Indian J. Genet., 76(4): 626-630 (2016)
DOI: 10.5958/0975-6906.2016.00077.8

Genetic improvement of food-grade soybean in India: Current status and future prospects

Akshay Talukdar* and M. Shivakumar[1]

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012; [1]ICAR-Indian Institute of Soybean Research, Indore 452 001, Madhya Pradesh

(Received: November 2016; Revised: November 2016; Accepted: November 2016)

Abstract

Soybean is the most important oilseed crop in India. It contains 40-45% quality protein and 18-22% oil besides phyto-chemicals essential for human health. Soymeal, which contains more than 50% protein, is largely exported at lower price to import pulse-protein at higher cost. Popularization of soy-based foods can facilitate eradication of protein-hunger and energy malnutrition prevalent in India. Elimination of anti-nutritional factors and off-flavor from soybean seeds will enhance liking of soy-based food by the consumers. With incorporation of consumer-preferred flavor, vegetable soybean can be a good food for the people. Reduction of phytase from seed will enhance protein and iron nutrition in the consumers. Molecular breeding and genome-editing techniques can be utilized properly to achieve these goals in shorter period of time.

Key words: Food-grade soybean, anti-nutritional factor, phytase, beany-flavor

Introduction

On global basis, over 65 % of food protein and over 80 % of food energy is supplied by plants. Legumes are the richest source of food protein from plants. (Ali 2009). Among the legumes, soybean is the most important source of protein (38-45%). Besides, it is a valuable source of vegetable oil (18-22%) that contains optimum dietary essential amino acids profile for human and animal nutrition. Soybean oil is the major source of naturally derived tocopherols, the antioxidant molecules that act as free radical quenchers preventing lipid peroxidation in a biological system and vegetable oil products (Table 1). Consumption of soy-based foods reduces cancer, blood serum cholesterol, osteoporosis and heart diseases. Isoflavones, lecithin and oligosaccharides are the other nutraceutical components present in soybean (Bora 2014). Presence of various phytochemicals beneficial for human being has made soybean truly to be called as 'functional food'. Therefore, enhancement of the nutritional components has become an important breeding objective in soybean worldwide.

Table 1. Nutritional value of soybean oil

Component	Amount (per 100 g)
Energy	884 Kcal
Iron	0.02 mg
Vitamin E (alpha tocopherol)	8.18 mg
Vitamin K (phylloquinone)	183.9 µg
Saturated fatty acid (SFA)	15.251 g
Mono-unsaturated fatty acid (MUFA)	22.727 g
Poly-unsaturated fatty acid (PUFA)	57.333 g

Source: USDA National Nutrient Database (https://ndb.nal.usda.gov)

Soybean production and consumption

India is the 5th largest producer of soybean in the world. Historically, soybean is a new crop in India; its commercial cultivation was started only during 1968-70 when it occupied merely 30,000 ha with annual production of 14,000 ton. Currently, it is cultivated in more than 11 mha with production of about 11 mt (Table 2). Major contributing states are Madhya Pradesh (50.32%), Maharashtra (34.29%), Rajasthan (8.33%), Andhra Pradesh (2.20%), Karnataka (2.30%) and others (2.57%) (www.dacnet.nic.in). India

*Corresponding author's e-mail: akshay.talukdar1@gmail.com
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com; http://epubs.icar.org.in/journal/index.php/IJGPB

Table 2. Area, production and yield of soybean in India since 1970

Year	Area (mha)	Production (mt)	Yield (kg/ha)
1970-71	0.03	0.01	426
1971-72	0.03	0.01	426
1981-82	0.48	0.35	741
1991-92	3.18	2.49	782
2001-02	6.34	5.96	940
2011-12	10.11	12.21	1208
2012-13	10.84	14.67	1353
2013-14	11.72	11.86	1012
2014-15*	11.09	10.53	950

Source: Directorate of Economics and Statistics; Department of Agriculture, Cooperation and Farmers Welfare, Govt. of India. www.dacnet.nic.in; *: 4th advance estimate

witnessed highest growth of soybean production and productivity during 1985-1999 (‘Golden period’) followed by a period of decline during 2000-2003, which recovered again during 2004-2012 (‘period of renewed growth’). It is having the 2nd highest growth rate after cotton in India; however, it has potential to even surpass cotton in next 30-40 years (Chand, 2014).

