Grain Micronutrients in Pigeonpea: Genetic Improvement Using Modern Breeding Approaches

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Contents

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Abstract

The green revolution increased crop productivity and significantly reduced starvation and protein malnutrition. However, this caused micronutrient depleted soil, thereby responsible for widespread deficiencies of plant nutrients. Legumes are the important constituents of traditional healthy diets worldwide and second in agricultural importance after cereals. On a worldwide scale, pigeonpea ranks sixth among all legume crops and is India's second most important legume. Biofortification is the process of enhancing the nutrient value of crops using conventional selective breeding and agronomic approaches or via genetically modifying them. In many Indian states, the seeds of pigeonpea serve as a protein-rich pulse and are consumed in many forms including grain, vegetable and fodder. A variety of nutrients are present in the seeds, including carbohydrates, fats, protein, vitamins, minerals, and also some secondary metabolites. Pigeonpea exhibited various ethnomedicinal and pharmacological properties, and it has a long history of ethnobotanical use. Conventional breeding programs are utilized to develop nutritionally improved cultivars, although the success of such a program is very slow due to restricted gene pool and linkage drag. The exploitation of breeding-based approaches along with supportive interdisciplinary research and development have been utilized for biofortified pigeonpea development. Some transgenic approaches were also undertaken for nutritional improvement and antibody production. Further improvement in those approaches and genomic technologies will enhance the nutritional quality of pigeonpea.

Keywords

Genomics-assisted breeding · Health related traits · Molecular markers · Nutraceuticals · Quantitative trait loci · Whole genome sequence

1 Introduction

Pulses hold a salient position in Indian Agriculture. India tops the list for being the largest producer and consumer of pulses in the world, contributing about 25% to the global pulse or grain legumes production (Saxena et al. 2019). One such important grain legume, which has originated from the Indian subcontinent, is the pigeonpea (*Cajanus cajan* (L.) Millspaugh). Predominantly grown in rain-fed conditions, pigeonpea is a considerable source of protein to rural and urban households in Asia and Africa. It augments and enhances the soil through symbiotic nitrogen fixation and revitalizes the soil by recycling of soil nutrients, releasing soil-bound phosphorus, and addition of organic matter (Pahwa et al. 2013). Moreover, it is a great source for additional nitrogen supply to the subsequent crops. According to studies, pigeonpea releases roughly 40 kg/ha of residual nitrogen in the crop fields. All these properties cooperatively make pigeonpea a supreme crop for sustainable agriculture, around the equatorial regions of India. About three-fourths of the total

Indian production of pigeonpea is derived from Gujarat, Karnataka, Maharashtra, Madhya Pradesh, Andhra Pradesh, and Uttar Pradesh. In Barbados, pigeons were fed with these pigeonpea seeds grown on barren lands; this justifies the name. Being a short-day plant, it has a longevity of 3–4 years. Plough pan is formed below the normal ploughing zone and is a compact soil layer, which reduces the productivity of the land. The long tap roots of pigeonpea are prominently known as "biological plough" because of their ability to break plough pan.

The pigeonpea seeds consist of three structural features – cotyledons, seed coat, and embryo. The embryo is rich in albumin, globulin, and the cotyledons have high carbohydrate content, along with calcium and iron (Figs. 1 and 2). The albumin has affluent number of amino acids rich in sulphur; which encompasses methionine and cystine. Other amino acids like glycine, lysine, alanine, and aspartic acid are also present. Methionine is a limiting essential amino acid and hence, it is a beneficial factor under nutrition. The pigeonpea seed coat majorly contains amino acids like serine, proline, threonine, and glycine (Saxena et al. 2019).

The pigeonpea seeds are an integral part of Indian diet. The dry seeds are dehusked and split into cotyledons which are commonly cooked as "dal." In many Indian states, the green seeds serve as a protein-rich vegetable. To garner highest seed yield and utmost nutritional quality, the green pods must be harvested at an appropriate stage. An inverse relationship was observed between the starch content and the sugar-protein contents. In the developing seeds, there is a drop in the sugar and protein content and a rapid elevation in the starch content whereas, iron, zinc, calcium, magnesium, and copper contents were found to be more or less unchanged during seed development in pigeonpea.

Pigeonpea also holds certain antinutritional factors. Polyphenols such as tannins and phenols, oligosaccharides, lectins, enzyme inhibitors like chymotrypsin and trypsin are some of the above mentioned factors (Toklu et al. 2021). Trypsin and chymotrypsin inhibitors are expressed only in the seeds. Whole seeds without dehulling are also consumed in many countries. Cooking of pigeonpea also plays a significant role which affects its nutritional features. The seeds are large in size, absorb more water, and have high nitrogen content, which makes it a quick cooking dal. Cooking not only enhances the bioavailability of certain nutrients, it also destroys certain antinutritional components. For instance, starch digestibility is improved by cooking whereas there is a drop in the measure of oligosaccharides. Heat destroys thiamine and riboflavin, but niacin content remains unchanged during roasting and cooking of pigeonpea seeds. Methionine and lysine content decreases upon roasting, whereas there are reports on increased methionine upon boiling.

Pigeonpea possesses many herbal properties which are essentially described in folk medicine and used to treat numerous human illnesses (Salehi et al. 2019). Pneumonia, bronchitis, coughs can be cured using floral extracts of pigeonpea. It can also be employed to treat respiratory infections, menstrual distress, and dysentery. Dried seeds have the ability to ease difficulties like headache and vertigo, whereas fresh seeds help to diminish urinary incontinence, as well as other kidney disorders. The seed extracts aid in curing sickle cell anemia, by impeding the

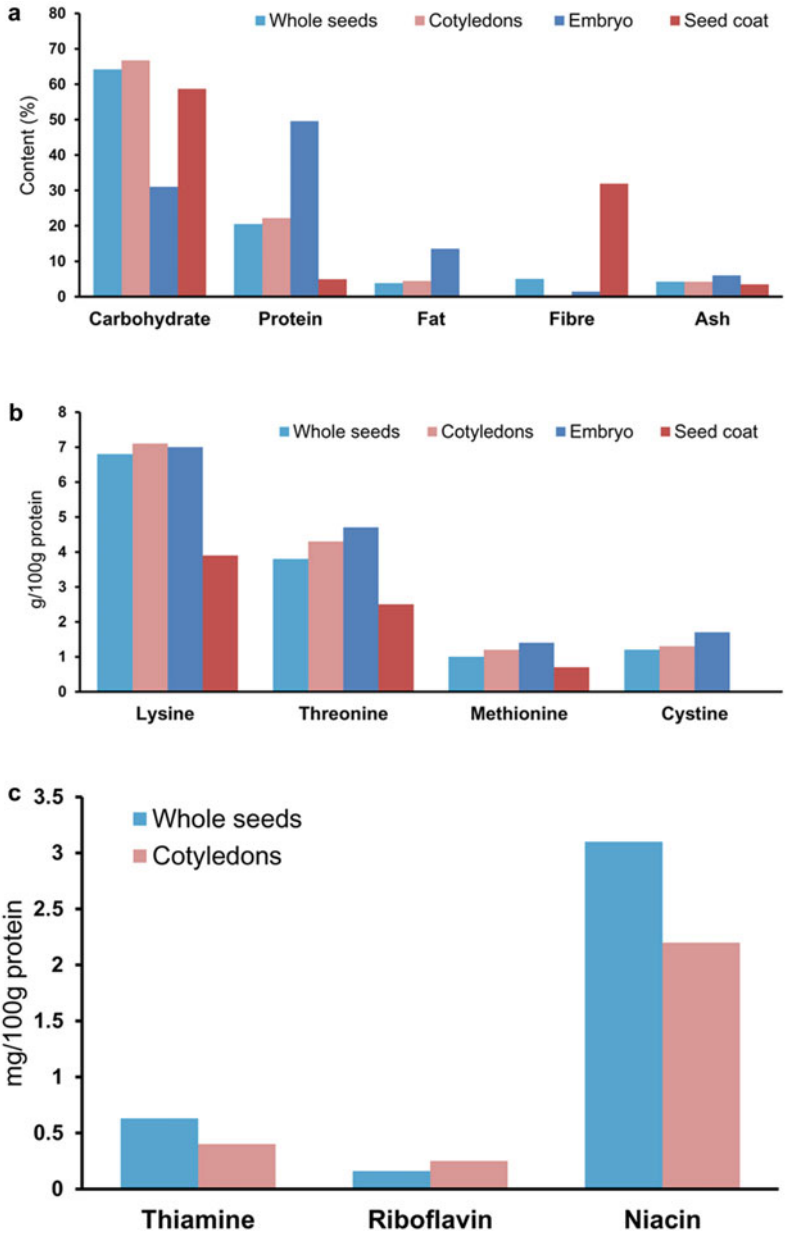

Fig. 1 Overall nutrient composition and their distribution in pigeonpea. (**a**) Nutrients in mature pigeonpea. (**b**) Major amino acids in mature pigeonpea. (**c**) Vitamins in mature pigeonpea. (**d**) Minerals and trace elements. (**e**) Protein fractions in dry pigeonpea seeds (Saxena et al. 2002)

