



Plant Archives

Journal homepage: <http://www.plantarchives.org>

DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.052>

EXPLORING THE POTENTIAL AND VERSALITY OF NUTRIENT RICH LEGUME ADZUKI BEAN : A REVIEW

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(Date of Receiving : 28-07-2024; Date of Acceptance : 29-09-2024)

ABSTRACT

Adzuki bean is an excellent source of nutraceutical properties used both as food and medicines, which makes it singular among other legumes. Adzuki has similar protein content to other more common cereals like wheat, but is relatively richer than other cereals in the essential amino acid lysine. Adzuki is also a good source of essential fatty acids, fiber, minerals (especially calcium and iron), and phytochemicals such as polyphenols and phytates. Existing studies on nutrition and health benefits of adzuki bean are limited since they fail to take into account differences in adzuki varieties and growing conditions. Nevertheless, the studies undertaken so far confirm adzuki's excellent nutrient profile and suggest it has considerable potential globally to be a functional food for health promotion and disease prevention. Adzuki bean is one of the earliest domesticated crops, being cultivated in more than 30 countries of world including East Asia however, first time the scientific crop improvement study was taken place in Japan. Its landraces are still grown in Japan with high production, which are predominantly large, red-seeded genotypes. Being an important pulse, its diverse germplasm has been conserved world over including Indian National Genebank, where a total of 201 accessions (98 are exotic, 103 indigenous and 9 of its wild relative *V. angularis* var. *nipponensis*) are conserved for their utilization in crop improvement programmes. Nutritional richness, crossability potentials, adaptation and domestication, biochemical traits as well as botanical characters of both wild and cultivated adzuki bean have been discussed in this review. Additionally, potential health benefits that could be associated with higher consumption of adzuki are also highlighted.

Keywords : Germplasm, Potential legume, Nutraceutical, Genebank.

Introduction

Adzuki bean {*Vigna angularis* (Willd.) Ohwi and Ohashi}, belongs to subgenus *Ceratotropis* of Fabaceae family is an annual legume domesticated in East Asia (Tomooka *et al.*, 2014; Yang *et al.*, 2015). This species seems to have the highest level of cold-tolerance among cultivated *Vigna* species (Takahashi *et al.*, 2017). It is well suited to monsoon-influenced humid sub-tropical, sub-tropical highland climate with dry winters (Rubatzky and Yamaguchi 1997) and grown widely in China, Japan and Korea, while has been introduced recently in other continents. It is a self-pollinating diploid plant species with 22 chromosomes ($2n=2x=22$), has papilionaceous flower;

while seed shape varies from cylindrical to cordate with a seed coat colour ranging from wine red, creamy, black or mottled. The varieties in Northeast Asia are mainly with red seed coat colour but other variants such as gray, white, black, and multifariously mottled varieties have also been reported. In East Asia it is grown as a traditional pulse crop, while used as a source of protein for human nutrition especially in developing and underdeveloped countries.

Adzuki bean is also popularly named as 'red beans' or 'red mung bean', used in many confectionery items as a prevalent ingredient. It is the second most important legume crop in Japan after soybean, while in India, it is sporadically grown as a pulse in higher

reaches of both eastern and western Himalayas (Takahashi *et al.*, 2017). High yielding varieties of adzuki bean are found in Japan (Arora, 2014), where it is predominantly used in traditional Japanese confections - a sweetened paste or *wagashi* and serves as filling in different sweet dishes. Adzuki bean is considered as a nutraceutical crop, serves as a source of carbohydrates, protein, vitamins, minerals and dietary fibers (Tjahjadi *et al.*, 1988). Though, it also contains some anti-nutritional factors like phytates, α -galactosides and trypsin inhibitors while their concentrations differ widely among the different cultivars (Sharma *et al.*, 2019). In India, grains are boiled and eaten as pulse and tender pods as vegetable. The physical characteristics of adzuki bean, like those of other grains and seeds of pulses are essential for harvesting, cleaning, separation, handling, aeration, drying, storing, milling, cooking and germination (Hsieh *et al.*, 1999). About 60 varieties of adzuki bean are present on the basis of their seed size ranging from 5 to 8 mm in length and 3 to 5 mm in width. These may be divided into two main categories (i) small sized seeds, which are regular seeds, also called Erimo type. It is used to process Ann, which can be eaten as a sweet and is used as a filler; and (ii) large sized seeds, also known as Dainagon, which is used in preparation of several traditional confectioneries and sweet soups.

Speciation and domestication: *Vigna angularis* var. *nipponensis*, a close progenitor of adzuki bean is spread across the Japan, Korea, China, Nepal and Bhutan (Yang *et al.*, 2015). Literature specifies that around 50,000 years ago speciation between *V. angularis* var. *nipponensis* and *V. angularis* var. *angularis* was occurred. Archaeologists estimated its domestication around 3000 BC, while bold seeded types were supposed to occur during the Bronze Age or Iron Age. Literature also indicates that Adzuki bean was domesticated in China ~12,000 year ago (Liu *et al.*, 2013). Thus, many workers have recommended its multiple domestication (Yamaguchi 1992; Kaga *et al.*, 2008; Kang 2015; Lee *et al.*, 2013; Lee 2015; Yang *et al.*, 2015). *V. nakashimae* and *V. nepalensis* are another probable progenitor of adzuki bean are broadly scattered across the East Asia and Himalayan belt (Tomooka *et al.*, 2002). With respect to Indian perspective, its cultivation is narrowed to the North-western and North eastern hilly zones. Its production was sporadic in the state of Himachal Pradesh (Kangra, Chamba, Mandi and Sirmour districts). Under human production, adzuki plants produces lesser but longer pods.