Soybean in India is primarily used for production of oil (75-80%). About 10% of soybean is used as seed and merely 10-15% is used as direct food. Common soy-food includes nuggets, soy-milk, soy-paneer (tofu), yogurt, soy-sauce, roasted-soy, soy-drink, soy-based bakery product *viz*., biscuits, muffins etc. Soy-protein is also finding place in cosmetic and health industries. However, in India, utilization of soybean as a source of quality protein for human consumption is not getting due attention yet.

In India, 60-70% of the population is vegetarian who meets their protein requirement from the plant source, pulses in particular. However, a large chunk of the Indian population (40% or so) who lives below poverty line cannot afford pulses for its high prices. It was reported that about 30% of Indian household consumes <70% of their energy needed. Therefore, protein and calorie malnutrition is rampant in India. Soybean can be of great help in this case to address the issue of protein malnutrition.

India produces about 5.5 mt of de-oiled cake (DOC) or soymeal annually. More than 90% of the DOC, which contains about 50% protein is exported @Rs. 35,000/- per ton earning more than Rs. 175,000 million. From this, it can be worked out that India is exporting valuable soy-protein @Rs. 70.00 per kilogram (kg). On the other hand, India is importing about 2.5mt of pulses @Rs. 90,000/- per ton costing Rs. 360/- per kg of protein. Thus, India is spending 5 times higher prices in importing pulse-protein as against export of quality soy-protein (Ali, 2014). Through proper processing, if DOC is converted to usable food products for human consumption, it can sufficiently address the country’s problem of protein malnutrition.

Seed-, feed- and food-grade soybean

Commercially, soybean can be categorized as i) seed-grade soybean, ii) feed-grade soybean, and iii) food-grade soybean (http://thefoodiefarmer.blogspot.in/2011/12/many-faces-of-soy.html). The seed-grade soybean is used as ‘seed’, i.e. to grow the next crop. To be used as seed, it has to fulfill certain purity standard as fixed by the state authority. The feed-grade soybean is meant exclusively for consumption by the farm animals. Nutritionally, it should have at least 36% protein, which when feed to the farm animal meets part of their daily requirement for protein. The food-grade soybean on the other hand, contains protein higher than feed-grade soybean; it usually ranges between 42-45%. It helps in making better quality soy-based food products. Usually, food-grade soybeans are used for production of soy-milk, tofu or soy-paneer, soy-sprouts and other soy-based food items. Like other plants, soybean too synthesizes a range of secondary metabolites for their adaptation and self-protection, called anti-nutritional factor (ANF). From nutritional point of view, the ANFs are considered as harmful and toxic as it interferes with normal growth, reproduction and health. Therefore, soybean, in general and food-grade soybeans, in particular, are expected to be free from anti-nutritional factors or allergens that may cause harm to the consumers.

Vegetable soybean

Botanically, vegetable soybean is similar to other soybeans (*Glycine max* L. Merr.). The only difference is that it is harvested before full maturity when the pods are green (R6 stage), and seeds are bigger and sweeter. In East Asia, it has been in cultivation for centuries. Immature pods are boiled in mildly salted water and the extracted seeds are consumed as fresh vegetable. Nutritionally, it is highly rich in protein, mono-unsaturated fatty acid, vitamin C, fiber, iron, zinc, calcium, phosphorous, folate, magnesium, potassium, tocopherol and anti-cancer isoflavones (Esler, 2011; Keatinge et al. 2011). It also has pleasant flavor and soft texture and easier to cook. Cooked vegetable soybean has the highest net protein utilization value (NPU: ratio of amino acid converted to protein) among all soy-products. Vegetable soybean also has 60% more calcium and twice the phosphorus and potassium levels of green peas, India's most commonly consumed fresh legumes (https://www.gov.uk/government/case-studies/dfid).

Vegetable soybean has short growth duration (65-75 days) and easily fit in any cropping sequences. It produces both consumable soybean and usable fodder or green manure (Shanmugasundaram 2004). Vegetable soybean can be cultivated in mixed cropping systems; it not only increases yield but improves the companion crops too. *Rhizobium* bacteria symbiotically fix the atmospheric nitrogen in the soybean roots and reduce the needs for fertilizer. In India, grain soybean intercropping has proven to increase yields. In southern India, intercropping of soybean with sorghum, cotton, sugarcane, pigeonpea, or peanut was found to be beneficial (Trikha 1983). Thus, there is good potential for vegetable soybean to fit into most cropping niches in India.