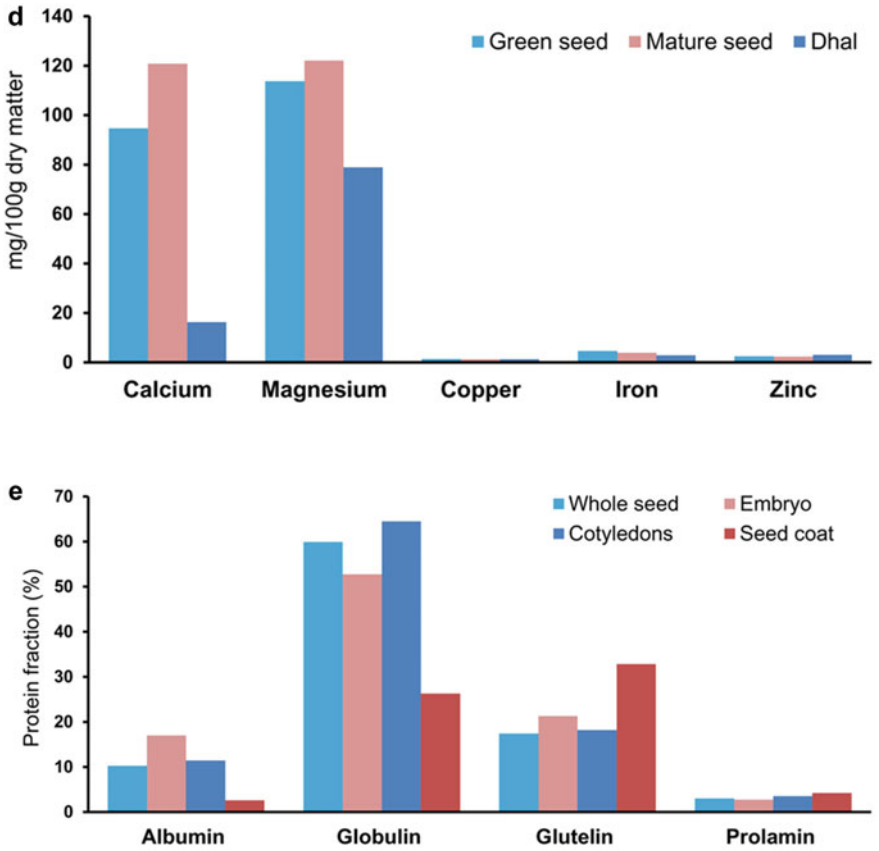

Fig. 1 (continued)

sickling of erythrocytes. According to some reports, dried pigeonpea roots could be used as anthelmintic, sedative, vulnerary, expectorant, and alexiteric.

2 Limitations in Conventional Breeding and Rationale of Nutritional Genomics

Improving the yield quantity, nutritional quality, and maintenance of genotype stability are the primary approaches to fulfil the demands of the population. Conventional breeding practices coupled with genomics-based selection approaches need to be employed to fight the threats offered by climate change and increasing population (Singh et al. 2020).

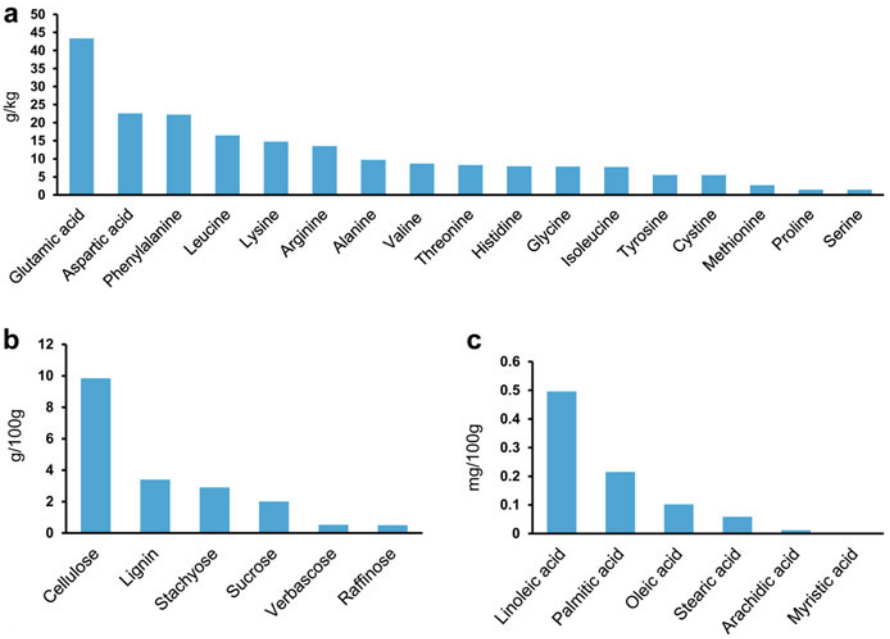

Fig. 2 Detailed nutrient composition of pigeonpea. (**a**) Amino acid composition (Ade-Omowaye et al. 2015). (**b**) Carbohydrate profile (Apata 2008). (**c**) Fatty acid profile (Ade-Omowaye et al. 2015)

Traditional plant breeding methods include the recognition and development of improved parental lines that has quality nutrient content, hybridization with elite genotypes, followed by selection of hybrids over a number of generations to get commercially established cultivars showing required nutritional properties. Additional considerations include quantitative trait complexity and the difficulty of selection of desirable trait because of low heritability. As a result, traditional methods take longer to grow a new and improved variety. Advancement in omics techniques in combination with breeding programs have a lot of potential to contribute for nutritional quality improvement in pigeonpea (Singh et al. 2020). Some of the constraints related to nutritional improvement of pigeonpea are detailed in the next few paragraphs.

Limited diversity within the basic pool of genes was revealed by a polymorphism study of sampled *Cajanus* accessions. Breeders have no choice but to use species and sub-species from secondary, tertiary, and quaternary gene pools through conventional and marker assisted selection techniques. Despite of vast genetic diversity of wild relatives, there is limitation of incorporation of them in breeding program because of lack of accurate information on the availability of desirable features and the necessity for extensive research whenever they are used. Poor agronomic traits in combination with partial characterization of relatively few wild relatives are responsible for lag in genetic improvement of pigeonpea (Saxena et al. 2014).

Pigeonpea is a short-day plant (Vales et al. 2012). A pivotal regulator of flower induction is the interaction of the photoperiod with day and night temperature. Hence, beyond 30° northern and southern latitudes, the cultivation of pigeonpea is restricted. (Saxena 2008). There is an inverse correlation between earliness and photosensitivity which confirms limited success of breeding programs in photo-insensitive and late maturing cultivars. Low-temperature in combination with photoperiod and sensitivity limit the cultivation of this crop in higher altitudes and latitudes (Vales et al. 2012). This is restricting the use of pigeonpea in alternative cropping systems (Vales et al. 2012).

The transfer of the genes of interest into the elite cultivar is highly interfered by the association of unwanted phenotypes with certain nutritional traits. As an example, transferring the genes involved in high protein accumulation was tried from *C. scarabaeoides* and *C. albicans* to the cultivars of pigeonpea. The selection of the desired genotype, high in productivity and protein yield, was obtained only after some 12–14 generations (Saxena and Sawargaonkar 2015).

3 Medicinal Properties of Pigeonpea

Pigeonpea had been used extensively in traditional medicine. In addition, the plant has also exhibited a wide array of pharmacological properties. This section will describe the various ethnomedicinal and pharmacological properties of pigeonpea along with a brief illustration about the selected chemical constituents present in the plant.

3.1 Ethnomedicinal Uses

The *Garo* tribal community of Netrakona district of Bangladesh uses pigeonpea as a remedy for diabetes. The seed paste of this plant is used as a stimulant while the leaf juice is used for the treatment of diabetes (Rahmatullah et al. 2009). In Trinidad and Tobago, the plant is used to treat food poisoning and is considered as colic. It is also used to treat constipation (Lans 2007). In Cote D'Ivoire, extraction from the leaves and stem are utilized for the treating of anemia, skin disease, and wounds (Koné et al. 2011). In Benin, the similar preparation is used for the treatment of candidiasis (Fanou et al. 2020). The local communities of south western Uganda use the juice of the leaves for the treatment of ear disease (Gumisiriza et al. 2019). In south west Nigeria, the leaves of the plants are used for treating malaria (Olorunnisola et al. 2013).

3.2 Active Principles of Pigeonpea

Chemical analysis revealed high quantities of flavonoids and stilbenes in the leaves of pigeonpea. Saponins, a significant quantity of tannins, and modest amounts of reducing sugars, resin, and terpenoids were also reported from the plant (Pal et al. 2011). Pigeonpea flavonoids can be found in a variety of plant organs. There are 27 flavonoids

present. Among them flavones, isoflavones, and flavonols have been noticed in six, eight, and four numbers respectively. Besides them two anthocyanins and several flavanones, isoflavanones are also recorded, along with a solitary chalcone (Nix et al. 2015). Table 1 illustrates the selected flavonoids present in the plant. Apart from these, the plant contains stilbenes in the form of longistylene A (Wu et al. 2020) and longistylene C (Wang et al. 2011). *Cajanus* lactone and cajaninstilbene acid (Wu et al. 2009) along with pinostrobin have also been reported from the leaves of the plant (Patel and Bhutani 2014).

3.3 Pharmacological Uses of Pigeonpea

Since ancient times, different portions of pigeonpea have been used for their biological activity, and some of them have experimental grounds for acceptability. Aside from their use in traditional medicine, there have been various studies on pigeonpea's biological and pharmacological properties (Table 2).

3.3.1 Antibacterial Activity

The antibacterial activity of pigeonpea has been explored in a number of studies. In one experiment it was shown that the ethyl acetate leaf extraction contains naringenin that inhibited growth of *Salmonella typhi* and Staphylococcus *aureus* indicating its potential in the treatment of typhoid (Agus et al. 2017). It was shown that organic solvents extractions and water extracts were inhibiting *Escherichia coli, Staphylococcus aureus* growth, whereas *Klebsiella pneumonae* was inhibited by the extracts of organic solvents only. In addition, the minimum concentration of extract to inhibit *E. coli* was recorded as 0.125–0.25 mg/ml; to inhibit *S. aureus* it was found to be 0.125 mg/ml and that of *Salmonella typhi* was to be 0.0325–0.0625 mg/ml (Okigbo and Omodamiro 2007).

3.3.2 Antifungal Activity

Antifungal activity of the plant was evaluated using ethanolic extract of leaf and root. It was observed that extracts inhibited growth of *Candida albicans* and *Candida tropicalis*. Tannins, flavonoids, and alkaloids in extracts from both organs was discovered to have clinically significant antifungal activity (Brito et al. 2012).

3.3.3 Antiviral Activity

One study looked at the activity of water and ethanolic extracts against the measles virus as well as its toxic effect to embryonated chicken eggs. The in vivo assay using stem extraction in water provided a Log(2) titre of 0.1, and when the assay was done in vitro, a 100% suppression of cytopathic effect was observed in cell lines of Hep-2. Hemagglutination titration revealed a decrease in viral content ($p = 0.05$) at all concentrations of the extracts (Nwodo et al. 2011).

