**Winged bean: an underutilized tropical legume on the path of improvement, to help mitigate food and nutrition security**

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**Abstract**

The utilization of most legumes for human consumption in the present day is low relative to cereal crops. Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is a valuable legume due to the presence of soybean-equivalent nutrients. Early work identified that winged bean has favorable agronomic features suitable for cultivation in the tropics with high average yield, and foods prepared from winged bean serve as an effective measure to meet the protein demand of the consumer. The seed oil meets all the required edibility parameters and is comparable with any other good-quality edible oil on the market. Recent work utilizing genomic, transcriptomic and metabolomic data are starting to reveal more about this crop, meaning it is now easier to genetically improve this plant for further utilization. This review reports the efforts undertaken and underway for the improvement of this crop for cultivation, commercialization and consumption perspectives.

**Key words:** Winged bean, legume, protein-demand, food security, *Psophocarpus*, anti-nutrients**.**

**1. Introduction**

The gap between the nutritional needs of the human population and the quantity of food supply has now become a global threat. To bridge this gap, efforts need to be made to identify and evaluate under-exploited food sources (Kuyper et al., 2017). The majority of calories consumed worldwide come from just 20 crops comprising cereals, legumes, roots and other food crops, yet thousands of species worldwide are consumed. In recent years, changes in consumer preference and agricultural practices as well as cash crop alternatives have led to a number of food species falling into disuse and becoming underutilized over a period of time. Further, little attempt has been undertaken for improvement and mainstreaming of these plants for further use over the crops with a longstanding commercial value. The presence of certain anti-nutrients (metabolites which adversely impairs the digestibility of the nutrients in the body) in several minor crops has led to their decline in use, despite other benefits. As a result, there is often a long time gap between the initial studies of these underutilized crops and any modern explorations. However, these currently underutilized species are often of high nutrient value and/or have extreme tolerances to abiotic stresses, and could be explored to be included in more mainstream cultivation and consumption practices. Major crops, i.e. those in mainstream cultivation dominating international markets, are relatively nutrient poor and show poor resilience to climatic perturbations (Lin, 2011). It is becoming clear that supplementing major crops(and the products derived from them) with these underutilized or minor crops of nutritive and stress tolerance importance, is a viable strategy for addressing the issue of nutrition security.

As protein-calorie malnutrition is prevalent in many developing countries of the tropics and sub-tropics, incorporation of other crops into the diet will act as a safety net to meet the challenges of malnutrition and address the issues of nutrient-deficiency. Enhancement and improvement of protein supply to meet the demand of the growing population necessitates the utilization of unconventional protein sources for further use. However, over the last few decades, legume yields have scarcely changed and total production has remained stable (Foyer et al., 2016). As a result, per capita consumption has dropped. A lower proportion of legumes in the diet were cited as a cause of higher rates of protein malnutrition and this trend is in a constant state of increase (Polak et al., 2015). So, at this point in time, it is crucial to prioritize the investigation and improvement of underutilized crops with the goal of introducing new foods with high and complementary nutritional value (Dawson et al., 2019).

Legumes (Fabaceae) are second only to grasses (Poaceae) in terms of their importance to humans. There are 670-750 genera and 18,000 to 19,000 species of legumes and this includes dozens, if not hundreds, of important grain, pasture and agro-forestry species (Polhill and Raven, 1981). Grain and forage legumes are grown on some 180 million Ha., i.e. about 12-15% of the earth’s arable land surface. They account for 27% of the World’s primary crop production with grain legumes alone contributing 33% of the dietary protein nitrogen needs of humans (Vance, 2001). In both the old and the new worlds, legume consumption has evolved in conjunction with cereals. However, the underutilized food legumes are gaining importance recently and they are suggested for increasing the production and availability of food grains (Chimmad et al., 1998).

Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is one such underutilized legume. All parts of the plant, from the seeds and immature pods to the leaves, flowers and tuberous roots are edible. It is virtually the duplicate of soybean in terms of composition and nutritional value of the seed, because both contain a similar proportion of protein, oil, minerals, vitamins and essential amino acids (Amoo et al., 2011). Once processed, both have similar value of digestibility (National Research Council, 1981).The winged bean seed has a high protein content of 29-37%, and its amino acid composition is almost identical to soybean with methionine and cysteine being limiting amino acids (Okezie and Martin, 1980; Wyckoff et al.,1983). The true value of these tuberous roots became apparent when it was found that they are exceptionally rich in protein (Kortt and Caldwell, 1984; Lumen and Reyes, 1982).