Keatinge et al. (2011) has reported that vegetable soybean produces the highest yield of crop protein per unit area. Large scale adoption of this crop and consumption of soybean could fight protein and iron deficiency prevalent in India. Breeding effort made in this direction resulted in development of improved varieties of vegetable soybean. 'Himso1563' was the first vegetable soybean variety released by the Indian Council of Agricultural Research, New Delhi during 2001. It matures in 100-120 days with a production of 5t/ha. NRC105 is another genotypes of vegetable soybean developed from the ICAR-Indian Institute of Soybean Research, Indore. It becomes ready for picking the pods in 60 days with a production of 3.9t/ha.

Genetic improvement in food-grade soybean

To make soybean a house-hold food, a few key issues need to be addressed, which are discussed below:

Eating quality (taste, texture, flavor)

Indian consumers are very choosy, they have liking for certain tastes than others. When vegetable soybean was first given to the Indian consumers, their common opinion of disliking it was 'taste' (Esler, 2011). The vegetable soybean, in general, carries a flavor, called 'beany flavor' or 'grassy flavor'. Unlike East-Asian countries, where vegetable soybean has been consumed for centuries, Indian consumer did not like it because of its 'beany or grassy flavor'. During 2001-2010, Asian Vegetable Research and Development Centre (AVRDC), Taiwan introduced a few genotypes of vegetable soybean in Jharkhand (India). Most of the genotypes were rejected by the farmers primarily for the 'taste'. Subsequently, a genotype was selected and released for commercial cultivation as "Swarna Vasundhara". It became popular for its taste and production. Vegetable soybean with typical 'basmati flavor' (an aromatic rice flavor) has also been identified to suit the taste of the consumer. Genotypes with high levels of sucrose, aspartic acid, glutamic acid and alanine found to have acceptable taste (Esler, 2011).

Biochemical analysis has established that production of 'beany flavor' in soybean or soy-based products is primarily due to the lipoxygenase or the oxidative rancidity of unsaturated fatty acids (Gardner, 1985; Lee et al. 2003). Plant lipids are sequentially degraded into volatile and non-volatile compounds by a series of enzymes via the lipoxygenase pathway, which catalyses the hydroperoxidation of polyunsaturated fatty acids to form the aldehyde and alcohols that are responsible for the grassy-beany flavor (Iassonova et al. 2009). NRC109 and NRC110 are two genotypes of soybean which are reported to be free from off-flavor (Kumar 2013). Since donor for null allele for lipoxygenase has been identified, markers linked to the target allele has been developed, transfer of the allele to other varieties is possible through marker assisted backcross breeding approach. Department of Biotechnology, Govt. of India has approved a project in this direction to make 4-5 popular varieties of soybean free from off-flavor, the project is going on.

Masuda (1991) reported that sucrose, glutamic acid and alanine are the major chemical compounds related to taste in soybean. Boiling induces ketones and furans and other volatiles to enhance characteristic flavor of vegetable soybean. Similarly, the components of the flower-like flavor in vegetable soybean come from cis. Jasmine, (Z)-3-hexenyl-acetate, linalool and acetophenone. Kumar et al. (2011) observed that boiling under pressure causes significantly higher decline for daidzein and glycitein but lower loss of Vitamin C and sucrose. Boiling treatments also deactivated lipoxygenases and trypsin inhibitor.

Farming techniques and locality also influence eating quality and contents of various nutritional factors in soybean. In case of vegetable soybean, early maturing summer varieties were received poorly by the consumers (Kokobun 1991). Kumar et al. (2011) reported that genotype, location and genotype x location interaction significantly influences protein, oil and unsaturated fatty acids *viz*., oleic, linoleic and linolenic acid. Therefore, breeding should be attempted to develop location-specific varieties. Further, nutritional status of the locality where the crop is grown also influences quality and yield of soybean. Stage and time of harvest of green soybean affects quality; sucrose increases in the early stages of green soybean growth, peaking at or after around 35 days after flowering. Furthermore, sucrose levels seem to specifically peak during the mid to late afternoon (Masuda 1991). Sugar content has a negative correlation with oil content. Therefore, oil concentration can be used as indirect selection criteria for sweet vegetable soybean.