Table 1 Selected flavonoids isolated from pigeonpea

Types	Name	IUPAC name	Source plant organ	References
Flavones	Apigenin	5,7,4′-trihydroxyflavone	Leaves	Fu et al. 2008
	Luteolin	5,7, 3′,4′-tetrahydroxyflavone	Leaves	
	Vitexin	Apigenin 8-C-glucoside	Leaves	Fu et al. 2007
	Isovitexin	Apigenin 6-C-glucoside	Leaves	
	Orientin	Luteolin 8-C-glucoside	Leaves	Wei et al. 2013
Isoflavones	Biochanin A	5,7-Dihydroxy-4′-methoxyisoflavone	Leaves and roots	Duker-Eshun et al. 2004
	Cajanin	5, 2′,4′-Trihydroxy-7-methoxyisoflavone	Seed and etiolated stems	Dahiya 1987
	Genistein	5,7,4′-Trihydroxyisoflavone	Roots/root, bark, and etiolated stems	Duker-Eshun et al. 2004
	2′-Hydroxygenistein	5,7,2′,4′-Tetrahydroxyisoflavone	Roots/root, bark, and etiolated stems	Duker-Eshun et al. 2004
Flavonols	Quercetin	3,5,7,3′,4′-Pentahydroxyflavone	Leaves	Zu et al. 2006
	Isoquercitrin	Quercetin 3-β-D-glucoside	Pod surface	Green et al. 2003
	Isorhamnetin	3′-Methoxyquercetin	Leaves	Zu et al. 2006
Flavanones	Naringenin	5,7,4′-Trihydroxyflavanone	Leaves	Wei et al. 2013
	Pinostrobin	5-Hydroxy-7-methoxyflavanone	Leaves	Wei et al. 2013
Isoflavanone	Cajanol	5,4′-Dihydroxy-7,2′-dimethoxyisoflavanone	Roots	Luo et al. 2010
	Cajanone		Roots	Dahiya 1991
Chalcone	Pinostrobin	2′,6′-Dihydroxy-4′-methoxychalcone	Leaves	Patel and Bhutani 2014

Table 2 Pharmacological activities of pigeonpea

S. no.	Pharmacological activity	Parts	Form used	Active principle involved	References
1.	Antibacterial activity	Leaf	Ethyl acetate fraction of leaf extract	Naringenin	Agus et al. 2017
2.		Leaf	Petroleum ether, ethanol, and chloroform/ methanol mixture extracts (organic) Aqueous extract		Okigbo and Omodamiro 2007
3.	Antifungal activity	Leaf Root	Ethanolic extract of leaf and root		Brito et al. 2012
4.	Antiviral activity	Leaf Stem Root	Hot water and ethanol extract of leaf, stem, and root		Nwodo et al. 2011
5.	Antimalarial activity	Leaf	Methanol extract Column chromatographic technique with organic solvent systems used to isolate compound	Cajachalcone	Ajaiyeoba et al. 2013
6.		Root	Ethanolic extract of roots	Longistylin A and C, and betulinic acid	Duker-Eshun et al. 2004
7.	Antidiabetic activity	Root	Methanolic extract of roots		Nahar et al. 2014
8.		Leaf	Methnolic extract		Ezike et al. 2010
9.	Hypocholesterolemic effect	Leaf	Ethanolic extract followed by extraction with hexane and dichloro ethane	Cajanin, Longistylin C, and Longistylin A	Luo et al. 2008
10.	Hypolipidemic effect	Leaf	Methanolic extract		Akinloye and Solanke 2011

11.	Neuractive activity	Leaf	Ethanolic extract followed by partitioning and column chromatography using organic solvents	Pinostrobin	Nicholson et al. 2010
12.	Antihelminthic activity	Leaf	Extraction with petroleum ether, ethyl acetate, ethanol, and water		Khan et al. 2015
13.	Hepatoprotective	Leaf	Ethanolic extract		Iweala et al. 2019
14.	Anti-inflammatory activity	Root	Hot water and ethanolic extract extract		Vo et al. 2020
15.	Anti-inflammatory and antinociceptive activities	Seed	Hexane extracts	Quercetin-3-*O*-β-D-glucopyranoside, Orientin, Vitexin, Quercetin, Luteolin, Apigenin, Isorhamnetin	Hassan et al. 2016
16.	Anticancer activity	Root	Pure compound	Cajanol	Luo et al. 2010
17.		Root	Pure compound	Cajanin	Fu et al. 2015
18.		Stem, roots	Aqueous extract		Teixeira et al. 2021
19.	Antioxidant activity	Leaf	Aqueous extract Ethanol extract Petroleum ether extract Ethyl acetate fraction *n*-Butanol fraction	Cajaninstilbene acid Pinostrobin Vitexin Orientin	Wu et al. 2009

3.3.4 Antimalarial Activity

Antimalarial activity of the plant was determined in vitro utilizing *Plasmodium falciparum* (K1) which is a multiresistant strain. This variant was used in the parasite lactate dehydrogenase assay employing bioassay-fractionation of the pigeonpea leaf extraction in methanol. Various chromatographic techniques were used to isolate the compound, and spectroscopy was used to determine its structure. The physiologically active ingredient from the ethyl acetate fraction was identified as a cajachalcone also known as 2′,6′-dihydroxy-4-methoxy chalcone. The IC_{50} of cajachalcone was 2.0 μg/ml (7.4 μM). *Plasmodium falciparum* was inhibited by the extracts containing active principle (Ajaiyeoba et al. 2013). In another study, it was observed that in vitro assays performed with the *Plasmodium falciparum* strain 3D7 that shows chloroquine-sensitivity was moderately strong for various compounds like betulinic acid, longistylin A and C, stilbenes (Duker-Eshun et al. 2004).

3.3.5 Antidiabetic Activity

The antidiabetic activity of the methanolic root extract was monitored using alloxan-applied mice with diabetes for 5 days. This indicated that upon oral ingestion of extracts of plant at various doses of body weight (200–400 mg/kg), there was a significant reduction in serum fasting glucose in diabetic mice induced with alloxan (Nahar et al. 2014). Some studies demonstrated that when alloxan applied mice, showing diabetes, were administered with 400–600 mg/kg of methanolic extract, the fasting blood sugar reduced with maximum effect between 4 and 6 h (Ezike et al. 2010).

3.3.6 Hypocholesterolemic Effect

Hypocholesterolemic effect of the leaf extraction of pigeonpea was evaluated on diet-induced hypercholesterolemic mice. Excessive levels of serum and cholesterol from liver were significantly lessened by the 200 mg/kg plant extract after 4 weeks pretreatment, comparing to the model, by nearly 31% and 23% ($p = 0.01$), respectively. The proportions of serum and liver triglycerides were also minimized by 23% and 14%, respectively. During this time, LDL cholesterol from serum reduced by almost 53% ($p = 0.01$), whereas superoxide dismutase activity from serum rose by nearly 21%. The body weight and atherogenic index were both significantly lowered. mRNA transcript accumulation of HMG-CoA reductase, LDL-receptor, and CYP7A1were dramatically increased in mice given 200 mg/kg/day of plant extract, but the hypercholesterolemic diet repressed those expressions (Luo et al. 2008).

3.3.7 Hypolipidemic Effect

Methanolic extraction from leaves of the plant was tested for its hypolipidemic effect. The result showed a significant ($p = 0.05$) reduction in cholesterol, serum triglyceride, HDL, LDL, cholesterol, and blood glucose. The extract also reduced the functionality of aspartate transaminase and alanine transaminase along with reduction in levels of creatinine, urea and malondialdehyde levels in alloxan induced hyperglycemic mice (Akinloye and Solanke 2011).

3.3.8 Neuroactive Activity

Pinostrobin, from pigeonpea, was studied in vitro for its neuroactive characteristics and was found to inhibit voltage-gated sodium channels ($IC_{50} = 23$ μM). This study was based on the previously known background about pinostrobin, which has the capacity to reduce the depolarization effects of a certain selective activator of sodium channels called veratridine, in the brain synaptonemal complex of mice. This compound had nil effect on synaptoneurosomes resting membrane-potential. Pinostrobin's pharmacological profile is similar to that of depressive medications that block sodium channels (Nicholson et al. 2010).

3.3.9 Anthelminthic Activity

Antihelminthic activity was assessed using the ethanolic and aqueous extract of the pigeonpea. The results suggest that, aqueous extraction has anthelmintic action for paralyzing and killing Indian earthworm *Pheritima posthuma* for a long period at 5 mg concentration, whereas the ethanolic extract has paralysis and death in a short time at the same dosage (Khan et al. 2015).

3.3.10 Hepatoprotective Activity

The hepatoprotective activity of the plant was studied with respect to hepatotoxicity in male wistar rats. *N*-Nitrosodiethylamine (NDEA) induced hepatotoxicity which was reversed by the ethanolic extract of the leaf of the plant. The results indicated that pigeonpea-treated groups had considerably ($p = 0.05$) lower alanine and aspartate aminotransferases levels and significantly ($p = 0.05$) higher glutathione *S*-transferase, superoxide dismutase, glutathione, albumin, and catalase levels (Iweala et al. 2019).

3.3.11 Anti-inflammatory Activity

The anti-inflammatory activity of pigeonpea was evaluated in an in vitro experiment using RAW 264.7 cells. The results confirmed that 95% ethanolic extract of the roots dramatically reduced intracellular reactive oxygen species and increased superoxide dismutase and catalase activity. EECR95 induced nuclear factor (NF) erythroid 2-related factor 2/antioxidant protein heme oxygenase-1 and hindered nuclear factor kappa B (NF-B) signaling pathways, resulting in antioxidant and anti-inflammatory properties, according to mechanism studies (Vo et al. 2020). In another experiment, albino rats were used as experimentation models to study the anti-inflammatory and antinociceptive activities of the plant seeds. The results indicated that in hexane extract of seeds, twenty-one unsaponifiable chemicals (including various phytols, stigmasterol, 2,6-di-(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadiene-1-one, campesterol, and sitosterol) as well as fatty acids described mostly as palmitic acids and 9,12-octadecadienoic, almost 12 in numbers were found. Quercetin, Orientin, Luteolin, Quercetin-3-*O*-D-Glucopyranoside, Vitexin, Apigenin, and Isorhamnetin are all found in the n-butanolic extraction part. Three hours after carrageenan challenge, the hexane extract (200 and 400 mg/kg) reduced carrageenan induced inflammatory effects by a significant 85% and 95%, respectively. This was associated by a reduction in TNF- and IL-6 levels of 11% and 20%, 8% and 13%, respectively, as well as a significant reduction in IgG serum quantity. In

addition, hexane fraction (200 and 400 mg/kg) reduced writings by 61 and 83%, respectively (Hassan et al. 2016).