Masefield (1973) was the first to predict the agricultural potential of the winged bean, and in the early 1970s and 80s, a national program on winged bean was developed in the Philippines, Indonesia and Sri Lanka mainly focusing on its introduction, cultivation and agronomic aspects. Hence a large amount of preliminary research was carried out at this time. More recently, an increased understanding of nutritional, anti-nutritional and chemical properties of this legume has been established (Lepcha et al., 2017), but investigations of the genetic and genomic variation and characterization of this species for further improvement and utilization are still in their infancy. Bioactive peptides with angiotensin-I-converting enzyme (ACE) inhibitor and anti-oxidative properties, from winged bean seed protein were recently developed into nano-liposomal particles (Chay et al., 2015; Wan Mohtar et al., 2014),which could be used in biofortification. Additionally it has been suggested that the extract of mature winged bean seeds inhibit wrinkle formation (Shibata et al., 2011).The winged bean protein is safe in terms of genotoxicity and cytotoxicity and hence has the potential for the use in pharmaceutical and food industries as functional ingredient. With all these points in mind, the winged bean has sparked the interest of many scientists recently (Chen et al., 2015).

However, winged bean remains underutilized, possibly due to its low productivity, long growing duration (six to eight months from seed to seed) and indeterminate growth habit. Breeding for improved cultivars, targeting traits related to agricultural production, such as increased harvest index, yield, controlled growth habit, adaptability to extreme climate-conditions and reduced anti-nutrients, could significantly be accelerated through the application of biotechnological tools (Lepcha et al., 2017). The winged bean genome is comparably small (~1.22 Gb) and diploid (2n=2Х=18) (Harder, 1992; Vatanparast et al., 2016), therefore is amenable to manipulation for further genetic improvement.

**2.Food and nutritional value of winged bean**

The fresh leaves, flowers, green pods, seeds and the tuberous roots of winged bean are rich in protein and are eaten either raw or cooked. When picked young, the pods lack noticeable fiber and make a succulent green vegetable. It may be steamed, boiled, stir-fried or pickled to make it crispy, chewy delicacy. The leaves of winged bean are cooked and eaten like spinach and are rich in vitamin A. This high nutritional value and potential economic importance has attracted worldwide attention in the last decades (Claydon, 1975; May, 1977; Prakash et al., 2001; Figure 3).

Mature seeds contain 15-18% oil, similar to that of soybean. It has fairly good amounts of phosphorus and iron (Figure 4) and various vitamins (Figure 5). The amino acid composition of the flour from the seeds reported relatively high amounts of lysine, aspartic acid, glutamic acid and leucine and relatively low amounts of the sulfur-containing amino acids (Figure 6), a pattern similar to that of soybean (Mnembuka and Eggum, 1995). Nevertheless, there is variation amongst varieties (Prakash et al., 1987) and winged bean has greater amounts of some essential amino acids (valine, leucine, histidine, and lysine) than soybean (Mutia and Uchida, 1994; Figure 6).

The fatty acid composition of winged beans is comparable to peanut (*Arachis hypogaea*, or groundnut; Figure 7) and it contains higher amounts of behenic and parinaric acid. Triglycerides showed a similar profile of fatty acids to those of whole lipid: the major fatty acids are palmitic (10.9%), stearic (4.5%), oleic (37.1%), linoleic (19.0%), eicosenoic (3.6%), behenic (18.5%) and lignoceric (4.2%) acids (Mohanty et al. 2014). Compared to soybean and corn oil, winged bean oil contains long chain fatty acids and a fairly small amount of polyunsaturated fatty acids (Figure 7) which is favorable for oil stability against auto-oxidation (Homma et al., 1983).Long chain saturated fatty acids, such as behenic and lignoceric acids, were found in relatively high amounts as compared with other edible seed oils (Mohanty et al., 2014). These findings indicate that winged bean seed is a good source of oil of favorable quality (Harding et al., 1978; Higuchi et al., 1982; Ibuki et al., 1983). Winged bean oil was found to contain tri-acylglycerols of larger ECN (Equivalent Carbon Number) value than soybean oil (Omachi et al., 1987).