Anti-nutritional factors

Soybean contains several anti-nutritional factors among which trypsin inhibitor (TI), phytase are the most important ones. Kunitz trypsin inhibitor (KTI), which constitutes more than 80% of the total TI are primarily responsible for improper digestibility of soybean, if consumed un-processed. Although KTI is heat labile, yet heat inactivation process is neither fully effective, nor economic. Genetic elimination of KTI is the most effective way of making soybean free from KTI (Moraes et al. 2006). The gene for KTI has been mapped and markers linked to the null allele have been reported. A massive project has been initiated involving Indian Institute of Soybean Research (IISR), Indore (MP), Indian Agricultural Research Institute (IARI), New Delhi and Agharkar Research Institute (ARI), Pune to convert 5 popular soybean varieties viz. JS 97-52, NRC 7, JS 93-05, MACS 450, DS 97-12 to KTI-free genotypes. The KTI-free lines have already been developed and undergoing testing for phenotypic and agronomic performances (Talukdar et al. 2014; Kumar et al. 2015; Shivakumar et al. 2016). Two genotypes *viz*., NRC101 and NRC102, which are KTI-free, have been developed at IISR, Indore.

Phytase

Soybean seeds contain phosphorus in the form of inositol hexaphosphate commonly known as phytate (Raboy, 2007). Besides sequestering inorganic phosphate, phytate may also chelate divalent cations such as Fe, thereby decreasing their availability. It also contributes toward water pollution by eutrophication as the phosphate-rich waste discharges in to water bodies. So, reduction of seed phytase can enhance mineral and protein bio-availability in soybean.

Microarray-based gene expression profiling of phytic acid biosynthesis pathway indicated step-wise regulation of 8 genes *viz*., myo-inositol-3-phosphate-synthaes (MIPS), Inositol phosphate kinase (IPK1-4) etc. Gene silencing constructs were used to silence *GmIKP1* and *GmIPK2* through seed specific vicilin/conglycinin promoters (IARI Annual Report, 2015-16). Similarly, mutation breeding through gamma-irradiation was also been attempted to develop plants with reduced or zero phytase.

Future prospects

Cultivation of soybean is primarily confined to Madhya Pradesh and its neighboring states. In order to increase its production and to popularize it as an alternative source of pulses-proteins, cultivation of soybean needs to be expanded to non-traditional areas such as North-Eastern India. Moreover, it can be grown in rice-growing belts of North-Western part of India provided its profits become competitive to that of rice. Duration of the crops also needs to be addressed so that it can be fitted to various cropping sequences prevalent in different parts of the country.

To make the food-grade soybean a house hold food, initiative needs to be taken at mission mode to address the quality of protein and oil. Program needs to be strengthened to increase concentration of oleic acid in oil with corresponding decrease in poly-unsaturated fatty acid, primarily linolenic acid. Some progress has been made in this direction at Indian Institute of Soybean Research, Indore; however more concerted effort is needed. Proper attention should be given to reduce anti-nutritional factors present in the seeds. Projects to convert some popular varieties in to KTI-free varieties have shown promise, so more varieties need to be converted to make it soy-industry and consumer friendly. Elimination of the off-flavor and phytase will improve food value of soybean immensely. It will make vegetable soybean a strong competitor to pea. Molecular breeding approaches including genome-editing techniques may be applied to achieve these goals. Popularization of soybean food and soy-based products among the Indian population will facilitate eradication of protein-hunger and energy malnutrition in India.

Conclusion

In order to meet the protein requirement for the large mass of Indian population, soybean can be a powerful alternative to the pulses, which are already in shortage in production. Soybean bears the potentiality to double its production through area expansion and increase in yield per unit area. Development of high yielding food-grade soybean varieties and their popularization will facilitate making of soybean as a household food. Production of more soy-based products and their distribution through public distribution system (PDS), mead-day meal and such other similar programs would help fight the protein and energy-malnutrition prevalent in India.

Declaration

The authors declare no conflict of interest.