The exact place of adzuki bean domestication is not known yet. However, it exists as a crop complex in

Japan where cultivated, wild, and weedy forms can be traced (Vaughan *et al.*, 2004; Kaga *et al.*, 2008). Additionally, carbonized adzuki bean seeds dating back 4,000 years ago have been discovered in archaeological sites in China, Korea, and Japan (Maeda 1987; Yano *et al.*, 2004; Crawford, 2006). Thus, Japan is considered as one of the possible places where adzuki bean might be domesticated. In comparison with its presumed wild ancestor, cultivated adzuki bean shows numerous differences in morphological and physiological traits probably associated with human selection during domestication. These differences, collectively called the domestication syndrome, resulted through selection process of several thousand years of adaptation to cultivated environments as well as ability to fulfil the human nutritional requirements and preferences (Hawkes, 1983).

In various parts of Japan, where wild and cultivated types are in sympatric speciation, plants with variable phenotypes are commonly found (Kaga *et al.*, 2004). Although wild and cultivated adzuki beans are mainly self-pollinated, however outcrossing (at 1% rate) between these forms has also been observed (Yamamoto *et al.*, 2006). Some plants in wild populations have been shown to have genes from cultivated adzuki bean (Wang *et al.*, 2004), which indicates that natural crossing among this crop complex is a regular phenomenon. According to Ellstrand *et al.* (2013), the population dynamics of introgressed wild populations are largely affected by the fitness of hybrids and their derivatives in natural conditions. During the process of domestication, its wild forms and selections from wild populations might have hybridized and resulted into variation, subjected to further cycles of human selection (Harlan, 1966). Through human selection, wild plants became crops and adapted to cultivated habitats, but exhibit reduced fitness when growing in natural habitats. However, it is not known to what extent and what factors contribute to crop genes which created disadvantage to plants in natural conditions, and why weedy forms with many cultivated genes could persist in natural conditions (Gressel, 2005). It is a known fact that the persistence or extinction of crop genes in wild populations is dependent on whether crop genes, which confer advantages or disadvantages to hybrid fitness outside of the cultivated environment. In addition, genetic drift may also play an important role in the survival of crop genes in wild populations.

Adzuki bean was the foremost crop plant in which scientifically crop improvement was taken place in Japan. Its landraces are still grown in Japan with high production in Hokkaido prefecture, which are

predominantly large, red-seeded genotypes. The Japanese farmers also grow adzuki beans on a small scale in kitchen gardens for home consumption. Whereas, wild adzuki bean is a climbing, annual, herbaceous plant generally having black-mottled seeds is commonly found in Japan on the other main islands (Honshu, Kyushu, and Shikoku), besides Hokkaido. It is generally habituated in disturbed lands such as outskirts of the field, riverbanks, and pavement verges. Landraces across the Japan might have accumulated alleles as a result of natural introgression with wild adzuki bean and through farmer selection. Adzuki bean holds a position of second most important food legume after soy beans (Danjiao *et al.*, 2015). There are more than 30 countries in the world that grow adzuki bean, although China, Japan and South Korea are the main growers, while China has the largest area (approximately 2500 hectares) under cultivation and is also considered as the highest producer of adzuki beans in the world.

Vernacular names: The term adzuki is a transcription of the Japanese name. They also have a Chinese lend word *shozu*, verbally it means "small bean", its corresponding term "large bean" (*daizu*) was termed for soybean. In Chinese, it is called as *xiaodou*, *hongdou*, *chidou* which has the undertone "red bean", as virtually all Chinese varieties are unvaryingly red colored; In English it is called as red bean; In Korean it is entitled as *pat* but has disparities with *kong* bean; Vietnamese know adzuki bean as *daudo*; while In India, it is called with many names in different places as *red chori* (Punjab); *ghurush*, *rains*, *dhoulash*, *sontha*, *chhota-sontha* (Uttarakhand); *rogi*, *kau*, *lalmansh* (Himachal Pradesh); *adua*, *perin*, *shakpu* (Arunachal Pradesh); *rato-masham*, *lal-masham* (Sikkim) and *wuzul moong* (Jammu and Kashmir).

Distribution: Adzuki bean is an important legume among the twelve most important legume crops, belonging to genus *Vigna*. Earlier it was kept under the genus *Phaseolus*, but has recently been transferred to the genus *Vigna* with the related pulses such as mung bean, urd bean, moth bean and rice bean (Lumpkin and McClary 1994). It is widely grown in China, Japan, South Korea, Vietnam, Myanmar and Taiwan, while sporadically also cultivated in Indian Himalayas (Arunachal Pradesh, Himachal Pradesh, Uttarakhand and Sikkim), Nepal and Bhutan. It is introduced in USA in 1978 but due to less yield could not be grown widely (Ohwi, 1953; Tateishi, 1996; Tomooka *et al.*; 2002; Kaga *et al.*, 2008).