**2.1. Winged bean protein isolates and concentrates**

Protein isolates prepared by Dench (1982) from winged bean seeds contained protein in the range of 73-87% and possessed low-bulk densities and high-fat. Concentrate containing 71.5% protein on a dry weight basis has been extracted from winged bean seeds by Sathe et al.,(1982). This concentrate had lower tannins and inhibitors than full-fat winged bean flour (Kantha and Erdman, 1984) . Winged bean protein concentrate had higher water-holding capacity, oil-bearing capacity and emulsifying capacity (Makeri et al., 2017). Winged bean protein concentrate (Ochiai Yanagi, 1983) has potential to be used in foods that require high emulsion stability index, foaming capacity and foaming stability.

New food products have been developed from seeds of *P. tetragonolobus* in Thailand. Preparation of winged bean tempeh (Gandjar, 1978), winged bean milk, miso and curd and the formulation of weaning foods had been reported by several workers and is being carried out on a commercial scale in Thailand, Indonesia and Ghana. Furthermore, reports on the use of winged bean fermented milk “tairu” and pellets from seed cake and tuberous roots of animal feed was earlier reported by Ismail (Ismail,1981.). A coffee substitute from roasted seeds and a tobacco substitute from dried leaves of winged bean were even tested previously (National Research Council, 1981).

**2.2. Winged bean anti-nutrients**

Anti-nutrients comprise a diverse range of compounds which are often synthesized in legumes. They reduce bioavailability of other beneficial nutrients therefore the removal of these compounds by breeding or biotechnological interventions would be valuable for improvement of winged bean as well as other crops.

Jaffe and Korte (1976) report a lower level of activity of amylase inhibitor in winged bean seeds compared to soybean. Kantha and Erdman (1984) reported phytates to the level 1.0-1.7% and 0.05-0.2% in the cotyledons and hulls, respectively in winged bean. Other toxic compounds such as hemagglutinins, lectins, cyanide and tannin have also been reported in winged bean (Kortt, 1984, 1985; Kotaru, 1987; Pueppke, 1979). Unlike soybeans, the winged bean also contains specific chymotrypsin inhibitors and trypsin inhibitors (Khor et al., 1982; Kortt, 1979), with insecticidal property (Gatehouse et al., 1991).Several studies have reported that heat treatment improves the amino acid digestibility of winged bean (Ekpenyong and Borchers, 1980; Mutia and Uchida, 1994; Tan et al., 1984). This improvement may be partially due to the reduction of trypsin inhibitor content upon heating;*in vitro* protein digestibility correlates with decreased trypsin inhibitor and tannin levels in winged bean meal. Low trypsin and chymotrypsin inhibitor mutants have been uncovered in M5 mutation lines of *P. tetragonolobus* (Kothekar*et al.* 1996).

Two putative Kunitz-type chymotrypsin inhibitor genes (WCI2 and WCI5) have been isolated from winged bean, one of which, WCI5, is exclusively expressed in seeds. Further, the molecular evolution of chymotrypsin inhibitors of winged bean was studied by Mukhopadhyay(2000). Translation of both genes resulted in polypeptides of 207 amino acids with 86% sequence similarity. Importantly, the protein products of both strongly inhibit gut proteinases of Helicoverpa armigera larvae which damage many important crop plants, making these proteins and their genes promising candidates for enhancing plant defense (Telang et al., 2009).

Vatanparast et al*.,*(2016) recently identified 28 and 20 gene transcripts from the trypsin inhibitor gene family from Sri Lankan and Nigerian accessions respectively. Singh et al., (2017) also reported transcripts differentially expressed between high and low condensed tannin-containing lines of winged bean. These results provide a starting point for determining the genetic basis of anti-nutrients and could be used in breeding and other biotechnological intervention strategies to minimize the biosynthesis of these compounds.

**3. Genetic improvement of winged bean studies so far**

**3.1. Regeneration through various tissue explants**

Plant regeneration is a vital step for producing viable plants from transformed tissues. Among the modes of plant regeneration, organogenesis in winged bean has been achieved from callus cultures derived from epicotyls (Bottino et al., 1979; Mehta and Ram, 1981; Tran Thanh Van et al., 1986;Venketeswaran et al., 1990), leaves (Gregory et al., 1980) and protoplasts (Wilson et al., 1985), while somatic embryos have been induced from hypocotyl and epicotyl cultures (Naik et al., 2015; Venketeswaran et al., 1990). The somatic embryogenesis and plant regeneration from leaf-derived calli of winged bean was established by Ahmed et al.,(1996). Protoplasts have been isolated from winged bean suspension culture using a 2-mercaptoethanol-supplemented enzyme solution; these protoplasts were induced to divide and give rise to callus colonies, shoots were regenerated, and rooted and developed to maturity (Wilson et al., 1985).Excised seedling leaf segments of winged bean have achieved direct somatic embryogenesis under appropriate incubation conditions (Dutta Gupta et al., 1997;Singh et al., 2014).