3.3.12 Anticancer Activity

The anticancer activity of cajanol, an isoflavanone derived from pigeonpea roots, was noted in a study using breast cancer cell lines from human (MCF-7). Cajanol suppressed MCF-7 cell growth depending upon dose- and time-specificity. After 24 h of treatment, the IC_{50} value was 83.42 μM, reached 58.32 μM after 48 h, and reduced to 54.05 μM after 72 h. Cajanol used a ROS-mediated mitochondria-dependent route to inhibit the cell cycle in the G2 and M stage and cause programmed cell death. Cajanol blocked the expression of Bcl-2 expression and elevated expression of the Bax gene, which led to the rupture of the outer mitochondrial membrane and resulted in cytochrome c liberation, as experimented through Western blot. The induction of the caspase-9 and caspase-3 cascades was linked to mitochondrial cytochrome c release, while active-caspase-3 was engaged in PARP cleavage (Luo et al. 2010). Another research showed cajanin stilbene acid obtained from the plant were investigated for its anticancer properties. Cajanin caused apoptosis and G2/M inhibition in a concentration-specified manner. Matrix Metalloproteinases was degraded, Bax level was increased, Bcl-2 was decreased, and caspase-3 was induced. BRCA-specific DNA impairment responsive pathways as well as cell cycle-controlling chromosome replicative pathways were both impacted by cajanin stilbene acid, according to microarray profiling (Fu et al. 2015). Other study indicated that the fractions of stem and root extracts inhibited melanoma proteases and generated cellular toxicity in SK-MEL-28 cells, cultured in vitro (Teixeira et al. 2021).

3.3.13 Antioxidant Activity

In a study, the antioxidative nature of pigeonpea from aqueous and ethanolic leaf extracts, as well as ethyl acetate, n-butanol, petroleum ether, and water fractions, as well as the four main compounds separated from the ethanol extract, namely pinostrobin, cajaninstilbene acid, orientin, and vitexin were investigated by a DPPH radical-scavenging assay. An IC_{50} value of 194.98 μg/ml, the ethyl acetate fraction had the highest scavenging power among the four fractions. Pinostrobin and vitexin were shown to have less effective radical-scavenging powers than cajaninstilbene acid (302.12 μg/ml) and orientin (316.21 μg/ml). The inhibition ratio (%) of the ethyl acetate fraction (94.13% ± 3.41%) was found to be the greatest in the beta-carotene-linoleic acid test, practically matching the inhibitory capability of the positive control BHT (93.89% ± 1.45%) at 4 mg/ml. When compared, cajaninstilbene (321.53 μg/ml) and orientin (444.61 μg/ml) had moderate antioxidant effects, while pinostrobin and vitexin both exhibited antioxidant activities at greater than 500 μg/ml (Wu et al. 2009).

4 Genetic Resources of Health-Related (HR) Genes

A large number of genetic resource accumulations, including genetic maps, molecular markers, whole-genome resequencing (WGRS) data, transcriptome assemblies, a reference genome sequence (Fig. 3) (Varshney et al. 2012) from multiple cultivars,

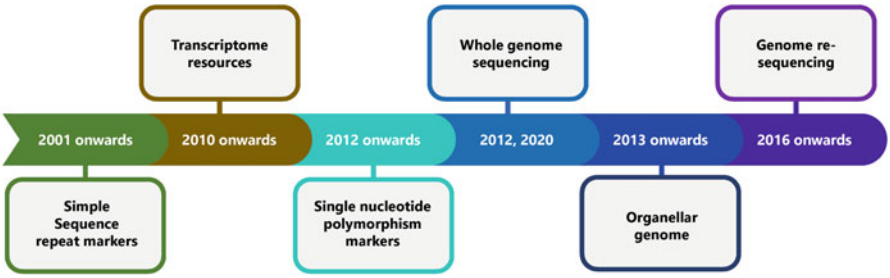

Fig. 3 Timeline of major genomic approaches adopted in pigeonpea

have become available in pigeonpea (Kumar et al. 2016; Varshney et al. 2017). These resources have aided in the creation of high-resolution genetic maps as well as efficient and expeditious genetic analysis of quantitative trait loci (QTLs) and genes regulating important nutritional traits in pigeonpea (Saxena et al. 2012).

Pigeonpea is an essential food source with amino acid rich plant protein for more than a billion people worldwide. However, genetic improvement for seed protein content (SPC) in the crop has acquired little concern in the past. The use of genomics-assisted breeding could aid in the acceleration of SPC genetic gain. Four genotypes of pigeonpea were taken for whole-genome resequencing data to recognize sequence-based markers and associated possible SPC genes (Obala et al. 2019). One hundred and eight sequence variations obtained from 57 genes were recognized by combining a common variant sieving methodology on already procured WGRS data with the gene functioning data concerning SPC. Subsequently, 17 of the 30 sequence variants when transformed into CAPS/dCAPS markers showed significant polymorphic traits between genotypes of low and high SPC. A significant ($p = 0.05$) co-segregation of 4 of the CAPS/dCAPS markers was observed with SPC when 16 polymorphic CAPS/dCAPS markers were tested on F_2 generation which is a cross of ICP 5529 and ICP 11605, former with high SCP and the latter with low SCP. In summary, mutations in four gene sequences gave rise to four markers and were suggested to be helpful in pigeonpea crop improvement programmes for enhancing/regulating SPC (Obala et al. 2019).

5 Classical Genetics and Traditional Breeding for HR Traits

Over the last two decades, many attempts have been made to create high-yielding cultivars by traditional breeding methods and advancements in biotechnology. These investigations have given information and understanding for creating superior pigeonpea varieties with many agronomically important quality characters and show great yield potential even in challenging agro-climatic settings. New cultivars with better nutritional content and boosting production potential have already been created using traditional plant-breeding techniques. To develop genotypes with the required nutritionally rich and agronomically superior features, classical plant breeding requires identifying and developing parental lines showing enhanced nutrition-rich content, crossing the latter with elite germplasm, and selection of the

segregating population for some generations (Pfeiffer and McClafferty 2007). Thus, it pertains to a much-extended time to procure a novel or better variety. The complications at genetic level of quantitative traits and low heritability are some bottlenecks that pose challenges for selecting superiors.

Due to a number of specific features, breeding of pigeonpea has proven to be more difficult than breeding other edible legumes. Pigeonpea is often cross-pollinated crop. Insect-aided natural outcrossing rates of 20–70% in pigeonpea, have restricted the application of effective selection and mating methods are available in self-pollinating species (Saxena and Sharma 1990). This crop's yield potential has gradually increased due to the employment of extensive hybridization, pure line breeding, population breeding along with mutation breeding hence create new pigeonpea varieties. Two genetic male-sterility (GMS) systems were found in pigeonpea to help with this bottleneck (Reddy et al. 1979). The GMS-based hybrids had a yield which was 30% more than that of nonhybrids but did not prove to be commercially viable because of its exorbitant production cost.

The alternative and more effective cytoplasmic-genetic male-sterility (CGMS) approach was created in response to the yield-jump seen in the GMS hybrids (Saxena and Kumar 2003). In 2004, India had its first cytoplasmic male sterility (CMS)-based hybrid GTH-1 available from ICRISAT's hybrid development programme in partnership with its partners. Furthermore, another CMS-based pigeonpea hybrid, ICPH 2671, was created in 2005 at ICRISAT utilizing *C. cajanifolius* (A4 cytoplasm) and has since been commercially available by Pravardhan Seeds under the name "Pushkal" for cultivation in various Indian states, including Maharashtra, Madhya Pradesh, Karnataka, and Andhra Pradesh. The expanded area cultivating pigeonpea hybrids is projected to result in higher crop yield and satisfying returns for farmers and pigeonpea production in a sustainable manner was possible. This will again be made feasible by ongoing attempts to breed resistance to biotic and abiotic challenges.

Besides breeding for yield, breeding for nutrition has always been the focus of pigeonpea breeders. Despite pigeonpea being the household dal, consuming every single day, the average protein requirement of an Indian adult is not met. Hence, a breeding programme was initiated back in 1982 at ICRISAT. ICRISAT's genebank houses 13,632 germplasm which has a protein range from 9% to 30% (Varshney et al. 2012). Protein content in pigeonpea is controlled by additive genetic action. Based on available information from the genebank, wild progenitors *C. scarabaeoids* (28.4%), *C. sericeous* (29.4%), and *C. albicans* (30.5%) were utilized to develop new protein lines. Accordingly, newly bred lines, called high protein lines (HPL) reported protein content up to 32%. These lines are in preliminary yield testing stage and serve as a donor for high protein trait in a breeding program. This twenty-first century has greater innovation in terms of protein. Protein based markets are worth USD 38 billion (2019) and is expected to grow at a rate of 9.1% from 2020 to 2027. Increasing traction towards plant-based protein (either as protein isolate or protein concentrates) is a greater opportunity for paradigm shift in nutritional breeding. Utilization of indigenous crops for protein source has been the current focus in Indian protein market. "Smart Protein" is a budding concept, pulses including pigeonpea is a part of this initiative. Harnessing the protein content of indigenous crops to be used as alternative protein

source without burdening the environment is the aim. With nonmeat, vegan, dairy-free, vegetarian, and ethical food systems in rise "smart protein" will be the future.