Wild and weedy forms: The existence of weed forms of adzuki bean was reported in Japan. Acclimatization in the humanoids' disturbed environments, old cultivars

escaping, and natural establishment from cultivar-wild hybrid derivatives are all contributing factors responsible for the extensive spread of its weedy forms. As opposed to its wild counterparts, adzuki beans grown from weeds are substituted for their cultivated counterparts and eaten as sweet beans, especially when pests attack was observed in the cultivated one. However, in adzuki bean cultivated fields, the weedy form was recognized as contamination and supposed to deteriorate the seed quality of adzuki bean cultivars (Yamaguchi, 1992). The other wild relative (*Vigna nepalensis*) of adzuki bean, having chromosome $2n=22$ is also found in India (Sikkim, Darjeeling and Assam) and East Nepal (Tateishi, 1985). The morphology of *V. nepalensis* is similar to that of *V. angularis* var. *nipponensis*, however the main differences between two taxa are in length of hilum, shape of bracteoles and pubescence of inflorescence rachis. Stipule of *V. nepalensis* is peltate, narrowly oblong or narrowly ovate, acute at the apex, 7.5 to 12.5 mm long, 1.5 to 4.5 mm wide. Flowers are golden yellow, 18 to 19 mm in diameter. Rachis are 2 to 6 cm long, glabrous. Bracteole is lanceolate or narrowly triangular-ovate, acute at the apex, 4 to 6 mm long, 1.5 to 1.8 mm wide, a little longer than or almost equal to the calyx. Pods pendulous, linear, cylindrical, 6 to 9 cm long, 4 to 4.5 mm wide, straight, glabrous. Seeds short cylindrical or ellipsoidal, greyish brown and variegated with black when matured, smooth, 3 to 4.5 mm x 2 to 3.2 mm x 1.8 to 2.6 mm. Hilum linear or oblong, 1.1 to 1.8 mm long, aril thin. Germination hypocotyl, primary leaf cordate with long petiole (Tateishi, 1985).

Cultivation: China was the world's greatest producer of adzuki beans in 2019–20, with approximately 670,000 ha of production acreage and 2601000 tonnes produced, next to Japan, which produces 140,000 tonnes annually (Anonymous, 2019). In addition to the US, South America, and India, the bean is also grown economically in New Zealand, Kongo, and Angola. The adzuki bean, which had an annual yield of about 100,000 tonnes in 1998, is the second-most significant legume in Japan, behind the soy bean. Japan is also the main consumer of adzuki beans, using roughly 140,000 t annually. Additionally, imports come from China, Korea, Colombia, Taiwan, the United States, Thailand, and Canada. Per area blowout the yields over a broad range is due to difference in cultivation intensity. Yield of 4 to 8 dt/ha is reported, but in Japan and China, the yield has attained a range between 20 and 30 dt/ha (Floridata Plant Encyclopedia 2016; Schuster 2016).

Ecological requirements: Adzuki bean is a forgiving crop that can thrive in regions with high rainfall and

flourish in any type of soil (Sharma *et al.*, 2019) that is ideally well-drained and range in pH from 5 to 7.5. Crop need soil temperatures above 6 to 10°C for germination, and the ideal temperature between 15 and 30°C for crop growth. Warm temperatures encourage vegetative growth, which makes their production less favourable. In the regions where it is grown, the average annual rainfall between 500 to 1750 mm is necessary, although the plant can tolerate droughts, however a considerable drop in productivity is anticipated. Fertilizer treatment varies greatly but tends to be similar to soybean. Up to 100 kg/ha of nitrogen can be fixed by rhizobia through nodulation (Schuster 2016; Floridata Plant Encyclopedia 2016).

Production, pests and diseases: The sowing of the adzuki bean is recommended in 2 to 3 cm depth in rows of 30 to 90 cm apart and 10 to 45 cm within the row. Broadcasting of seeds is rarely followed. The seed quantity required between 8 to 70 kg/ha. Initial vegetative growth is slow, therefore weed management is crucial, require intercultural operation from germination to flowering. The cultivation practices of many nations vary greatly. Adzuki beans are frequently planted in China as intercrops with maize, sorghum, and millet, whereas in Japan they are produced in accordance with crop cycles. Harvesting is done when moisture content of the seed drops below 16%. Generally, rice and adzuki bean multiple cropping systems are recommended, while maize and adzuki bean relay intercropping systems are also followed in some countries (Li *et al.*, 2017). The most important diseases and pests of the crop are brown stem rot (*Cadophora gregata*), phytophthora stem rot (*Phytophthora vignae*), and fusarium wilt (*Fusarium oxysporum*) (Kondo *et al.*, 2018). These are extremely serious biotic problems have been observed in adzuki bean crop. Since these are soil-borne, hence it is challenging to control them using chemical or cultural approaches. The best way to manage these diseases is through the cultivation of resistant cultivars only. Currently, Japan has developed resistant cultivars such as *Kita-no-otome* and *Syumari* to overcome the biotic problems. Furthermore, important pests of adzuki bean are adzuki pod worm (*Matsumuraeses phaseoli*), Japanese butterbur borer (*Petasites japonica*) and cutworm (*Agrotis spp*), which attack the crop. Bean weevil (*Callosobruchus chinensis*) is a most devastating storage pest of adzuki bean (Wang *et al.*, 2019).

Uses : Adzuki bean is one of the important cultivated pulse crops, which can be consumed as a fresh vegetable, dried grains similar to other pulses, also used as a fodder for livestock and for green manuring.