**3.2. Potential for mutation breeding in the improvement of winged bean**

Induced mutation, utilizing physical mutagens (gamma rays) and chemical mutagens (ethyl methane sulphonate), of winged bean has been carried out by Khan and Claydon(1975), Armachuclo and Bernardo (1981) and Kesavan and Khan (1978).These authors have reported various morphological mutations at different doses and concentrations of mutagens, for example vigorous growth, increased number of branches and increased pod-bearing ability. These investigations have been followed by Jugran et al., (1983),Tetteh and Opoku-Asiama (1986),Klu et al., (1989). Klu et al.,(1991) reported radiation-induced mutations for improved seed quality in winged bean and successfully isolated mutants with increased protein content, with clear implications for winged bean improvement.

Jugran et al.,(2001) also reported various morphological mutations, induced through gamma irradiation, with potential for future improvement of winged bean. They identified true-breeding mutants with violet-colored stems and calyxes (instead of the normal green) and early flowering and fruiting mutants. No other traits were modified indicating no obvious pleiotropic effects of the mutations. Nath et al., (1986) successfully induced chlorophyll mutation in winged bean after gamma irradiation. Suma-Bai and Sunil (1993) carried out radiation sensitivity analysis in various genotypes of winged bean, reporting that low doses of gamma rays stimulated the germination of winged bean and high doses of these rays (above 10Krad) inhibited germination. Kothekar et al.,(1996) studied the effects of gamma irradiation and induced low trypsin and chymotrypsin inhibitor mutants of winged bean. Klu et al.,(1997)successfully induced mutations in winged bean with low tannin content. The crude protein content of the lines generated by Dadke and Kothekar (2005) ranged from 26.45 per cent to 33.32 per cent in the seeds of these mutants, greater than would be expected from progeny of a single non-mutant. Klu and van Harten (2000) applied the induced mutation technique for overall trait-improvement and accelerated domestication of winged bean.

**3.3. The generation of large-scale genomic data for future genetic improvement**

Some studies have investigated the genetic basis of agronomic traits in winged bean. Erskine (1981) studied the role of various environmental factors, including differences between the seasons in night temperature, soil moisture status and light intensity, and their interactions for flowering and pigmentation in a diallel cross between seven genotypes for vegetative and phenological characters. Subsequently, De Silva and Omran (1986) reported heterosis with respect to the number of seeds per pod and grain yield in half-diallel cross between nine diverse genotypes of winged bean. An understanding of the genetic basis of these phenotypes needs more genetic studies to be carried out.

Early studies on the estimation of genetic diversity among winged bean accessions have attempted to use morphological and cytological markers (Armachuclo and Bernardo, 1981). Cytological studies revealed no chromosomal differences between winged bean and its congener *Psophocarpus scandens* (Endl.) (also 2n=2X=18; Harder, 1992). ISSR, RAPD (Chen et al*.,* 2015; Mohanty et al*.*, 2013) and SSR (Yang et al. 2018) molecular markers have been used by various workers for estimating the genetic variation within, and relationships between, various accessions of winged bean. The results indicate that the studied accessions have a close relationship and a narrow genetic background; and as in many crops, phenotypic variation in winged bean does not correlate with diversity at the molecular level (Chen et al.*,* 2015; Yang et al.*,*2018).

Large volumes of genomic data for winged bean are being generated by employing various sequencing platforms and have the potential to provide new resources for gene discovery, marker development and trait-association and corresponding studies for strengthening future marker-assisted breeding of this underutilized crop. Gene discovery in winged bean can leverage knowledge from other crops, and studies so far suggest that comparative analyses would be of relative ease to carry out. For example, comparison of the assembled contigs of winged bean by Vatanparastet al., (2016) against other legumes sequences available in the NCBI database, e.g. chickpea (*Cicer arietinum* L.), pigeon pea (*Cajanus cajan* (L.) Huth), soybean (*Glycine max* (L.) Merr.), common bean (*Phaseolus vulgaris* L.), *Medicago truncatula* Gaertn.and *Lotus japonicus* (Regel.) K.Larsen revealed that 15,558 of 16,115 contigs (96.5%) had significant similarity of sequences in one or more legume protein databases. About 90.5% of the 16,115 contigs had ≥80% sequence identity. The majority of the contigs (57.3%) were similar to *G. max* reflecting its genetic similarity.