Next nutritive trait is Fe and Zn. The recommended daily allowance (R.D.A.) of Fe for a child and an adult in India is 13 and 17 mg per-day, respectively. Whereas the R.D.A. of per-day Zn for a child and an adult is 7 and 12 mg. Nevertheless, a food proportion of 7 g a day per person in India, imparts a daily per capita iron intake of 14.93 mg, which is much lesser than R.D.A. With this backdrop, a baseline study of genetic variability was taken for Fe and Zn content in pigeonpea at ICRISAT. Accordingly, a range of 24.91–44.65 mg/kg seed for Fe content and 26.08–47.80 mg/kg seed for Zn content was noted. Both wet methods, as well as Energy-dispersive X-ray fluorescence technique, were used to calibrate and estimate whole seed Fe and Zn content. A breeding programme is halfway in fortifying for Zn and Fe in pigeonpea. Marker-assisted backcrossing is effectively carried out for forwarding the generation.

Recent development of early and photo-insensitive pigeonpea lines coupled with rapid-generation turnover methods has helped in fast-forwarding the generation. Interestingly, early genotypes are high in nutritional traits and is a win-win situation for introgression and generation advancement. Unlike the 1990s, three cropping seasons with year-round breeding can now be done. Conventional breeding coupled with genomic selections has increased the selection efficiency. Reduction in time taken for completion of a cropping season has increased the genetic gains in pigeonpea.

6 Genetic Diversity with Regard to HR Traits

Molecular markers play a pivotal role in genetic improvement program of any crop. These are used both in the genetic diversity assessments as well as trait-specific molecular mapping. Various kinds of molecular markers have been adopted in pigeonpea also including first generation restriction fragment length polymorphism (RFLP), and subsequently, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), and latest single-nucleotide polymorphism (SNP) (Saxena et al. 2014; Pazhamala et al. 2015) markers. Amongst these, SNP markers stand for ideal DNA marker owing to their higher abundance throughout the genome and high throughput estimation procedure, apart from other advantages of a codominant marker.

WGRS was given about 292 accessions to track the genetic diversity of pigeonpea. This included wild species, landraces, and breeding lines, yielding a total count of 17.2 million variations (Varshney et al. 2017). To discover how several candidate genes were related to agronomically significant variables, a GWAS was conducted. Sequence similarities exist between the genes functionally described in other plants for flowering time control, seed development, and pod dehiscence and the candidate genes for these features in pigeonpea. These polymorphic locations will help create high-density SNP arrays, genotyping of various mapping populations to create genetic maps, and identify the genomic areas underlying significant agronomic features. A total of 932 markers were used to create a condensed intraspecific pigeonpea linkage map, covering an overall adjusted map

length of 1411.83 cM to enhance chromosomal anchoring and to map the genes linked to useful agricultural traits. It contains 65 SSR marker loci, 319 RAD-SNPs, and 547 bead-array SNPs (Arora et al. 2017). The genetic advancement of pigeonpea could be sped up with the help of this information. Recently, two high-density Affymetrix Axiom genotyping chips have been created in pigeonpea to accelerate the genetic gain. A 56 K *Cajanus* SNP chip has been created to study the genetic variation across 103 pigeonpea lines (Saxena et al. 2018).

7 Molecular Mapping of HR Genes and QTLs

High-throughput genotyping applications have caused drastic improvements in the density of markers which were used to generate genetic maps of pigeonpea. These have been adopted in pigeonpea, too, for the last two decades. Several genotyping programs targeting the F_2 populations have resulted in high-density genomic maps to date (Arora et al. 2017; Saxena et al. 2017; Yadav et al. 2019). Such genetic resources were crucial to dissect the genomic design of agronomic traits in pigeonpea, including its nutritional appearances. Fine mapping of QTLs responsible for nutritive properties of pigeonpea is essentially required to generate superior cultivars/genotypes with potential well-being properties (Fig. 4).

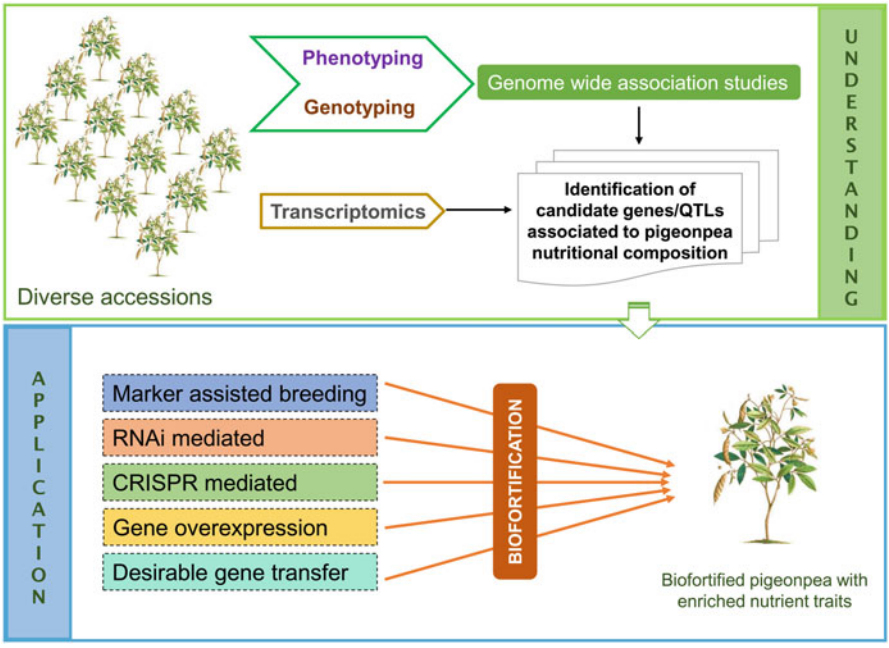

Fig. 4 Overview of concurrent genomic technologies for designing biofortification of pigeonpea

8 Marker-Assisted Breeding for HR Traits

In recent times, the availability of convenient library preparation methods and greater multiplexing capacity has facilitated the genotyping-by-sequencing (GBS) approach as a promising tool for the simultaneous discovery and characterization of numerous SNPs (Saxena et al. 2017). Whole-genome resequencing (WGRS) has become the latest high-throughput option for determining genetic variation and trait-linked marker discovery. Accordingly, an SNP array has been developed by resequencing diverse germplasm of pigeonpea with as many as 56,512 unique informative sequence variations (Saxena et al. 2018). Furthermore, identifying key agronomic traits associated with 1554 SNPs and 385 insertion/deletion (InDel) markers potentially enriched the genomic resource in pigeonpea toward marker-assisted selection. The WGRS-based first-generation HapMap of pigeonpea unveiled 5.5 million genome-wide variants (4.6 million SNPs and 0.7 million InDels) (Kumar et al. 2016). Using a different whole-genome resequencing method, candidate gene sequence-based markers in relation to seed protein content were recognized, using four pigeonpea genotypes (Obala et al. 2019). The first-generation HapMap in *Cajanus* spp. was created using the whole-genome resequencing (WGRS) method to develop genetic resources. In a panel comprising of 20 *Cajanus* spp., including 2 wild and 18 cultivated species, there are 5,465,676 genome-wide variants, comprising 4,686,422 SNPs and 779,254 InDels. These sequence variations make mapping the genomic areas underlying fundamental features possible.

9 Map-Based Cloning of HR Genes/QTLs

Pigeonpeas have a protein level of about 21%. However, because they contain less lysine than other legumes, they have poor nutritional value. Dihydrodipicolinate Synthase, or DHDPS, is a crucial regulator of lysine biosynthesis. The DHDPS genes is inactivated by even trace amounts of lysine via a feedback mechanism, as a result pigeonpea exhibits low levels of lysine. Hence, the pigeonpea was transformed with the mutant DHDPS gene (dhdps-r1 from *Nicotiana sylvestris*), since it is no longer responsive to the feedback inhibition by lysine. DHDPS activity was two to six times higher in transgenic pigeonpea, resulting in an 8.5-fold increase in the amount of free lysine in the seeds (Thu et al. 2007). Additionally, pigeonpea has been utilized in the creation of edible vaccinations. With a transformation efficiency of roughly 67%, the Rinderpest virus's haemagglutinin protein antigen was successfully produced in pigeonpea (Satyavathi et al. 2003). An Indian isolate of the Peste des Petits Ruminants (PPR) virus's hemagglutinin-neuraminidase gene (HN) has also been successfully converted and expressed in transgenic pigeonpea. Neuraminidase activity showed that HN protein was physiologically active in transgenic pigeonpea (Prasad et al. 2004).

10 Genomics-Aided Breeding for HR Traits

Conventionally identified QTLs controlling key agronomic traits in pigeonpea available so far (Bohra et al. 2019; Varshney et al. 2013) are inconvenient due to time challenges, cost, and labor faced by those low-throughput marker systems. The pitfalls of conventional marker systems can be overcome by employing high-density genome-wide marker systems. Genome-wide association study (GWAS) is one of the approaches that address the concern of low precision conventional QTL mapping. Instead, being independent of the biparental mapping population helps better understand the genomic background underlying complex phenotypic traits with higher resolution (Huang and Han 2014; Liu and Yan 2019). Accordingly, association mapping of diverse genotypes came out with the significant number of SSRs and SNPs throughout pigeonpea genome governing multiple traits of interest (Mir et al. 2014; Patil et al. 2017). The breakthrough GWAS of 286 resequenced pigeonpea accessions pinpointed numerous marker trait associations related to domestication and with prospects to breeding (Varshney et al. 2017). Nonetheless, more rigorous genotyping of potential accessions/cultivars and simultaneous high-resolution marker-trait association studies would still be required for the efficient next-generation genomics-assisted breeding programs in pigeonpea.

11 Transgenic Studies

Owing to properties such as rapid growth, lofty protein content, capacity to tolerate drought conditions, and a deep root system, pigeonpea is an economically essential crop. There is a huge breach created between the demand and supply of pigeonpea. This has been caused due to the explosion of population and the interplay of biotic and abiotic stresses affecting the growth of the crop. Biotic factors include certain insect pests, like *Helicoverpa armigera*; and some fungal diseases like Fusarium wilt. Abiotic stresses which lead to a drop in productivity include salinity and water logging. Other factor like extensive use of pesticides and herbicides which decreases soil fertility also effects the production of pigeonpea (Negi et al. 2021). Crop breeding has been the most traditional and well-established method of crop improvement. Plant breeding in pigeonpea is a laborious and time-consuming process. One of its main drawbacks is the restricted genetic diversity that results from gene loss during artificial selection. In order to resolve the issues and increase the pigeonpea production, several biotechnological approaches have been used. One of the most triumphant biotechnological approaches has been transgenic technology which removes the major breeding barriers. The development of transgenic technology has demonstrated remarkable success in pulse crop protection. It has also long-term supported research on the inclusion of agronomically advantageous traits, which improves crops and increases the world's population's access to high nutritious food (Saxena et al. 2016). The effective integration of many foreign genes using recombinant DNA technology has opened up new possibilities for the creation of tolerant pigeonpea cultivars with built-in resilience to survive biotic stress factors (Ghosh et al. 2014a).

