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RESEARCH ARTICLE

INSILICO ANALYSIS OF SOYA BEAN ALLERGENS AND THEIR CROSS REACTIVITY

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ABSTRACT

Allergy is a steadily increasing health problem for all age groups all over the world. Food allergies mostly against milk, eggs, peanut, *soya bean*, wheat, nuts, fruits, grass and pollens affect all age groups. Soya bean a member of the legume family, has universal distribution. Both the oil and the flour are used in many sorts of foods as it contains all the essential amino acids required by the human physiology. People allergic to soya and cross reactivity between the members of same family are likely to raise symptoms like excessive diarrhea, loss of appetite, gastro intestinal obstructions, itching, asthma, shortness of breath, muscular fatigue etc. The proteins sequence homologies have been used to identify prospective cross reactivity of the expressed protein belongs to such a family, it may be considered to have a higher probability to be an allergenic protein, and can be subsequently used in tracing the foods which contain the same type of protein/ amino acids. The outcome can be extrapolated in other food items for their allergenicity, cross reactivity, side effects etc. Incorporating this technique in the field of food technology can even enable in formulating food stuffs, specific for particular allergic individuals and thus facilitate allergic free diets.

INTRODUCTION

The purposes of food are to supply energy, promote growth, to supply force and heat, and to furnish material to repair the wear and tear which is constantly taking place in the body (Sampson 1999). Our nutritional status, health, physical and mental faculties depend on the nutrition (Sampson 2005). But some people show allergy to certain type of food substances. Allergy is a disorder of the immune system, often referred to as *atopy*. Allergy is an ever increasing health problem for all age groups (Vanek-Krebitz *et al.*, 1995; Scheurer *et al.*, 1995; Rabjohn *et al.*, 1999). Food allergy (FA) can be defined as an abnormal immunological response to food items that causes an adverse clinical reaction (Rabjohn *et al.*, 1999). Food allergy encompasses a range of disorders that result from adverse immune responses to dietary antigens, for someone with a food allergy, eating or swallowing even a tiny amount of a particular food can cause symptoms such as skin rash, nausea, vomiting, cramping, and diarrhea. Food allergies are mostly against milk, egg, peanut, *soya bean*, wheat, nuts, fruits, grass and even pollens grains.

The soya bean has many health benefits by virtue of proteins and isoflavones genistein and daidzein in it. The scientifically proven health benefits of soya beans include, reducing hormone-related cancers like breast and colon, low content of saturated fat and it is popular mainly because it is identical to fish, egg, meat and milk. Soya allergy implies adverse reactions to one or more of the proteins found in it, and involves the antibody immunoglobulin E (IgE) of the immune system (Jenkins *et al.*, 2005). Allergic reactions may be caused by ingestion of soya-containing foods or by inhalation of soya dust. Soya also contains a trypsin inhibitor which may result in respiratory hypersensitivity reactions. Early studies showed that replacing milk protein with finely ground soya bean in liquid diets led to an excessive diarrhea, loss of appetite, gastro intestinal obstructions, itching sensation, asthma, shortness of breath, muscular fatigue, high mortality, poor growth etc., (Breiteneder and Ebner 2000). People those who are allergic to soya, are more likely to develop allergy to certain other foods as well. This is because individuals allergic to a particular food may react to other foods that share a similar protein structure, and it is particularly true in foods of the same family (Ferreira *et al.*, 2004). The Soya bean confirm cross reactivity between members of the same family. It shows higher risk of allergy to peanut, green pea, lima bean, walnut, apple, tomato etc., (Vanek-Krebitz *et al.*, 1995; Ferreira *et al.*, 2004). A hypothesis states that late onset may be the result of individuals being sensitized by long-term exposure to environmental factors that contain proteins similar to those in the known triggers of allergenic response (Mari 2001; Burks *et al.*, 1997; Shin *et al.*, 1998). Recent studies have identified common molecular features of proteins from different sources, which could account for clinically important cross-reactivity (Schein *et al.*, 2005) and sensitivity (de Leon *et al.*, 2003; Eigenmann *et al.*, 1996). These allergies and their cross