Variety of dishes are made from adzuki bean, commonly sweetened before eating. For the purpose, red bean paste (anko), an indispensable ingredient in all cuisines, is made by boiling red beans with sugar. It is standard practice to spice dishes using ingredients like chestnut. Red bean paste is a common ingredient in many Chinese recipes, including red bean icecream, moon cakes, *baozi*, *tangyuan*, and *zongzi*. Additionally, it's used as a filler in Japanese confections including *anpan*, *dorayaki*, *imagawayaki*, *manj*, *monaka*, *anmitsu*, *taiyaki*, and *daifuku*. Adzuki beans are cooked with sugar and a little salt to create *hongdou tang*, the fluid version of the meal. Its grains are commonly consumed in the form of sprouts, or boiled in a hot beverage resembling tea. Red bean paste is a favorite+ garnish or filling for a variety of waffles, pastries, baked buns, and biscuits in some Asian cultures. On special occasions, rice with adzuki beans is customarily prepared in Japan. Its whole bean is used in *amanatto* and ice cream (such as in the 'Cream and Red Bean' product produced by IMEI) or as paste. In Japan, China and Korea, the people consume it daily as a dessert or snack and also during celebrations of traditional festival such as the Chinese New Year. In Japan, it is consumed as a whole bean, as boiled, and as sweetened bean paste, *ann* or “*an*”. Most common way of its consuming is in the form of snacks such as bakery products (buns and pastries) filled with *ann*, e.g., “*manju*” (wheat or rice flour cake with adzuki ‘*ann*’ filling), “*daifuku*” (glutinous rice cake with adzuki ‘*ann*’ filling), “*anpan*” (bun with adzuki *ann* filling) and “*monaka*” (baked pastry with adzuki ‘*ann*’ filling). Also famous for traditional confectionery, e.g., “*amanatto*” (whole hard candied or sugar coated adzuki), “*yokan*” (firm sweet adzuki paste set with agar- agar) and “*mizu-yokan*” (soft adzuki paste set with agar- agar). In the form of desserts, such as “*kakigori*” (shavings of ice with sugar syrup and occasionally with sweet bean topping and a scoop of ice cream). In curries, it is used as red bean soup, “*shiruko*” (sweet adzuki soup with dumplings, which are small sized rice cake/flattened corn starch balls).

Adzuki beans also have a long history of use in traditional medicine. They are used as a diuretic and to treat kidney diseases, antidote, dropsy and beriberi. The beans are also used for problems with pregnancy and birth, including threatened miscarriage and retained placenta, and they are used to encourage lactation. In addition, adzuki flour is made into shampoos and facial creams. Researchers have proved recently that there is sufficient evidence which support the use of adzuki bean in curing the type 2 diabetes (Kwan, 2023). Since they contain reasonable amounts of fibre, they have a very low glycemic index. It makes

them a perfect low glycemic index food for people with diabetes. Consuming adzuki bean which has a glycemic index 26, making them a low GI diet, can adequately control blood glucose levels in diabetics. However, still there are several research gaps in the current literature evaluating the effects of adzuki bean consumption on preventing and managing diabetes its effects on obesity, because most of the evidences of its usefulness in diabetes and obesity comes from animal studies, and these studies have not yet identified the underlying mechanisms by which adzuki bean impacts diabetes and obesity related outcomes. As most of the results suggest that adzuki bean can be regarded as a functional food to prevent and treat obesity and related complications. Since ancient times, adzuki beans have been popular for their wide range of health benefits. For years, they have been used in many sweet and savoury dishes in different forms. If consumed right, these beans can help prevent many health-related conditions. The usefulness of adzuki beans is not only due to its attractive nutritional profile but also because it has no gluten that is found in other common cereals. As more and more patients are diagnosed with gluten intolerance and celiac disease, the demand for gluten-free foods is also growing.

Adzuki bean gene pool: Genus *Vigna* includes seven pulse crops: mung bean [*V. radiata* (L.) R. Wilczek], azuki bean [*V. angularis* (Willd.) Ohwi & Ohashi], rice bean [*V. umbellata* (Thunb.) Ohwi & Ohashi], moth bean [*V. aconitifolia* (Jacq.) Marechal], cowpea [*V. unguiculata* (L.) Walp.], black gram [*V. mungo* (L.) Hepper], and tuber cowpea [*V. vexillata* (L.) A. Rich. (Pratap et al., 2014)]. Primary gene pool of *V. angularis* comprises of *V. angularis* var. *nipponensis* and wild types of *V. umbellata*. Secondary gene pool includes *V. dalzelliana*, *V. glabrescence* and *V. minima* and tertiary gene pool consists of *V. aconitifolia*, *V. mungo*, *V. radiata*, *V. trilobata* and *V. grandis* (Tripathi et al., 2020). The findings of the research conducted by Siriwardhane et al., (1991) reveals that wild species such as *V. nakashimae* and *V. angularis* var. *nipponensis* belongs to sub-genus *Ceratotropis*, which were cross compatible with adzuki bean in both the directions whereas, *V. riukiensis* was cross compatible only if used as male parent and similarly with *V. umbellata* crosses were successful if used as pollen parent and *V. angularis* as seed parent. *V. riukiensis* was cross compatible with both rice bean and adzuki bean, hence it can be used as bridge species to transfer the genes between them.