The availability of several transformation techniques has facilitated the production of effective transgenic crops in many crop species. Of them, *Agrobacterium tumefaciens*-mediated genetic transformation is the most practical and widely applied method on a variety of plants. Researchers employed genetic transformation technology to improve more than 15 cultivars of the pigeonpea by enhancing nutritional quality or by including resilience against various environmental factors. Transgenic pigeonpea has been developed by incorporating a variety of genes, including cowpea *protease inhibitor* (*CPI*), *Bacillus thuringiensis* endotoxins *cry1A(b), cry1Ab, cry1Aabc, cry1AcF, cry1Ac, cry2Aa, and cry1 E-C,* etc. This has elevated the toxicity against the lepidopteran insects (Nandini et al. 2022).

The antibiotic selection based in vitro tissue culture approaches showed numerous drawbacks despite extensive use, such as after successful transformation, small percentage of totipotent cells were able to survive, the selection pressure lowering the explants' overall capacity for regeneration, and inadequate rooting responses (Ghosh et al. 2014b). In 2008, Ramu et al. first introduced *in planta* transformation method which fully skipped the in vitro co-cultivation and selection process and produce a large number of transgenics. Ghosh et al. (2017) developed a unique shoot grafting technique to develop Cry1Ac and Cry2Aa transgenic pigeonpea lines with steady DNA integration up to the T_2 generation. Furthermore tissue culture independent technique was introduced by Ganguly et al. (2018) as plumular meristem transformation method with increasing transformation frequency and PCR based screening process.

11.1 Transgenic Pigeonpea Development for Biofortification

Various agronomically important genes has been discovered in well-characterized systems like *Arabidopsis*, tobacco, rice, pea, carrot, and other plants, and scientists were working to create transgenic pigeonpea plants that were resistant to biotic, abiotic stresses, and with good agronomic traits. (Banu et al. 2014).

The ability to fix nitrogen in the roots is one of the most significant crop-specific characteristics of pigeonpea. This attribute improves and increases soil fertility. However, due to its high fixation in soil and low mobility, availability of phosphorous is constrained. As an adaptive strategy, plants vary the number of lateral roots, develop excessively root hair, and exude organic acids, particularly citrate to alter the rhizosphere (Shen et al. 2005). In order to refine and upgrade P uptake, Transgenic pigeonpea was created by overexpressing *Daucus carota citrate synthase* (DcCs) gene from carrot (*Daucus carota*), under a constitutive and root specific promoter. In both P deficient and P available situations, transgenic pigeonpea lines overexpressing the DcCs gene demonstrated higher level citrate synthase production and enhanced root growth (Hussain et al. 2016).

Pigeonpea serves as an important source of protein, often high lysine content and complements the protein in cereals. Although during agricultural processing, lysine and tryptophan are lost in large amounts (Singh and Eggum 1984). Additionally, *Dihydrodipicolinate synthase* (*DHDPS*), the main enzyme of lysine biosynthesis pathway is also feedback-inhibited by lysine. Under the control of a phaseolin seed-

specific promoter, a mutant *dhdps-r1* gene from *Nicotiana sylvestris* that expresses a lysine insensitive enzyme was inserted into the pigeonpea genome by Thu et al. (2007) through particle bombardment and *Agrobacterium* mediated transformation. They examined 11 lines which showed two- to sixfold increase in DHDPS activity compared to wild type in immature seeds at a late stage of development. In comparison to control lines, the *dhdps-r1* overexpression increased the free lysine concentration in pigeonpea seeds by 1.6–8.5 times.

Proline is an important amino acid in plants functions as an osmoprotectant and is crucial for maintaining osmotic balance, safeguarding enzymes and subcellular structures, and raising cellular osmolarity, which provides the turgor required for cell expansion under stressful circumstances. The rate-limiting enzyme in the production of proline, *1-pyrroline-5-carboxylate synthetase* (*P5CS*), is also inhibited by proline through feedback inhibition. Surekha et al. (2014) inserted a mutated version of *P5CS* named *P5CSF129A* from *Vigna aconitifolia* into pigeonpea genome. This mutated *P5CSF129A* gene is indifferent of feedback control. T_0 transgenic generation showed higher proline accumulation than control plants. A significant improvement was seen in chlorophyll content and growth performance in T_1 lines alongside decreased levels of lipid peroxidation. The relative water content under high salinity also showed improvement. *Render pest virus* (*RPVH*) and *peste des petits ruminants' virus* (*PPRV-HN*) both are the causal agents of devastating diseases in cattle animals with very high mortality rate such as cattle plague and Peste des Petits Ruminants respectively. New vaccination methods were developed using pigeonpea transformation to strengthen the immune systems of sheep, goats, and bovids against those viruses as the existing live attenuated vaccines are heat labile. Satyavathi et al. (2003) developed pigeonpea line that express *Rinderpest virus's hemagglutinin* protein. T_1 Pigeonpea leaves had the highest expression of the *hemagglutinin* protein at 0.49% of the total soluble protein. The transgene was expressed in the offspring of the fertile transgenic plants. Prasad et al. (2004) successfully generated transgenic pigeonpea lines by inserting two *PPRV* surface glycoproteins, hemagglutinin-neuraminidase, and fusion protein using pBI121 binary vector. T_1 plants showed transgene's inheritance.

Extracellular enzymes, especially those that cause the proteolytic breakdown of proteins in host plants are secreted by many phytopathogenic bacteria and some insects and crucial for pathogenesis. Plants have many inhibitors that work against these proteolytic enzymes as a key line of defense against these diseases. One such inhibitor named *cowpea protease inhibitor* (*CPI*), isolated from cowpea was inserted into pigeonpea genome through *Agrobacterium* mediated transformation. Transgenic pigeonpea lines showed higher level of defense against the lepidopteran insects (Lawrence and Koundal 2001).

11.2 Biofortification Resources of Pigeonpea Used in Other Transgenic Crops

In pigeonpea, under biotic and abiotic stress conditions, complex signaling pathways were found to be activated, causing changes in gene expression, necessary for plants

to adapt and acclimate. One such gene named *Pigeonpea hybrid-proline-rich protein encoding gene* (*CcHyPRP*) was used to develop transgenic tolerance lines in rice by Mellacheruvu et al. (2016). *CcHyPRP* was cloned under an inducible *rd29A* promoter and a constitutive *CaMV35S* promoter. Four independent homozygous T4 lines for each rd29ACcHyPRP and CaMV35SCcHyPRP were developed, which revealed very high accumulation of proline and endochitinase. In comparison to the control lines, the CcHyPRP transgenics showed greater resistance to rice blast disease causing fungus *Magnaporthe grisea*. Transgenic rice was shown to have more bZIP and endochitinase transcripts and endochitinase activity than control plants. These T_4 lines also demonstrated excellent levels of tolerance to the main abiotic stimuli, including heat, salinity, and drought, as demonstrated by enhanced chlorophyll content, survival rate, biomass, root, and shoot growth, in comparison to the untransformed lines. Additionally, under various biotic and abiotic stress situations, transgenic rice lines had larger panicles and more grains in comparison. In comparison to the control, the *CcHyPRP* transgenics showed increased catalase and superoxide dismutase (SOD) enzyme activity as well as decreased malondialdehyde (MDA) levels.

12 Future Prospects

In the post-green revolution period, improving the nutritional value of pigeonpea has become crucial for reducing malnutrition issues in developing nations. Establishing desired genotypes will be aided by in-depth knowledge of the genes and QTLs related to nutritional quality and seed quality (Singh et al. 2020). In order to develop molecular techniques aiming at enhancing seed quality and other nutritionally related qualities in pigeonpea, it will be essential to identify the genes/QTLs controlling the quality traits. To define quality features, attention should be paid to locate genetically varied and nutritionally improved pigeonpea lines (Singh et al. 2020). In order to measure various phenotypic features, it is crucial to design a high-throughput phenotyping platform. Examples of techniques that will be impactful for high throughput phenotyping include picture-based computer vision phenotyping, image processing, and data extraction tools. All integrated approaches will improve the understanding of systems biology by providing information on gene function, genomic architecture, organization, biological pathways, and metabolic and regulatory networks (Fig. 4).

The world's problems with malnutrition can be addressed in a new way by utilizing and combining cutting-edge NGS "omics" technology to sequence vast populations, uncover the genetic basis of agronomically essential traits, and anticipate breeding value. Breeders will be aided to gather information on specific alleles of known genes involved in nutritional grain quality attributes to achieve this goal through the availability of gene-based markers and cutting-edge techniques. Genomic regions/genes can be found that are expected to influence seed quality and nutritional qualities of interest by genotyping and phenotyping for those traits utilizing associations and machine learning models, drawing on the collection and

use of numerous unrelated lines. When omics technologies are used in conjunction with breeding programmes, it is anticipated that the nutritional quality of pigeonpea will improve.