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reactivity can be traced using bioinformatics tools. The major allergenic proteins in soya bean have been isolated and sequences that react with IgE from patient sera have been identified (Lopez-Torrejon *et al.,* 2003; Wensing *et al.,* 2003). The in-house bioinformatics methods permit almost instantaneous sequence similarity searching, with direct connections to much larger databases, to determine what other sequences are similar to the amino acid sequence of an allergen, one can perform full-length sequences for similarity to the known allergens by selecting the FASTA and BLAST searches. With the help of these information's, Proteins similar to these allergens subsequently found in other food items that are known to elicit clinically significant responses in individuals with soy bean allergies (Gonzalez *et al.,* 1995), such as tree nuts (Mari 2001), pea nut (Jian Ye *et al.,* 2006), and legumes (Gijzen *et al.,* 2003; Gonzalez *et al.,* 2000). Using the above informations major allergic proteins and their cross reactivity can be deduced, which helps in finding others foods to which they are susceptible and thereby avoiding that food in advance.

MATERIALS AND METHODS

Bioinformatics is a multidisciplinary field of science, which uses techniques and methods from computer science, mathematics, and statistics to resolve questions in biological science (Kleine-Tebbe *et al.,* 2002). The bioinformatics techniques are employed to gain useful insights from the data generated in the research fields of biological science (Neudecker *et al.,* 2003). These bioinformatics tools/ technique have been employed to asses cross reactivity *in silico*, which incorporates several different methods for comparing the sequences of allergens within the database. The in-house bioinformatics methods permit almost instantaneous sequence similarity searching, with direct connections to much larger databases.

In the present study, the allergenic proteins of soya such as gly m 1, gly m 2, gly m 3, gly m 4, gly m 5, gly m 6, gly m Bd28k, gly m conglycinin, gly m glycinin G1,G2, gly m lectin, gly m TI have been deciphered. The sequences for these allergic proteins are retrieved in fasta format from NCBI. Local similarity search has been performed for the retrieved sequence using BLAST for finding the regions of local similarity between sequences.

Fasta sequence has been retrieved for multiple sequence alignment (MSA) (Guillaume Launay and Thomas Simonson. 2008) which aligns three or more biological sequences. MSA is performed using clustal w. In many cases, the input set of query sequences are assumed to have an evolutionary relationship by which they share a lineage and descended from a common ancestor. From the resulting MSA, sequence homology can be inferred and phylogenetic relationship among the Soya bean and other species can be inferred by the sequences shared evolutionary origins and dendogram.To view the graphical overview of the alignments and taxonomic relations of the blast hits, ExPASy (Expert Protein Analysis System) tool a proteomics server from the Swiss Institute of Bioinformatics (SIB) has been explored. The other leguminous plants and species of different family which contain same type of protein can be identified using the pfam data base, a large collection of protein families, each represented by multiple sequence alignments and hidden Markov models (HMMs).

RESULTS AND DISCUSSION

Soya bean shows higher risk of allergy many human subjects. The allergenic protein sequence is retrieved from NCBI / UNIPROT / SWISSPROT / PIR. Their gene bank id are shown below in Table 1.

Table 1. Accession numbers of soya allergen proteins

No	Allergen Proteins	Allergen Nomenclature	Gen Bank/ Swiss Prot/ Uniprot/ Pir Id
1	Gly m 1	Gly m 1.0101	AAB09252.1
		Gly m 1.0102	AAB09252.1
			P24337
2	Gly m 2		A57106
			AAA50175.1
3	Gly m 3	Gly m 3.0101	AJ223982.1
		Gly m 3.0102	AJ223981.1
4	Gly m 4		CAA42646.1
5	Gly m 5	Gly m 5.0101	P26987
		Gly m 5.0201	Q9FZP9
6	Gly m 6	Gly m 6.0101	P04776
		Gly m 6.0201	P04405
		Gly m 6.0301	P11828
		Gly m 6.0401	Q9SB11
		Gly m 6.0501	Q7GC77
7	Glym Bd28K		BAB21619.
8	Gly m conglycinin		CAA35691.1
			AAB23463.1
			AAB01374.1
			AAA33947.1
			AAA33966.1
9	Gly m glycinin G1		AAA33966.1
10	Gly m glycinin G2		CAA68460.1
11	Gly m lectin		AAA33983.1
12	Gly m TI		CAA56343.1
			CAA45777.1
			CAA45778.1
			AAB23482.1
			AAB23483.1
			AAB23464.1
			P01071