References

Ali Kahraman. 2009. Antinutritional factors in food grain legumes. Proceedings of the First International Symposium on Sustainable Development, June 9-10 2009, Sarajevo 40. Mustafa Onder Selcuk University, Agricultural Faculty, Department of Field Crops, Konya, TURKEY.

Ali Nawab. 2014. Potential and strategies for combating protein calorie malnutrition. In: Proceedings and Recommendation-Brain storming workshop on “Soybean for household food and nutritional security”, 21-22 March, 2014. New Delhi, India: 19-20.

Bora P. 2014. Anti-nutritional factors in foods and their effects. J. Academia & Industrial Res., **3**(6): 285-289.

Chand R. 2014. Policy reorientation for soybean production and marketing. In: Proceedings and Recommendation-Brain storming workshop on “Soybean for household food and nutritional security”, 21-22 March, 2014. New Delhi, India: 19-20.

Esler I. 2011. Prospects for vegetable soybean in India and its market acceptance. Report. AVRDC-The world vegetable centre. Pp-1-26.

Gardner H. W. 1985. Flavor and bitter tastes from oxidation of lipids by enzymes. B.D. Min, T.H. Smouse (Eds.), Flavor chemistry of fats and oils, The American Oil Chemists’ Society, Champaign, Illinois, pp. 189-206.

Iassonova D. R., Johnson L. A., Hammond E. G. and Beattie S. E. 2009. Evidence of an enzymatic source of off Flavors in lipoxygenase-null soybeans. J. Am. Oil Chem. Soc., **86**: 59-64.

Keatinge J. D. H., Easdown W. J., Yang R. Y., Chadha M. L. and Shanmmugasundaram S. 2011. Overcoming chronic malnutrition in a future warming world: The key importance of mungbean and vegetable soybean. AVRDC-The world vegetable centre.

Kokobun Makie. 1991. Cultural practices and cropping systems for vegetable soybean in Japan. In: Vegetable Soybean, Research Needs for Production and Quality Improvement. National Agriculture Research Centre, Japan. 53-60.

Kumar V., Rani A., Rawal R. and Mourya V. 2015. Marker assisted accelerated introgression of null allele of Kunitz trypsin inhibitor in soybean. Breeding Sci., **65**: 447-452. doi: 10.1270/jsbbs.65.447.

Kumar V., Rani A., Rawal R. and Husain S. M. 2013. Lipoxygenase-2-free Indian soybean (*Glycine max* L.) genotypes. Curr. Sci., **104**(5): 586-587.

Lee J. Y., Min S., Choe E. O. and Min D. B. 2003. Formation of volatile compounds in soy flour by singlet oxygen oxidation during storage under light. J. Food Sci., **68**: 1933-1937.

Moraes R., Soares C. B., Colombo L. R., Salla M. F. S., Barros J. G. A., Piovesan N. D., Barros E. G. and Moreira M. A. 2006. Assisted selection by specific DNA markers for genetic elimination of the kunitz trypsin inhibitor and lectin in soybean seeds. Euphytica, **149**: 221-226.

Raboy V. 2007. Seed phosphorous and the development of low-phytate crop. In: Inositol phosphates: Linking agriculture and the environment (Eds. B. L. Turner, A. E. Richardson and E. J. Mullany). CAB International, pp. 111-132.

Shanmugasundaram S. and Yan M. R. 2004. Global expansion of high value vegetable soybean. World Soybean Research Conference, p. 915-920.

Shivakumar M., Verma K., Talukdar Akshay, Lal S. K., Kumar A. and Mukherjee K. 2016. Introgression of null allele of Kunitz trypsin inhibitor through marker-assisted backcross breeding in soybean (*Glycine max* L. Merr.). BMC Genetics, DOI: 10.1186/s12863-016-0413-2.

Talukdar Akshay, Shivakumar M., Verma K., Kumar A., Mukherjee K. and Lal S. K. 2014. Genetic elimination of Kunitz trypsin inhibitor from DS9712, an Indian soybean variety. Indian J. Genet., **74**(4): 608-611.

Trikha R. N. 1983. The Potential of Soybean in Indian Cropping Systems. In: Soybean in Tropical and Subtropical Cropping Systems. G.B. Pant University of Agriculture and Technology, India.