

# Pigeonpea wild relatives: an emerging alternative for the management of pod borer, *Helicoverpa armigera*

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**Abstract** : Pigeonpea is one of the important legume crops globally and India occupies second place in production. However, stagnant productivity in pigeonpea due to various biotic and abiotic constraints has been a major problem. Among major yield limiting factors, the lepidopteran pod borer *H. armigera* assumes significance due to its crop damaging potential. Various pest control strategies were developed and are being practiced to manage *H. armigera* damage on pigeonpea. However, controlling *H. armigera* has been difficult due to its broad host range, high migratory behavior and its tendency to develop resistance against various phytotoxins and commercial insecticides. In addition to Bt-based transgenic approaches, efforts towards exploration of alternative strategies to mitigate pod borer damage are being made by scientists. In this aspect, exploitation of genetic variation present in crop wild relatives for crop improvement against insect pests has emerged as a promising strategy. The wild relatives of crop plants are bestowed with an array of resistance traits and hence can form an excellent source of resistance genes. It is therefore crucial to identify, understand and extrapolate the pod borer resistance mechanism to cultivated pigeonpea for the enrichment of genetic diversity and utility in crop improvement programmes.

**Key words:** Pigeon pea; wild relatives; *Helicoverpa armigera*; resistance response; insect resistance

Pigeonpea is considered as one of the important pulse crops in the world due to high protein content and its ability to grow under semi-arid regions. Though historians and scientists have debated that pigeonpea originated in Africa, the actual origin had been traced to be the peninsular land of India. This fact is strengthened by the presence of pigeonpea progenitor *Cajanus cajanifolius* and high range of crop diversity in India (Purseglove, 1968; Saxena, 1988; Van Der Maesen, 1980; Van Der Maesen, 1990). Furthermore, presence of linguistic and archaeological evidences, daily usage in cuisine and diversity in recipes support the argument of Indian origin (Kajale, 1974; Van Der Maesen, 1990).

Global production of pigeonpea is estimated to be 4.49 MT, out of which India accounts for around 63% of production in a total land area of 3.9 Mha (70%). Among the different pulses cultivated in India, 20% is attributed to pigeonpea making it the second most

important pulse crop (FAO, 2019). Pigeonpea growing areas in India are broadly classified into four different agro-ecological zones - north east plain zone, north west plain zone, central zone and south zone. Based on the agro ecological conditions and utilization, pigeonpea is grown as a mono crop, mixed crop and intercrop (Sameer Kumar *et al.*, 2014). Further, based on the crop maturity period, cultivar accessions were classified into super-early duration (<90 days), extra-early duration (<120 days), early duration (120-140 days), mid-early duration (140-160 days), medium duration (160-180) and long duration (>180 days) varieties (Sharma *et al.*, 2019; Saxena *et al.*, 2019).

Pigeonpea is one of the most important sources of dietary proteins for the Indian vegetarian population. In Indian cuisine, pigeonpea dry seeds are mostly used in dehulled split form (dhal). In some regions, green or tender pigeonpea is also eaten as a vegetable (Talari and Shakappa, 2018; Sarkar *et al.*, 2020). In

terms of nutritional value, pigeonpea is considered as an alternative and inexpensive protein source for the lower economy class people and vegetarian population in India as well as other under developing countries (Adeola *et al.*, 2017; Talari and Shakappa, 2018). Notably, total protein content in the raw pigeonpea seeds' is estimated to be around 20%. Furthermore, it is a good source of healthy carbohydrates, some essential vitamins including folate and important minerals like magnesium, zinc, calcium, phosphorous, potassium and iron. Besides having an array of nutrients, it is also enriched with different bioactive compounds, making it a crucial nutrient resource to fight malnutrition (Talari and Shakappa, 2018; Sarkar *et al.*, 2020). In most parts of the world, pigeonpea is cultivated only as a food crop, whereas in India and some parts of Africa it is utilized as a 'multi-purpose' crop (Daniel and Ong, 1990).

**Pigeonpea as a soil ameliorating agent:** Growing pigeonpea or other legume crop is one of the conventional and effective agronomy practice to fix atmospheric nitrogen into the soil and is mediated by the bacteria belonging to *Rhizobium* spp. that are present in the root nodules of plants. Apart from symbiosis, the remains of the crop improves soil organic matter and provides additional nitrogen (Daniel and Ong, 1990; Chikowo *et al.*, 2004). Similarly, it has the capability to grow under low phosphorous soil. Particularly, exudates from the root of pigeonpea have extraordinary ability to free the iron bound phosphorus. The root exudates increase the overall phosphorous availability for the crop and also benefits neighboring or subsequent crops cultivated in the soil (Sinclair, 2004).

