagromyza obtusa* Malloch) and pod wasp (*Tanaostigmodes cajaninae* La Salle). *Genet Resour Crop Evol* 50:817–824
- Sharma HC, Sujana G, Rao DM (2009) Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. *Arthropod-Plant Interact* 3:151–161
- Singh N, Tyagi RK, Pandey C (2013) Genetic resources of pigeonpea: conservation for use. National Bureau of Plant Genetic Resources (NBPGR), New Delhi, pp 1–49
- Singh M, Gautam NK, Rana MK, Dahiya OP, Dutta M, Bansal KC (2014) Pigeonpea genetic resources and its utilization in India, current status and future prospects. *J Plant Sci Res* 1:107
- Singh VK, Khan AW, Saxena RK, Kumar V, Kale SM, Sinha P, et al Kumar, S (2015) Next-generation sequencing for identification of candidate genes for Fusarium wilt and sterility mosaic disease in pigeonpea (*Cajanus cajan*). *Plant Biotechnol J*
- Srivastava N, Vadez V, Upadhyaya HD, Saxena KB (2006) Screening for intra and inter specific variability

- for salinity tolerance in pigeonpea (*Cajanus cajan* (L.) Millsp.) and its related wild species. *E-J SAT Agric Res Crop Improv* 2:1
- Subbarao GV (1988) Salinity tolerance in pigeonpea (*Cajanus cajan* (L.) Millsp.) and its wild relatives. PhD thesis, Indian Inst Technol, Kharagpur, India
- Subbarao GV, Johansen C, Jana MK, Rao JK (1990) Physiological basis of differences in salinity tolerance of pigeonpea and its related wild species. *J Plant Physiol* 137:64–71
- Subbarao GV, Johansen C, Jana MK, Rao JVDK (1991) Comparative salinity responses among pigeonpea genotypes and their wild relatives. *Crop Sci* 31:415–418
- Sujana G, Sharma HC, Rao DM (2008) Antixenosis and antibiosis components of resistance to pod borer *Helicoverpa armigera* in wild relatives of pigeonpea. *Int J Trop Insect Sci* 28:191–200
- Tang H, Sezen U, Paterson AH (2010) Domestication and plant genomes. *Curr Opin Plant Biol* 13:160–166
- Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277(5329):1063–1066
- Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdil P, Charrier A et al (1998) Several QTLs involved in osmotic-adjustment trait variation in barley (*Hordeum vulgare* L.). *Theor Appl Genet* 96:688–698
- Tikka SBS, Parmar LD, Chauhan RM (1997) First record of cytoplasmic-genic male sterility in pigeonpea (*Cajanus cajan* (L.) Millsp.) through wide hybridization. *GAU Res J*. 22:160–162
- Toker C, Mutlu N (2011) 16 Breeding for abiotic stresses. In: Pratap A, Kumar J (eds) *Biology and breeding of food legumes*, CABI Wallingford, Oxfordshire, UK, p 241
- Toker C, Lluch C, Tejera NA, Serraj R, Siddique KHM (2007) 23 Abiotic Stresses. Chickpea breeding and management. Chickpea breeding and management, CABI Wallingford, Oxfordshire, UK, p 474
- Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *J Exp Bot* 23(1):267–282
- Upadhyaya HD, Reddy KN, Gowda CLL, Singh S (2007) Phenotypic diversity in the pigeonpea (*Cajanus cajan*) core collection. *Genet Resour Crop Evol* 54:1167–1184
- Upadhyaya HD, Reddy KN, Shivali S, Varshney RK, Bhattacharjee R, Singh S et al (2011) Pigeonpea composite collection for enhanced utilization of germplasm in crop improvement programs. *Plant Genet Resour* 9:97–108
- Upadhyaya HD, Reddy KN, Singh S, Gowda CLL (2013) Phenotypic diversity in *Cajanus* species and identification of promising sources for agronomic traits and seed protein content. *Genet Resour Crop Evol* 60:639–659
- Van der Maesen LJG (1986) *Cajanus* DC and *Atylosia* W and A (Leguminosae). Agricultural University Wageningen papers 85–4:1–225
- Varshney RK (2011) Application of next generation sequencing and genotyping technologies to develop large-scale genomic resources in SAT legume crops. In: Acharya NG (ed) *National symposium on genomics and crop improvement: relevance and reservations*, Ranga Agricultural University, Rajendranagar, Hyderabad, 25–27 February 2010
- Varshney RK (2016) Exciting journey of 10 years from genomes to fields and markets: some success stories of genomics-assisted breeding in chickpea, pigeonpea and groundnut. *Plant Sci* 242:98–107
- Varshney RK, Nayak SN, May GD, Jackson SA (2009) Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends Biotechnol* 27:522–530
- Varshney RK, Penmetsa RV, Dutta S, Kulwal PL, Saxena R, Datta S et al (2010) Pigeonpea genomics initiative (PGI): an international effort to improve crop productivity of pigeonpea (*Cajanus cajan* L.). *Mol Breed* 26:393–408
- Varshney RK, Chen W, Li Y, Bharti AK, Saxena RK, Schlueter JA et al (2012) Draft genome sequence of pigeonpea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. *Nat Biotechnol* 30:83–89
- Varshney RK, Kudapa H, Pazhamala L, Chitikineni A, Thudi M, Bohra A, Ellis N et al (2015) Translational genomics in agriculture: some examples in grain legumes. *Crit Rev Plant Sci* 34:169–194
- Vaughan DA, Balazs E, Heslop-Harrison JS (2007) From crop domestication to super-domestication. *Ann Bot* 100:893–901
- Vavilov NI (1922) The law of homologous series in variation. *J Genet* 12(1):47–89
- Wanjari KB, Patil AN, Patel MC, Manjaya JG (2000) Male sterility derived from *Cajanus sericeus* and *Cajanus cajan*: Confusion of cytoplasmic male sterility with dominant genic male-sterility. *Euphytica* 115:59–64
- Warschefsky E, Penmetsa RV, Cook DR, von Wettberg EJ (2014) Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. *Am J Bot* 101(10):1791–1800
- Yeo AR, Flowers SA, Rao G, Welfare K, Senanayake N, Flowers TJ (1999) Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. *Plant, Cell & Environ* 22(5):559–565
- Yoo CY et al (2009) Regulation of transpiration to improve crop water use. *Crit Rev Plant Sci* 28:410–431
- Yu J, Holland JB, McMullen MD, Buckler ES (2008) Genetic design and statistical power of nested association mapping in maize. *Genetics* 178(1):539–551