References

Ade-Omowaye BIO, Tucker GA, Smetanska I (2015) Nutritional potential of nine underexploited legumes in Southwest Nigeria. Int Food Res J 22(2):798

Agus S, Achmadi SS, Mubarik NR (2017) Antibacterial activity of naringenin-rich fraction of pigeon pea leaves toward *Salmonella thypi*. Asian Pac J Trop Biomed 7(8):725–728

Ajaiyeoba EO, Ogbole OO, Abiodun OO, Ashidi JS, Houghton PJ, Wright CW (2013) Cajachalcone: an antimalarial compound from *Cajanus cajan* leaf extract. J Parasitol Res 2013:703781

Akinloye OA, Solanke OO (2011) Evaluation of hypolipidemic and potential antioxidant effects of pigeon pea (*Cajanus cajan* (L) Millsp.) leaves in alloxan-induced hyperglycemic rats. J Med Plant Res 5(12):2521–2524

Apata D (2008) Effect of cooking methods on available and unavailable carbohydrates of some tropical grain legumes. Afr J Biotechnol 7:2940–2945

Arora S, Mahato AK, Singh S, Mandal P, Bhutani S, Dutta S, Kumawat G, Singh BP, Chaudhary AK, Yadav R (2017) A high-density intraspecific SNP linkage map of pigeonpea (*Cajanas cajan* L. Millsp.). PLoS One 12:e0179747

Banu SA, Huda KMK, Tuteja N (2014) Isolation and functional characterisation of the promoter of a DEAD-box helicase Psp68 using *Agrobacterium*-mediated transient assay. Plant Signal Behav 9(6):e28992

Bohra A, Bharadwaj C, Radhakrishnan T, Singh NP, Varshney RK (2019) Translational genomics and molecular breeding for enhancing precision and efficiency in crop improvement programs: some examples in legumes. Indian J Genet 79:227–240

Brito SA, Rodrigues FF, Campos AR, da Costa JG (2012) Evaluation of the antifungal activity and modulation between *Cajanus cajan* (L.) Millsp. leaves and roots ethanolic extracts and conventional antifungals. Pharmacogn Mag 8(30):103–106

Dahiya JS (1987) Reversed-phase high-performance liquid chromatography of Cajanus cajan phytoalexins. J Chromatogr A 409(C):355–359

Dahiya JS (1991) Cajaflavanone and cajanone released from Cajanus cajan (L. Millsp.) roots induce nod genes of Bradyrhizobium sp. Plant Soil 134(2):297–304

Duker-Eshun G, Jaroszewski JW, Asomaning WA, Oppong-Boachie F, Brøgger Christensen S (2004) Antiplasmodial constituents of *Cajanus cajan*. Phytother Res 18(2):128–130

Ezike AC, Akah PA, Okoli CC, Okpala CB (2010) Experimental evidence for the antidiabetic activity of *Cajanus cajan* leaves in rats. J Basic Clin Pharm 1(2):81

Fanou BA, Klotoe JR, Fah L, Dougnon V, Koudokpon CH, Toko G, Loko F (2020) Ethnobotanical survey on plants used in the treatment of candidiasis in traditional markets of southern Benin. BMC Complement Med Ther 20(1):288

Fu YJ, Zu YG, Liu W, Hou CL, Chen LY, Li SM, Shi XG, Tong MH (2007) Preparative separation of vitexin and isovitexin from pigeonpea extracts with macroporous resins. J Chromatogr A 1139(2):206–213

Fu YJ, Liu W, Zu YG, Tong MH, Li SM, Yan MM, Efferth T, Luo H (2008) Enzyme assisted extraction of luteolin and apigenin from pigeonpea [Cajanus cajan (L.) Millsp.] leaves. Food Chemistry 111(2):508–512.

Fu Y, Kadioglu O, Wiench B, Wei Z, Gao C, Luo M, Gu C, Zu Y, Efferth T (2015) Cell cycle arrest and induction of apoptosis by cajanin stilbene acid from *Cajanus cajan* in breast cancer cells. Phytomedicine 22(4):462–468

Ganguly S, Ghosh G, Purohit A, Kundu Chaudhuri R, Chakraborti D (2018) Development of transgenic pigeonpea using high throughput plumular meristem transformation method. Plant Cell Tissue Organ Cult 135(1):73–83

Ghosh G, Purohit A, Chaudhuri RK, Chakraborti D (2014a) Advances in genetic transformation of important pulse crop pigeonpea. OA Biotechnol 12:5

Ghosh G, Purohit A, Ganguly S, Chaudhuri RK, Chakraborti D (2014b) In vitro shoot grafting on rootstock: an effective tool for *Agrobacterium*-mediated transformation of pigeonpea (*Cajanus cajan* (L.) Millsp.). Plant Biotechnol 31:301–308

Ghosh G, Ganguly S, Purohit A, Chaudhuri RK, Das S, Chakraborti D (2017) Transgenic pigeonpea events expressing Cry1Ac and Cry2Aa exhibit resistance to *Helicoverpa armigera*. Plant Cell Rep 36(7):1037–1051

Green PWC, Stevenson PC, Simmonds MSJ, Sharma HC (2003) Phenolic compounds on the pod-surface of pigeonpea, Cajanus cajan, mediate feeding behavior of Helicoverpa armigera larvae. J Chem Ecol 29(4):811–821

Gumisiriza H, Birungi G, Olet EA, Sesaazi CD (2019) Medicinal plant species used by local communities around Queen Elizabeth National Park, Maramagambo Central Forest Reserve and Ihimbo Central Forest Reserve, South western Uganda. J Ethnopharmacol 239:111926

Hassan EM, Matloub AA, Aboutabl ME, Ibrahim NA, Mohamed SM (2016) Assessment of anti-inflammatory, antinociceptive, immunomodulatory, and antioxidant activities of Cajanus cajan L. seeds cultivated in Egypt and its phytochemical composition. Pharmaceutical biology 54(8):1380–1391

Huang X, Han B (2014) Natural variations and genome-wide association studies in crop plants. Annu Rev Plant Biol 65:531–551

Hussain AI, Pavithra HV, Sreevathsa R, Nataraja KN, Babu N (2016) Development of transgenic pigeonpea (*Cajanus cajan* L. Millsp.) overexpressing citrate synthase gene for high phosphorus uptake. Indian J Exp Biol 54(8):493–501

Iweala EE, Evbakhavbokun WO, Maduagwu EN (2019) Antioxidant and hepatoprotective effect of *Cajanus cajan* in *N*-nitrosodiethylamine-induced liver damage. Sci Pharm 87(3):24

Khan R, Soni LK, Jain S (2015) Anthelmintic activity of leaves of *Cajanus cajan* Linn on Indian earthworm. Asian J Pharm Health Sci 5(4):1327–1330

Koné WM, Koffi AG, Bomisso EL, Tra Bi FH (2011) Ethnomedical study and iron content of some medicinal herbs used in traditional medicine in Cote d'Ivoire for the treatment of anaemia. Afr J Tradit Complement Altern Med 9(1):81–87

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 14(8):1673–1681

Lans C (2007) Comparison of plants used for skin and stomach problems in Trinidad and Tobago with Asian ethnomedicine. J Ethnobiol Ethnomed 3:3

Lawrence PK, Koundal KR (2001) *Agrobacterium tumefaciens* mediated transformation of pigeonpea (*Cajanus cajan* L. Millsp.) and molecular analysis of regenerated plants. Curr Sci 80:1428–1432

Liu HJ, Yan J (2019) Crop genome-wide association study: a harvest of biological relevance. Plant J 97:8–18

Luo QF, Sun L, Si JY, Chen DH (2008) Hypocholesterolemic effect of stilbenes containing extract-fraction from *Cajanus cajan* L. on diet-induced hypercholesterolemia in mice. Phytomedicine 15(11):932–939

Luo M, Liu X, Zu Y, Fu Y, Zhang S, Yao L, Efferth T (2010) Cajanol, a novel anticancer agent from pigeonpea [*Cajanus cajan* (L.) Millsp.] roots, induces apoptosis in human breast cancer cells through a ROS-mediated mitochondrial pathway. Chem Biol Interact 188(1):151–160

Mellacheruvu S, Tamirisa S, Vudem DR, Khareedu VR (2016) Pigeonpea hybrid-proline-rich protein (CcHyPRP) confers biotic and abiotic stress tolerance in transgenic rice. Front Plant Sci 6:1167

Mir RR, Kudapa H, Srikanth S, Saxena RK, Sharma A, Azam S, Saxena K, Varma Penmetsa R, Varshney RK (2014) Candidate gene analysis for determinacy in pigeonpea (*Cajanus* spp.). Theor Appl Genet 127:2663–2678

Nahar L, Nasrin F, Zahan R, Haque A, Haque E, Mosaddik A (2014) Comparative study of antidiabetic activity of *Cajanus cajan* and *Tamarindus indica* in alloxan-induced diabetic mice with a reference to in vitro antioxidant activity. Pharmacognosy Res 6(2):180–187

Nandini B, Reddy UG, Mallikarjuna BP, Manu B, Vaijayanthi PV, Ashwini M, Chakraborti D (2022) Genomic design for abiotic stress resistance in pigeonpea. In: Kole C (ed) Genomic design for abiotic stress resistant pulse crops. Springer, Cham, pp 169–248

Negi J, Rathinam M, Sreevathsa R, Kumar PA (2021) Transgenic pigeonpea [*Cajanus cajan* (L). Millsp.]. In: Kavi Kishor PB, Rajam MV, Pullaiah T (eds) Genetically modified crops: current status, prospects and challenges, vol 1. Springer, Singapore, pp 79–96

Nicholson RA, David LS, Pan RL, Liu XM (2010) Pinostrobin from *Cajanus cajan* (L.) Millsp. inhibits sodium channel-activated sdepolarisation of mouse brain synaptoneurosomes. Fitoterapia 81(7):826–829

Nix A, Paull CA, Colgrave M (2015) The flavonoid profile of pigeonpea, Cajanus cajan: a review. SpringerPlus 4:1–6

Nwodo UU, Ngene AA, Iroegbu CU, Onyedikachi OA, Chigor VN, Okoh AI (2011) In vivo evaluation of the antiviral activity of *Cajanus cajan* on measles virus. Arch Virol 156(9):1551–1557

Obala J, Saxena RK, Singh VK, Kumar CV, Saxena KB, Tongoona P, Sibiya J, Varshney RK (2019) Development of sequence-based markers for seed protein content in pigeonpea. Mol Genet Genomics 294:57–68

Okigbo RN, Omodamiro OD (2007) Antimicrobial effect of leaf extracts of pigeon pea (*Cajanus cajan* (L.) Millsp.) on some human pathogens. J Herbs Spices Med Plants 12(1-2):117–127

Olorunnisola OS, Adetutu A, Balogun EA, Afolayan AJ (2013) Ethnobotanical survey of medicinal plants used in the treatment of malaria in Ogbomoso, Southwest Nigeria. J Ethnopharmacol 150 (1):71–78