Botany and physiology: The adzuki bean has annual growth habit and rarely it is biennial, bushy erect or twining herb, usually the height of the plant ranged

between 30-90 cm. The stem is pubescent and stems are green in colour and purple pigmentation can also be observed in certain plants. It has a taproot system, can fix atmospheric nitrogen through root nodules with nodulation for nitrogen fixation. The depth of root ranged from 40-50 cm from the point of seed sprout. It has trifoliate pinnate leaves that are alternately arranged along the stem and attached to the stem by long petiole. Its ovate leaflets are 5-10 cm long and 5-8 cm broad. The flowers of adzuki bean are papilionaceous and are bright yellow in colour, which are arranged on axillary false raceme, consisting of 6-10 flowers. Its pods are cylindrical, smooth and thin-walled. The young pods are green in colour turning white, grey or brown on maturity. The size of pod is between 5-13 cm x 0.5 cm, containing 2-14 grains per pod. Pod shattering is common at the time of maturity and makes harvesting difficult. The grains are smooth and sub-cylindrical, 5.0 to 9.1 mm long, 4.0 to 6.3 mm broad, and 4.1 to 6.0 mm thick (FAO, 2016; Lumpkin et al., 2016; Schuster, 2016; Wu and Thunlin, 2016). The seed coat colours varied from maroon to blue-black mottled and creamy white (Fuller, 2007; Schuster, 2016). Seedlings emergence of adzuki bean is hypogeal and requires 7 to 20 days to emerge. As compared to other legumes, growth of the plants is slow. Flowering lasts in 30-40 days. Adzuki bean plants generally take 80 to 120 days for maturity, depending upon the variety and growing environmental conditions.

National and international status of adzuki bean accessions: In India, All India Coordinated Research Network on Potential Crop (AICRNPC) conducts research on adzuki bean at centers located in Himalayan region. A total of 201 accessions of adzuki bean are conserved at National Genebank, New Delhi, India. Of which, 98 are exotic and 103 are indigenous collections which include 9 accessions of its wild relative *V. angularis* var. *nipponensis*. Adzuki bean germplasm collections are also conserved by many other countries of the world in their Gene banks which include Japan, Republic of Korea, China, Taiwan, USA, North Korea, Philippines, Russia, Nepal, France etc. (<https://www.genesys-pgr.org/a/overview/v2GrK91wmaV>). Among these countries, China holds largest number (Above 5000) of accessions in the Institute of Crop Germplasm Resources (CAAS), Beijing, which is followed by Japan (2300 accessions) in National Agriculture and Food Research Organization. World Vegetable Centre (formerly known as AVRDC: Asian Vegetable Research and Development Centre) is the only international organization working on adzuki bean, which holds 2,387 germplasm collections.

Agro-morphological characterization

One of the first crops in Japan to undergo a scientific agricultural development programme was the adzuki bean (Kaga *et al.*, 2008). The yield, consistency of the bean colour, and the maturation time are key breeding factors. For the purposes of fodder and green manure, separate cultivars with smaller seeds and higher biomass are bred (Schuster, 2016). There are cultivars that have been locally adapted in China, Japan, Korea, and Taiwan and India (HPAU-51, Milan Local, Grams Local-2 and Totru Local). In adzuki bean improvement programme, important economic traits that are directly contributing to yield are as seed yield per plant, seeds per pod and number of pods per plant are primary determining characteristics while additionally taking into consideration, the number of primary branches and pod length. Plant height, 100-seed weight and other traits for breeding new varieties are having excellent inclusive properties (Danjiao *et al.*, 2015). According to Xu *et al.* (2000), the wild and weedy relatives of the adzuki bean exhibit increased genetic diversity, which may be a helpful characteristic for crop improvement. Adzuki bean is mostly autogamous, however, cross pollination has been documented in substantial amounts. It is fully fertile with its cultigens; consequently, it can be used directly in breeding schemes. However, detrimental linkage drag frequently confuses the introgression of favorable features from the wild. Because they closely resemble the cultigens while providing more diversity than the cultigens, intermediate, weedy, or semi-weedy types are better candidates for breeding than wild adzuki bean.

Wang *et al.* (2001) conducted a replicated yield trial in Australia and screened a total of 168 accessions representing subset of core collection of Chinese adzuki bean landraces. For each accession along with the check variety, yield components, grain yield and phenotypic parameters were recorded. B2172 from Anhui was identified as highest yielding accession followed by three more accessions (B2180 and B2158 from Anhui and B2760 from Henan Province) exceeded the best performing checks. As a percentage of the trial mean, the expression ranges for the important quantitative traits were recorded as follows: 111% for total height, 144% for pods/ha, 218% for seed number/ha, 43% for complete maturity, 79% for flowering, 80% for initial maturity, 176% for synchronous maturation, and 118% for 100-seed weight.

Redden *et al.* (2009) characterized a set of core collection containing 231 accessions at multilocation. Genotypic and environmental interactions was high in

South China, where material was characterized for late maturity and small seed size, and north China for earliness. The germplasm from the provinces of mid-north China had the highest diversity. Yoon *et al.* (2012) in order to compile fundamental information for the advancement of crop breeding, 150 samples of the *V. angularis* germplasm collected from Korea were characterized for various agronomic traits. Out of 150 accessions, eight accessions with different seed coat colors were observed, among them 66.5% were with dark brown colour. Days to seed maturity was recorded between 89-142 days with average of 115 days. Number of pods per plant showed range of 5-132.5 pods with mean of 28.5 pods. 100 seed weight ranged between 5.7-23.0 g with mean value of 12.9 g. Germplasm are the vital source for resistant or tolerant genes. To identify salt tolerant lines, Yoshida *et al.*, (2016) screened 74 germplasm along with 145 germplasm of seven species of compatible wild relatives. They identified one accession of *V. riukiensis* (JP205833) and one accession of *V. nakashimae* (JP107879) as salt tolerant germplasm.