The study by Singh et al., (2017), on the leaf transcriptomes of contrasting condensed tannin-containing lines of winged bean, reported the maximum number of contigs from C2H2 family of transcription factors (TFs), followed by the WRKY family. These TFs were found more frequently in higher condensed tannin containing lines as compared to the low condensed tannin containing lines of winged bean, which could be worthy of follow-up for future breeding of low anti-nutrient winged bean. In addition to the study by Singh et al*.,*(2017), other transcriptome sequences have been generated from leaves (Chapman, 2015) and from a range of tissues (Wong et al., 2017). Transcriptome mining has revealed thousands of single nucleotide polymorphisms and simple sequence repeat (SSR) markers in winged bean (Chapman, 2015;Vatanparast et al., 2016). Singh et al.,(2017) resolved ca. 2000 SSR-containing loci, and Wong et al*.,*(2017) identified over 9000. All four studies reported an abundance of tri-nucleotide repeat-containing SSRs, which is not unexpected given that contraction or expansion of these repeats would not interrupt the coding region of the transcript in which the SSR was found.

Comparison of the four underutilized legume transcriptomes generated by Chapman (2015) allowed the identification of cross-legume Conserved Ortholog Sequence (COS) markers, i.e. putatively orthologous transcripts across the four species. These markers could be used for primer design and subsequent phylogenetic or comparative mapping investigations. These investigations reflect the utility of transcriptome sequences for facilitating further genetic studies concerning crop origins, the partitioning of genetic diversity and comparative genetic mapping.

The development of these molecular markers has the potential to expedite breeding for improved varieties, termed molecular breeding (Moose and Mumm, 2008). In this, researchers begin by identifying genomic regions of interest, for example those containing positive alleles for yield or stress tolerance, using quantitative trait locus mapping or association mapping. They then identify molecular markers linked to these regions which are then genotyped in the crossed progeny. Individuals with the correct combination of markers are then retained in the population and the others removed, saving time to wait until the plants are adult to phenotype them, and reducing the amount of space needed to grow entire population of plants to adulthood, only to discard the majority. For relatively slow-growing crops such as winged bean this may be particularly important going forward.

**3.4. Genome editing and the future of genetic manipulation**

Significant progress has been made in last several years in the targeted genome-editing technologies including ZFNs, TALENs and CRISPR-Cas system (Nemudryi et al., 2014).Use of the CRISPR-Cas9 system towards the study of symbiotic nitrogen fixation (SNF) in legumes, and the engineering of this pathway into non-legume crops to reduce the dependence on the use of nitrogen fertilizers has been proposed (Wang et al., 2017). The successful application of CRISPR-Cas9 mediated genome editing of specific genes in soybean(Cai et al., 2015), proves the potential to target specific genes responsible for biosynthesis of anti-nutrients, including condensed tannin or inhibitors in winged bean (Figure 8). This will help in adopting the plant for further cultivation, consumption and commercialization and will provide a safety-net against the nutrition deficiency.

**4. Conclusion**

Underutilized legumes with high-quality protein and calories that are not extensively utilized as food need to be targeted for improvement. This will help to address the food and nutrition issues of the population. As a very limited number of legumes, dry beans, legume seeds or grain legumes are used on a large scale for human food at present; the focus should be to improve underutilized legumes for human consumption and use and we proposed winged bean as a model for this. In this paper we review some past, present and future suggestions for methods to improve these crops through various biotechnological, breeding and agronomic practices to address the productivity, yield and climate resilient traits without compromising the bioavailability of molecules in this underutilized legume. Successful development of a somatic embryogenesis will lead to the ability to edit genes of interest through *Agrobacterium*-mediated transformation. Induced mutations have the ability to induce variability and increase the rate of domestication as is evident from this paper. Induced mutagenesis has produced several positive results in winged bean. Tolerance to stress, diseases and pests as well as the presence of anti-nutrient factors (saponins, lectins, inhibitors and tannins) are the traits which need be addressed. Once the genetic bases of these traits is revealed, genome-editing techniques of the genes responsible will expedite the development of desirable genetic variants. The genome-edited plant ideotype with suitable traits, i.e. low anti-nutrients and fortified protein-rich edible parts of the plant, has the potential to provide household nutrition security to those at risk of hunger and malnutrition.

**Declaration of interest**

The authors have no conflict of interest to declare.

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