**As cattle feed and fodder:** Pigeonpea leaves and pods are rich in protein; green leaves and young pods are often used as cattle feed. However, dried plant portions are also stored and used as cattle fodder in off-season (Daniel and Ong, 1990). Furthermore, pigeonpea seed powder is also used as an alternative to fish meal to balance the protein content (Adeola *et al.*, 2017; Talari and Shakappa, 2018). Besides, significant improvement in cattle growth has been reported from pigeonpea-cattle grazing trail suggesting the role of pigeonpea as a forage crop (Ayenan *et al.*, 2017).

**Other domestic uses:** As pigeonpea has strong and woody stems, the dried stems are widely used as fuel in Indian villages and a few African countries (Daniel and Ong, 1990). Additionally, various parts of pigeonpea are being used as a traditional medicine in India, China, and several African countries (Talari and Shakappa, 2018).

### **Yield gap and major constraints for stagnated productivity in pigeonpea**

In India, based on agro climatic zones, different varieties are being cultivated which vary in duration and productivity. The expected yield of commercial pigeonpea varieties could range from 1500-3000 kg/ha, with the actual yield lingering around 700 kg/ha (Sameer Kumar *et al.*, 2014). The huge yield gap in pigeonpea production is due to a range of abiotic and biotic factors (Sharma *et al.*, 2010; Umeshia *et al.*, 2017).

**Abiotic constraints:** Pigeonpea experiences various abiotic stress factors during its life cycle, which includes, moisture stress (waterlogging or drought), temperature stress, and salinity stress. Among them, moisture stress is predominant because pigeonpea is majorly cultivated under rainfed agriculture. In general, excessive or absence of rainfall is common in rainfed agriculture. Comparably, waterlogging is a predominant issue in rainfed agriculture as even a short span of waterlogging (2-3 days) is enough to cause a drastic yield loss or crop loss (Choudhary *et al.*, 2011). In India, waterlogging alone is responsible for an annual yield loss of 25-30% (Sultana, 2010). In a nutshell, pigeonpea cultivation is recommended in areas that receive low rainfall, owing to its inherent ability to tolerate high degree of drought. However, low soil moisture in early seedlings and reproductive stage (terminal drought) adversely affects the productivity (Lopez *et al.*, 1996). Excess salt accumulation on soil surfaces is responsible for the salinity stress. Presence of enormous salt in the soil leads to accumulation of toxic free radicals inside the plant cells, and promotes excessive uptake of sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) ions from the soil, which collectively causes cytotoxicity (Deshpande and Nimbalkar, 1982). Salinity stress has been reported to prolong the 50% of flowering of the

crop by approximately 2-3 weeks, which substantially reduces the pod weight and count (Promila and Kumar, 1982).

**Biotic constraints:** During vegetative to reproductive stages, pigeonpea is infected by various phytopathogens and insects. Among the phytopathogens, those belonging to *Fusarium* spp., *Phytophthora* spp. and sterility mosaic virus pose serious threats to crop productivity (Sharma *et al.*, 2010). *Fusarium* wilt occurs 65 days after sowing (DAS) and the disease severity increases at peak vegetative stage of the crop (180 DAS; Sharma *et al.*, 2010). Additionally, occurrence of drought along with wilt increases the pathogen virulence and leads to substantial yield losses (Sinha *et al.*, 2017). *Phytophthora* blight and sterility mosaic disease infect during the early vegetative stage of the crop (Sharma *et al.*, 2010). Notably, *Phytophthora* blight affects young seedlings (within 60 DAS) and kills within 3 days leading to 100% crop loss. The disease is generally encountered after 3-5 continuous rainy days. Since pigeonpea is a rain fed crop, it is highly prone to get infected by *Phytophthora* blight (Sharma *et al.*, 2010). Similarly, sterility mosaic disease (SMD), which also appears at early vegetative stages is caused by pigeonpea sterility mosaic virus (PSMV), which is transmitted through the mite species *Aceria cajani*. The extent of yield loss caused by SMD varies according to the age of the plant. The infection at an early vegetative stage (before 40 DAS) could result in 95-100% yield loss, whereas, in the later stages yield loss ranges from 27-97 % (Kannaiyan *et al.*, 1984).

Insect pests are critical factors which cause huge loss to pigeonpea seed production. Further, presence of high protein content in seeds and leaves attracts an array of insects. So far, more than 200 insect species have been found to feed on pigeonpea of which 34 are a potential menace not only for pigeonpea but for other crops as well (Lal and Katti, 1998). These insects are oligophagous to polyphagous with different feeding behaviors. Out of these, two polyphagous lepidopteran pests, *H. armigera* and *M. vitrata* are major constraints for stagnated productivity (Wadaskar *et al.*, 2013). *H. armigera* or pod borer is the most devastating among them, which can cause about 80-100% crop losses (Sharma *et al.*, 2010).