Chu *et al.* (2021) done genetic analysis for the seed coat colour using phenotype and chi square test in adzuki bean and found that a single R locus determines the seed coat colour, hence ivory colour was recessive to red colour. However, red colour was recessive to brown, light brown, golden brown, black, mottled black on red and mottled black on grey. Except black mottle on grey which was controlled by two loci, all other colours were controlled by single locus. Hu *et al.* (2022) grown a collection of 27 wild relatives and 448 cultivated adzuki beans at two different locations in China (Nanning and Nanyang), which were phenotyped for yield and yield-related traits. It was observed that for the majority of variables, significant variation was seen across the germplasm, locations, and the genotype \times environment interaction. High heritability was observed for the number of branches per plant (63.9%), weight of 100 seeds (68.0%) and number of seeds per pod (77.8%). Principal component analysis (PCA) reduced a total of 10 traits into 3 comprehensive factors, with the top three principal component factors accounting for 72.31% of the total variability. All the 475 adzuki bean accessions were divided into five distinct groups using cluster analysis.

Desta *et al.* (2023) investigated adzuki bean genetic diversity using 18 agro-morphological traits. They studied 252 germplasm line assembled from Japan, China, and Korea. Among the qualitative traits recorded by them, wide variability was recorded for pod color, seed coat color and leaf shape. Compared to Korean and Japanese adzuki bean accessions, Chinese

accessions required less days to bloom (DF, 58.22 days) and maturity (DM, 107.13 days) ($p < 0.05$). Comparing Japanese adzuki beans with Korean and Chinese adzuki beans, the average number of pods per plant and the 100-seed weight were high, however the variation of each was not statistically significant. In comparison to control (checks), over 29.76% of the accessions had flowered that bloomed early, 3.97% had premature blooms, 21.43% produced more pods per plant and 3.97% yielded more seeds per pod. From this investigation, analyzed adzuki bean accessions have shown good genetic diversity hence resulted into identification of ten high yielding and ten early maturing accessions.

Molecular diversity analysis

Germplasm characterization using molecular markers gained importance due to speedy and quality data generating ability. Genetic diversity can be best estimated by DNA markers viz., AFLP, RAPD, RFLP, SSR, SNPs etc., particularly simple sequence repeat markers are the most widely used markers predominantly due to their abundance in the genome, ability to differentiate heterozygotes, reproducible etc., and many workers have been used molecular markers for conducting diversity analysis in adzuki bean, are elaborated in this session. Xu *et al.*, (2000) conducted molecular characterization of three forms of *V. angularis*, cultivated, wild and weedy using RAPD markers to know the genetic variations, and studied their relationships. The wild form shown the high genetic variance ($H_g = 0.132$; $G_D = 0.388$), in comparison to weedy ($H_g = 0.124$; $G_D = 0.341$) and cultivated forms ($H_g = 0.079$; $G_D = 0.274$). In both weedy and the wild populations, there was considerable intra-population genetic variation. Among evaluated populations of all groups in *V. angularis* complex, genetic variation was higher across populations than within populations. Wang *et al.* (2004) have developed 50 SSR primers pair after screening of selected eight primers for polymorphism. They discovered complex spatial pattern of population structure. Though adzuki bean is predominantly an autogamous species, however out of 20 genotypes tested, three individuals in the population showed heterozygosity, implies the outcrossing and gene flow between the individuals.

Use of 50 AFLP markers established the genetic variation and relationships among members of the azuki bean complex, including wild (*V. angularis* var. *nipponensis*), cultivated and weedy types (*V. nakashimae*), and also rice bean (*V. umbellata*) from Korea. Out of the 462 fragments amplified using seven primer combinations, 333 (72.1%) were polymorphic.

Within each species, there were 70 polymorphic fragments in the adzuki bean complex and 41 in *V. nakashimae*, however, in the rice bean polymorphism was not observed. With respect to *V. nakashimae*, the adzuki bean complex, and rice beans, Jaccard's similarity matrix was separated into three groups. Compared to adzuki bean and *V. nakashimae*, the relationship between the rice bean and azuki bean is closer (Yoon *et al.*, 2007). Xu *et al.* (2008) in their investigations used 13 polymorphic SSR primers to evaluate genetic diversity in 616 adzuki bean accessions including its wild relatives from eight Asian countries to develop core collections. The most varied and genetically unique cultivars of adzuki bean were from Japan, China, and Korea, indicating a lengthy and largely isolated history of cultivation in each nation. The cultivated adzuki bean from Bhutan and eastern Nepal were similar to one another but somewhat different from other varieties. The findings suggests that Himalayan germplasm may represent a new gene source for adzuki bean breeding. Similar studies using SSR markers were conducted to evaluate the genetic diversity of 366 *V. angularis* core collections of China by Ning *et al.* (2009). Out of 106 markers, they found 13 markers as polymorphic and used for the studies. *V. umbellata* had 4 to 9 alleles per locus, with a mean value of 10.2 alleles. The average polymorphism information content (PIC), which measures the proportion of polymorphic loci, was 0.561.