Pahwa K, Ghai N, Bedi S (2013) Pigeonpea: a potential multipurpose crop. Rastriya Krishi 8:3

Pal D, Mishra P, Sachan N, Ghosh AK (2011) Biological activities and medicinal properties of *Cajanus cajan* (L.) Millsp. J Adv Pharm Technol Res 2(4):207

Patel NK, Bhutani KK (2014) Pinostrobin and *Cajanus* lactone isolated from *Cajanus cajan* (L.) leaves inhibits TNF-α and IL-1β production: in vitro and in vivo experimentation. Phytomedicine 21(7):946–953

Patil PG, Dubey J, Bohra A, Mishra RK, Saabale PR, Das A, Rathore M, Singh NP (2017) Association mapping to discover significant marker-trait associations for resistance against Fusarium wilt variant 2 in pigeonpea [*Cajanus cajan* (L.) Millspaugh] using SSR marker. J Appl Genet 58:307–319

Pazhamala L, Saxena RK, Singh VK, Sameerkumar CV, Kumar V, Sinha P, Patel K, Obala J, Kaoneka SR, Tongoona P, Shimelis HA (2015) Genomics-assisted breeding for boosting crop improvement in pigeonpea (*Cajanus cajan*). Front Plant Sci 17(6):50

Pfeiffer WH, McClafferty B (2007) HarvestPlus: breeding crops for better nutrition. Crop Sci 47:S-88–S-105

Prasad V, Satyavathi VV, Sanjaya, Valli KM, Khandelwal A, Shaila MS, Lakshmi Sita G (2004) Expression of biologically active hemagglutinin-neuraminidase protein of Peste des petits ruminants virus in transgenic pigeonpea [*Cajanus cajan* L. Millsp.]. Plant Sci 166:199–205

Rahmatullah M, Mukti IJ, Haque AK, Mollik MA, Parvin K, Jahan R, Chowdhury MH, Rahman T (2009) An ethnobotanical survey and pharmacological evaluation of medicinal plants used by the Garo tribal community living in Netrakona district, Bangladesh. Adv Nat Appl Sci 3(3):402–418

Reddy LJ, Green JM, Singh U, Bisen SS, Jambunathan R (1979) Seed protein studies on *Cajanus cajan*, *Atylosia* spp. and some hybrid derivatives. In: Proceedings of the international symposium on seed protein improvement in cereals and grain legumes, vol II. International Atomic Energy Agency, Vienna, pp 105–117

Salehi B, Ata AV, Anil Kumar N, Sharopov F, Ramirez-Alarcon K, Ruiz-Ortega A, Sharifi-Rad J (2019) Antidiabetic potential of medicinal plants and their active components. Biomolecules 9 (10):551

Satyavathi VV, Prasad V, Khandelwal A, Shaila MS, Lakshmi Sita G (2003) Expression of hemagglutinin protein of Rinderpest virus in transgenic pigeon pea (*Cajanus cajan* (L.) Millsp.) plants. Plant Cell Rep 21:651–658

Saxena KB (2008) Genetic improvement of pigeon pea – a review. Trop Plant Biol 1(2):159–178

Saxena KB, Kumar RV (2003) Development of a cytoplasmic nuclear male-sterility system in pigeonpea using *C. scarabaeoides* (L.) Thouars. Indian J Genet Plant Breed 63:225–229

Saxena KB, Sawargaonkar SL (2015) Genetic enhancement of seed proteins in pigeonpea – methodologies, accomplishments, and opportunities. Int J Sci Res 4(5):03–07

Saxena KB, Sharma D (1990) Pigeonpea: genetics. In: Nene YL, Hall SD, Sheila VK (eds) The pigeonpea. CAB International, Wallingford, pp 137–157

Saxena KB, Kumar RV, Rao PV (2002) Pigeonpea nutrition and its improvement. J Crop Prod 5(1/2):227–260

Saxena RK, Varma Penmetsa R, Upadhyaya HD, Kumar A, Carrasquilla-Garcia N, Schlueter JA, Farmer A, Whaley AM, Sarma BK, May GD (2012) Large-scale development of cost-effective single-nucleotide polymorphism marker assays for genetic mapping in pigeonpea and comparative mapping in legumes. DNA Res 19:449–461

Saxena RK, Von Wettberg E, Upadhyaya HD, Sanchez V, Songok S, Saxena K, Kimurto P, Varshney RK (2014) Genetic diversity and demographic history of *Cajanus* spp. illustrated from genome-wide SNPs. PLoS One 9:e88568

Saxena KB, Tikle AN, Kumar RV, Choudhary AK, Bahadur B (2016) Nectarivore-aided shybridisation and its exploitation for productivity enhancement in pigeonpea. Int J Sci Res Publ 6(08):321–331

Saxena RK, Kale SM, Kumar V, Parupali S, Joshi S, Singh V, Garg V, Das RR, Sharma M, Yamini KN (2017) Genotyping-by-sequencing of three mapping populations for identification of candidate genomic regions for resistance to sterility mosaic disease in pigeonpea. Sci Rep 7: 1–10

Saxena RK, Rathore A, Bohra A, Yadav P, Das RR, Khan AW, Singh VK, Chitikineni A, Singh IP, Kumar CS (2018) Development and application of high-density Axiom *Cajanus* SNP array with 56K SNPs to understand the genome architecture of released cultivars and founder genotypes. Plant Genome 11:180005

Saxena RK, Saxena KB, Varshney RK (2019) Pigeonpea (*Cajanus cajan* L. Millsp.): an ideal crop for sustainable agriculture. In: Al-Khayri J, Jain S, Johnson D (eds) Advances in plant breeding strategies: legumes. Springer, Cham, pp 409–429

Shen J, Li H, Neumann G, Zhang F (2005) Nutrient uptake, cluster root formation and exudation of protons and citrate in *Lupinus albus* as affected by localised supply of phosphorus in a split-root system. Plant Sci 168(3):837–845

Singh U, Eggum BO (1984) Factors affecting the protein quality of pigeonpea (*Cajanus cajan* L.). Plant Food Hum Nutr 34(4):273–283

Singh N, Rai V, Singh NK (2020) Multi-omics strategies and prospects to enhance seed quality and nutritional traits in pigeonpea. Nucleus 63:249–256

Surekha CH, Kumari KN, Aruna LV, Suneetha G, Arundhati A, Kavi Kishor PB (2014) Expression of the *Vigna aconitifolia* P5CSF129A gene in transgenic pigeonpea enhances proline accumulation and salt tolerance. Plant Cell Tissue Organ Cult 116(1):27–36

Teixeira EM, Silva-López RE, Da Silva BR, Fontão AP, Sampaio AL (2021) *Cajanus cajan* (L.) Millsp. aqueous extracts against melanoma cell line and their proteases. Eur J Med Plants 32(2): 1–14

Thu TT, Dewaele E, Trung LQ, Claeys M, Jacobs M, Angenon G (2007) Increasing lysine levels in pigeonpea (*Cajanus cajan* (L.) Millsp.) seeds through genetic engineering. Plant Cell Tissue Organ Cult 91(2):35–143

Toklu F, Sen Gupta D, Karaköy T, Özkan H (2021) Bioactives and nutraceuticals in food legumes: nutritional perspective. In: Breeding for enhanced nutrition and bio-active compounds in food legumes. Springer, Cham, pp 229–245

Vales MI, Srivastava RK, Sultana R, Singh S, Singh I, Singh G, Saxena KB (2012) Breeding for earliness in pigeonpea: development of new determinate and nondeterminate lines. Crop Sci 52 (6):2507–2516

Varshney RK, Chen W, Li Y, Bharti AK, Saxena RK, Schlueter JA, Donoghue MT, Azam S, Fan G, Whaley AM (2012) Draft genome sequence of pigeonpea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. Nat Biotechnol 30:83

Varshney RK, Mohan SM, Gaur PM, Gangarao N, Pandey MK, Bohra A, Sawargaonkar SL, Chitikineni A, Kimurto PK, Janila P (2013) Achievements and prospects of genomics-assisted breeding in three legume crops of the semi-arid tropics. Biotechnol Adv 31:1120–1134

Varshney RK, Saxena RK, Upadhyaya HD, Khan AW, Yu Y, Kim C, Rathore A, Kim D, Kim J, An S (2017) Whole-genome resequencing of 292 pigeonpea accessions identifies genomic regions associated with domestication and agronomic traits. Nat Genet 49:1082–1088

Vo TT, Yang NC, Yang SE, Chen CL, Wu CH, Song TY (2020) Effects of *Cajanus cajan* (L.) Millsp. roots extracts on the antioxidant and anti-inflammatory activities. Chin J Physiol 63(3): 137–148

Wang D, Xiao M, Li Y, Shen X, Lu Y, Liu K, Li Z, Hu Y (2011) Determination of longistylin A and longistylin C in *Cajanus cajan*. Zhongguo Zhong Yao Za Zhi 36(19):2680–2683

Wei ZF, Luo M, Zhao CJ, Li CY, Gu CB, Wang W, Zu YG, Efferth T, Fu YJ (2013) UV-induced changes of active components and antioxidant activity in postharvest pigeon pea [Cajanus cajan (L.) Millsp.] leaves. J Agric Food Chem 61(6):1165–1171

Wu J, Li B, Xiao W, Hu J, Xie J, Yuan J, Wang L (2020) Longistylin A, a natural stilbene isolated from the leaves of *Cajanus cajan*, exhibits significant anti-MRSA activity. Int J Antimicrob Agents 55(1):105821. (Erratum in: Int J Antimicrob Agents 58(2):106392, 2021)

Yadav P, Saxena KB, Hingane A, Kumar CV, Kandalkar VS, Varshney RK, Saxena RK (2019) An "Axiom *Cajanus* SNP array" based high density genetic map and QTL mapping for high-selfing flower and seed quality traits in pigeonpea. BMC Genomics 20:1–10

Zu YG, Fu YJ, Liu W, Hou CL, Kong Y (2006) Simultaneous determination of four flavonoids in pigeonpea Cajanus cajan (L.) Millsp. leaves using RP-LC-DAD. Chromatographia 63(9–10):499–505