## ***H. armigera*: a major insect pest of pigeonpea that threatens productivity**

Polyphagous insect pests have always been major threats to crop productivity due to their wide range of host specificity. *H. armigera* has a host range of more than 300 plant species across 68 families (Datasheet *H. armigera*: <https://www.cabi.org/isc/datasheet/26757>). The lifecycle of *H. armigera* is comprised of four stages viz., egg, larva, pupa and moth (adult). For the completion of one lifecycle from egg to moth (Fig. 1) it takes 4-6 weeks in summer, and 8-12 weeks in winter. They spend majority of lifespan in the caterpillar stage, during which it feeds voraciously. This feeding behavior of the caterpillar and crop damaging potential makes *H. armigera* the most important pest for pulse crops cultivated worldwide (Pomari-Fernandes *et al.*, 2015). In general, *H. armigera* adults lay eggs on the leaf surface of the host plants. The young caterpillar feeds on leaves and moves to plant reproductive parts i.e. fruits, bolls, pods etc. (Pomari-Fernandes *et al.*, 2015). Freshly hatched neonates prefer terminal leaves of pigeonpea, which are more soft and tender. However, later instars feed almost on all reproductive organs including seeds, which leads to substantial yield losses (Sharma *et al.*, 2010).

Controlling *H. armigera* through chemical pesticides is a commonly followed practice. However, it is known to possess the tendency to develop resistance towards various host plant toxins and commercial pesticides (Pearce *et al.*, 2017). Furthermore, resistance to insecticides in *H. armigera* is attributed to the presence of a large number of gene families involved in detoxification of xenobiotics (Pearce *et al.*, 2017). Enzymes belonging to Cytochrome P450s (CYPs) superfamily are recognized as important factors for insecticide resistance (Tian *et al.*, 2017; Wang *et al.*, 2018). In insects, these enzymes play a vital role in xenobiotics and other photochemical metabolism. Particularly, occurrence of sequence/expression polymorphism in this gene super family has been correlated with insecticide resistance (Wang *et al.*, 2018). Around 30 % of globally commercialized insecticides are targeted against *H. armigera*, which has put a high selection pressure on the insect to

develop resistance against pesticides of different chemical formulae (Ahmad, 2007). As compared to other species of *Helicoverpa*, *H. armigera* population is endemic (DPI&F, 2005) due to which they tend to retain the developed insecticidal resistance trait across generations. However, insect pest species that migrate to different geographical locations would lose the developed insecticidal resistance traits in the further generation (DPI&F, 2005). Insecticide resistance development in *H. armigera* and ecological impact of continuous usage of synthetic chemical insecticides has created a need to look for alternative approaches to control the pest attack.

### Crop improvement in pigeonpea for the management of pod borer

As an alternative to chemical pesticides, improving host plant resistance or tolerance level against target insect pests is a tangible approach. In this direction, efforts were made in ICRISAT, India to identify *H. armigera*-resistant accessions. However, screening of 14,000 pigeonpea accessions identified only low to moderate level of resistance against pod borer. It is therefore necessary to look for alternative sources for pod borer resistance (Reed and Lateef, 1990).

Advent of transgenic technology facilitated integration

of foreign genes into the targeted organism. In plant species, this technology was first successfully demonstrated in tobacco in 1983 (Fraley *et al.*, 1983). Further, cotton transgenics expressing insecticidal crystal (cry) protein from *Bacillus thuringiensis* (*Bt*) was approved for commercialization in United States in 1996 (Bilal *et al.*, 2012). In India, *Bt* cotton expressing Cry1Ac protein was introduced in the year 2002, which was developed for resistance against cotton boll worm *H. armigera* (Bilal *et al.*, 2012). Notably, adaptation of *Bt* cotton accelerated Indian cotton production, owing to which India became the leading cotton producing country in the world (ISAAA, 2017). Successful outcome of *Bt* cotton gave a positive signal for the utilization of *Bt* insecticidal genes in other agronomically important crops. After accomplishing encouraging results in many crops, *Bt* insecticidal genes have also been utilized for development of podborer resistance in pigeonpea (Table 1). Although efficacy of *Bt* genes was proved in various food crops, the propensity of *H. armigera* to resist *Bt* genes and hurdles in social acceptance of GM food crops, resulted in the need to look for other options.