Zhao *et al.* (2011) have employed 11 highly polymorphic SSR markers to investigate the genetic diversity in 558 wild, semi-wild and cultivated adzuki bean accessions assembled from Bhutan, Japan, China, South Korea, and Myanmar. A total of 86 polymorphic bands were produced. One set of SSR primers yielded an average of 7.28 polymorphic bands. In wild, semi-wild, and cultivated adzuki beans, specific bands could be seen. Chinese cultivars and landraces predominately produced specific bands of cultivated azuki bean. Wild accessions from China, Bhutan, and South Japan, produced specific bands. Analysis displayed wild, semi-wild and domesticated were genetically dispersed in the decreasing sequence. The genetic link between semi-wild accessions and wild adzuki bean showed closer association. In accordance with genetic distance, a cluster analysis was used and it divided 558 accessions into five major groups.

One hundred fifty-eight applicable core collections of adzuki bean germplasm and 12 wild relatives' genetic diversity was examined by Li-xia *et al.* (2012) using 85 SSR markers. With a mean of 5.81 alleles for each locus, 493 alleles were discovered, and 73.02% of these had distribution frequencies below

five percent. Amongst wild and domesticated adzuki bean germplasm, there are discrepancies in the allele distributions, with the wild genotype having a greater degree of allelic variation. Chen *et al.* (2015a) have developed and validated EST-SSR markers from the transcriptome of *V. angularis*. As potential molecular markers, 7,947 EST-SSRs were identified. Among them, 500 SSR markers were chosen at random for validation, and 296 of those gave reproducible amplicons with 38 polymorphic markers, which were screened with 32 adzuki bean genotypes chosen from various regions of China. These developed 38 SSR polymorphic markers which are valuable in genetic diversity analysis of adzuki bean and other related *Vigna* species.

Chen *et al.* (2015b) have developed a total of 53 new genomic-SSR 38 (gSSR) and EST-SSR (eSSR 15) markers. Using 110 markers (53 new and 57 already existing markers), 261 adzuki bean accessions were genotyped. These accessions were grouped into ten different clusters. The accessions from North China were found distinct as compared to the accessions from South China. Based on POPGEN analysis, 110 markers detected 607 alleles. There were between 2-17 alleles per locus, with an average of 5.52 alleles per marker. The range of the observed heterozygosity (H_o), with a mean of 0.0880, was 0-0.448. The polymorphic information content (PIC) has a mean of 0.4149 and a range of 0.0236 to 0.8904. For gSSR markers average PIC was 0.5081 while that of eSSR markers was 0.2382. In species like *V. umbellata*,

V. mungo and *V. radiata*, cross-species transferability of SSR loci in the *V. angularis* is found to be in excess of two-thirds (Chaitieng *et al.*, 2006). SSR loci in *Vigna* have a greater cross-species transferability rate because of their higher genetic homology. Particularly in genus *Vigna*, the cross-species transferability of SSR loci is likely to be significantly more successful (Jasrotia *et al.*, 2019). Zhao *et al.* (2022) studied the genetic diversity analysis of 23 adzuki bean and 23 mung bean accessions using DNA fingerprinting construction with the help of 38 pairs of SSR markers. In 2019, 46 germplasm were used to extract the young leaves. Among 38 primers 18 primers showed polymorphism and showed 0.830 average PIC value. Adzuki bean and mung bean accessions were grouped into four and six categories respectively based on hierarchical cluster analysis. With the help of nine digital fingerprints, constructed by core primers adzuki bean germplasm resources could be distinguished.

Biochemical analysis (nutritional evaluation)

Adzuki beans that are properly cooked have 66% water, 25% carbs, 7% food fibre, 8% protein, and very little fat (Table 1 A and B). Cooked beans contain 128 calories per 100 grammes of reference content, as well as moderate to high amounts (10% or more of the Daily Value, DV) of vitamin B9 folate (30% DV), and a number of dietary minerals (11% to 27% DV). Adzuki bean is the rich source of protein, iron, zinc and flavonoids. Considering its low calorie and fat level, high digestible protein content and abundance of bioactive chemicals, it has been referred to as the "red pearl" of beans (Kitano-Okada *et al.*, 2012; Kramer *et al.*, 2012).

Gohara *et al.*, (2016), found that all adzuki bean cultivars showed poly-unsaturated fatty acids prevalence and nutritional indices and ratios considered adequate for biological system maintenance of a healthy organism. Adzuki bean sprouts are consumed as food and herbal medicine in Chinese folk (Li *et al.*, 2011). As an herbal medicine, adzuki bean has been practiced since the Tang Dynasty of China to maintain health and to control weight, is also known as weight loss bean (Liu *et al.*, 2017). Adzuki beans cultivars showed different antioxidant activities and cytoprotective effects according to the concentration and composition of phenolic compounds. Protein and phenols present in adzuki bean act as anti-diabetic (Yao *et al.*, 2014). Considering their antioxidant characteristics that support good health, the bioactive elements found in seed coat to reduce stress and inflammation during hypertension thus, have gained a lot of attention (Lin and Lai, 2006; Mukai and Sato, 2011).