### Utility of pigeonpea wild relatives in crop improvement against *H. armigera*

In the scenario of escalating food demand, scarcity

**Table 1. Exploitation of *Bt* ICPs for development of pod borer resistance in pigeonpea**

Pigeonpea Cultivar	Name of the Cry gene	References
Pusa 992	<i>cry2Aa</i>	Singh <i>et al.</i> , 2018
UPAS 120	<i>cry2Aa, cry1Ac</i>	Ghosh <i>et al.</i> , 2017
PAU 881	<i>cry1Ac</i>	Kaur <i>et al.</i> , 2016
Asha	<i>cry1abc</i>	Das <i>et al.</i> , 2016
TTB7	<i>cry1AcF</i>	Ramu <i>et al.</i> , 2012
JKPL	<i>cry1Ac</i>	Krishna <i>et al.</i> , 2011
ICPL 87	<i>cry1ab</i>	Sharma <i>et al.</i> , 2006
ICPL 87	<i>cry1E-C</i>	Surekha <i>et al.</i> , 2005



of resources, cultivable land and impending climate change impact created the necessity for effectual crop improvement programmes. Understanding the crop genetic diversity between the different species present within a genus would form a solid platform to identify novel alleles (Khan *et al.*, 2020). The genus *Cajanus* totally consists of 34 species among which *C. cajan* is the only cultivar, while the remaining are wild relatives. PWRs are progenitors of *C. cajan*, which are known to be bestowed with various important agronomic traits that were lost during domestication (Kassa *et al.*, 2012). Deciphering molecular signatures of wild relatives would not only provide information about the mechanism behind desired traits, but also would allow us to broaden the genetic diversity of the crop (Khan *et al.*, 2020).

Particularly, the geographical hotspots rich in diversity of *Cajanus* species are focused in India followed by North Australia and African countries (Khoury *et al.*, 2015). In past decades, substantial efforts have been made by ICRISAT, India, for the characterization of pigeonpea wild accessions (Sujana *et al.*, 2008; Sharma *et al.*, 2009; Parde *et al.*, 2012). Research showed that, PWRs possess enormous potential to provide valuable traits such as tolerance to abiotic stresses including salt tolerance, resistance to pests and diseases, high protein content, rapid seedling growth, photo-insensitivity, cleistogamy, super-early flowering and cytoplasmic male sterility (Mallikarjuna *et al.*, 2006; Sujana *et al.*, 2008; Pazhamala *et al.*, 2015; Muñoz *et al.*, 2017).

### **Pod borer resistance in PWRs**

Pod borer resistance in PWRs are known to be arbitrated by both biochemical and physical barriers. Initial screening performed in Indian PWRs proved that pod borer resistance is linked with biochemical composition and morphological variation present in the pod wall (Sujana *et al.*, 2008; Sharma *et al.*, 2009; Choudhary *et al.*, 2013).

### **Biochemical basis of pod borer resistance**

When plants recognize the herbivore attack by herbivore-associated molecular pattern (HAMP) (Steinbrenner *et al.*, 2020), followed by the activation

of phytohormones, especially jasmonic acid, it leads to the activation of jasmonic acid (JA) signaling network. JA signaling is known for the wound and herbivore mediated defense response, which triggers the accumulation of toxic metabolites and /or deterrents and other digestive reducers against insect herbivores (Kessler and Baldwin, 2002). In plants, the biochemical compounds produced in response to herbivory are classified into antibiosis and antixenosis based on their activity. If the plant metabolites possess inhibitory effect on insect growth and development, it is known as antibiosis, and, if they lead to non-preference for oviposition, it is known as antixenosis (Sujana *et al.*, 2008).

Immense efforts were made by Sujana *et al.* (2008), to evaluate the PWRs for their antibiosis and antixenosis properties in 29 accessions belonging to 13 species from different gene pools. Among them, *C. acutifolius* (ICPW 1), *C. albicans* (ICPW 13 and 14), *C. sericeus* (ICPW 159 and 160), *C. platycarpus* (ICPW 68), *C. scarabaeoides* (ICPW 83, 90, 94, 125, 137, 141 and 280), *Paracalyx scariosa* (ICPW 207) and *Rhynchosia aurea* (ICPW210) were found to express high levels of antixenosis, in both choice and no-choice experiments. Further, incorporation of lyophilized pod and leaf powder in diet caused significant effects on larval growth, i.e., reduction in larval weight, prolonged post embryonic development and prolonged pupal and larval growth period. The high level of antibiosis property was reported in *C. acutifolius*, *C. lineatus*, *C. sericeus*, *C. scarabaeoides*, *C. platycarpus*, *P. scariosa* and *R. aurea* wild accessions.

Further, information acquired from literature depicted that variation in biochemical composition such as total soluble protein, total soluble sugars, and total condensed tannins are associated with pod borer resistance in PWRs (Choudhary *et al.*, 2013). High amount of soluble sugars, low amount of polyphenols and low amount of condensed tannins in cultivated pigeonpea pods were associated with pod borer susceptibility (Sharma *et al.*, 2009; Choudhary *et al.*, 2013).

### **Digestive reducers**





















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