Owing to its rich nutrients, it has been widely used in foods like paste in pastries, desserts, adzuki rice, porridge, ice cream and adzuki milk, playing an important role in Asian descent food consumption and international bean trade. In addition, adzuki bean has function of eliminating dampness, detoxification, hypoglycemic and weight loss. In Chinese traditional medicine, adzuki bean is used as diuretics, antidotes and treatment of beriberi (Danjiao *et al.*, 2015). They nevertheless contain antinutritional elements which include trypsin inhibitors, α -galactosides, and phytates, while the quantities of each vary greatly amongst adzuki bean varieties (Sharma *et al.*, 2019). Shi *et al.*, (2017) studied 17 Chinese adzuki bean cultivars for biochemical content range which revealed that protein content varied between 22.9 to 25.4%, starch 44.53 to 53.92%, total flavonoid content 53.54 to 70.41 mg/g, antioxidant content 0.45 to 3.62 μ MTrolox/g and total phenol content 2.11 to 2.75 mg/g. He also studied

correlation between the different biochemical parameters and found positive correlation between phenol and antioxidant. Bhatt *et al.*, (2021) also studied the biochemical content of adzuki bean and its wild relative *V. angularis* var. *nipponensis* and found the difference in quantities of biochemical content. In biochemical analysis of *V. angularis* var. *nipponensis* and adzuki bean, sugar content ranged from 2.51 to 3.3% and 3.26 to 4.26%, phenol 204.9 to 839.33 mg/100g and 486.7 to 648.6 mg/100g, starch 23.1 to 30.2% and 34.20 to 34.85%, phytate 1.06 to 1.29% and 1.34 to 1.35%, fat 0.70 to 0.94% and 0.59 to 0.82%, ash 4.38 to 6.13% and 4.25 to 5.25%, protein 21.24 to 25.24% and 21.64 to 22.87% and moisture 10.6 to 11.6% and 10.91 to 11.26% respectively. Johnson *et al.*, (2022) characterized adzuki bean accessions for important biochemical constituents and discovered that protein content of seeds varied from 23.1 to 27.9%, seed coat from 10.7 to 16.7 % and leaves from 19.5 to 24%.

Table 1 A: Nutritional value of adzuki bean as per 100g as per USDA nutritional database

Nutritional value per 100 g				
Vitamins	Qty.	%DV	Minerals	Quantity
Thiamine (B1)	0.12 mg	10%	Energy	536 kJ (128 kcal)
Riboflavin (B2)	0.06 mg	5%	Carbohydrates	24.8 g
Niacin (B3)	0.72 mg	5%	Dietary fiber	7.3 g
Pantothenic acid (B5)	0.43 mg	9%	Fat	0.1 g
Vitamin B6	0.1 mg	8%	Protein	7.5 g
Folate (B9)	121 µg	30%	-	-
Vitamin C	0 mg	0%	-	-

Table 1 B: Adzuki bean minerals value per 100g as per USDA nutritional database

Minerals	Quantity	%DV [†]
Calcium	28 mg	3%
Copper	0.3 mg	15%
Iron	2 mg	15%
Magnesium	52 mg	15%
Manganese	0.57 mg	27%
Phosphorus	168 mg	24%
Potassium	532 mg	11%
Selenium	1.2 µg	2%
Sodium	8 mg	1%
Zinc	1.8 mg	19%
Water	66 g	

µg=micrograms, mg = milligrams

The most important compound in the aroma of the cooked adzuki is maltol, which adds a sugary and caramel-like flavor. The beany flavor is caused by the presence of alkanols similar to those of cooked soybeans, such as 1-hexanol and 3-methyl-1-butanol

(Bi *et al.*, 2021). Seed coat colour of adzuki varieties varies between light brown to dark red. This is caused by the presence of a pigment belonging to the class of mono-glycosidic anthocyanins (Chu *et al.*, 2021).

Similarly, Deepika *et al.* (2023) has also characterized 100 accessions of adzuki bean conserved in National genebank of India. The variability in different traits observed was as follows: moisture content ranged from 7.5-13.3 g/100 g, ash content 1.8-4.2 g/100 g, protein content from 18.0–23.9 g/100 g, starch content from 31.0- 43.9 g /100 g, total soluble sugar content from 3.0-8.2 g/100 g, phytic acid content from 0.65-1.43 g/100 g, phenol content from 0.01-0.59 g/100g, and antioxidant activity from 11.4-19.7 mg/100g GAE. Among the accessions, IC341955 (23.7%) and EC15256 (23.6%) were notable for their exceptionally high protein content, while IC341955 (19.2 mg/g GAE) and IC341957 (19.1 mg/g GAE) exhibited enhanced antioxidant activity. These accessions can be used in breeding of adzuki bean.

Conclusion

The identification of the functional components of adzuki bean can increase the demand for this crop. Thus, new high-yielding cultivars that produce high-quality beans must be developed using accessions conserved in different genebanks of world. Though Adzuki bean can be considered as an important pulse however, various researchable issues like its grain contain antinutritional elements like trypsin inhibitors, α -galactosides, and phytates needs to be resolved. In the future, more work is needed to verify the anti-obesity mechanism of adzuki bean supplementation and identify the specific bioactive components involved. Biochemical study of adzuki bean germplasm is necessary to identify desired material for fulfilment of requirement of protein and other minerals useful for nutritional security and to diversify our food basket. However, more research is needed to examine the effects of adzuki bean supplementation on T2D-related outcomes in human subjects. Moreover, molecular study of adzuki bean germplasm is essential to develop useful markers to conduct related studies.

Acknowledgements: The authors acknowledge the support received from HOD-DGE, HOD-DGE&PC, HOD-DGR, HOD-DGC, Professor PGR, and the PG School ICAR-IARI and Director, ICAR-National Bureau of Plant Genetic Resources in carrying out this study

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