Fig.1 Multiple Sequence Alignment of Gly M conglycinin 4

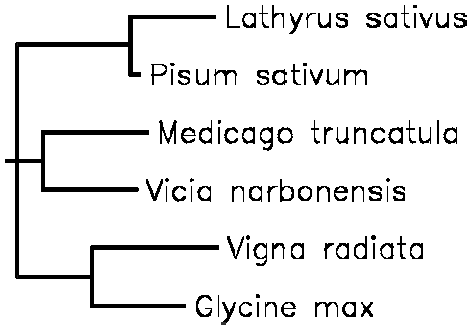

Fig.2. Dendogram of Gly M Conglycinin 4

Table 2. Conserved domains of allergen proteins

Allergenic proteins	Iso allergens	Region / site	CDD(conservd domains)
	Gly m 1.0101	REGION	72937
	Gly m 1.0102	Single peptide	157580
		region	30292
		site	30292
GLY M 1		site	30292
		region	155813
GLY M 2		region	144812
	Gly m 3.0101	CDS (1..396)	
GLY M 3	Gly m 3.0102	CDS (1..396)	
GLY M 4		REGION	158860
	Gly m 5.0101	region	158422
	Gly m 5.0201	region	158422
GLY M 5	Gly m 5.0301	Region 223…346	58422
	Gly m 5.0302	Region 425… 583	158422
	Gly m 6.0101	Region(36.. 190)	158422
		Region (332..472)	158422
	Gly m 6.0201	Region (33..183)	158422
		Region (322..462)	158422
	Gly m 6.0301	Region (36..187)	158422
GLY M 6		Region (318..458)	158422
	Gly m 6.0401	Region (37..193)	158422
		Region (397..536)	158422
	Gly m 6.0501	Region (38..193)	158422
		Region (363..503)	158422
		Region (37..166)	158422
BD 28 K GLYCINE MAX		Region (280..426)	158422
		Region (207..330)	158422
		Region (409..567)	158422
		Region (46..169)	158422
GLY M CONGLYCININ		Region (243..401)	158422
		Region (223..365)	158422
		Region (445..601)	158422
		Region (23..180)	158422
		Region (36..190)	158422
GLY M GLYCININ G1		Region (332..472)	158422
		Region (33…183)	158422
GLY M GLYCININ G2		Region (322..462)	158422
GLY M LECTIN		Region (34..261)	143910
		Region (28..199)	29140
		Site(87,92)	29140
		Region (28..200)	29140
		Site (85,90)	29140
		Region (28..200)	29140
		Site (85.90)	29140
		Region(28..197)	29140
GLY M TI		Site (86,91)	29140
		Region (28..198)	29140
		Site(86,91)	29140
		Region (27..199)	29140
		Site (84,89)	29140
		Region (3..175)	29140
		Site (60,65)	29140

From the multiple sequence alignment (MSA) it is found that most of the species proteins have similar conserved and identical regions, from the resulting MSA sequence the phylogenetic relationship of Soya bean and other species are inferred using the dendogram. Figure 1, Figure 2. When the conserved domains (CDD) regions are analyzed for the above mentioned allergenic proteins, it is observed that the regions are identical in most of the proteins. The CDD regions for proteins are showm below in Table 2. From the above results, it is conformed that soya bean allergic protein are present in others species such as tomato, apple, barley, mango, Coconut, peanut, cashew, sesame, rice, cotton, walnut, hazelnut, brazil nut, buck wheat, etc.

Conclusion

The bioinformatics tools and techniques can be used to find the individuals major allergenic proteins and their cross reactivity, which helps in finding other food items that are known to elicit clinically significant responses in individuals with soya bean allergies. It is found that those individuals who are allergic to Soya are also allergic to above mentioned food, this is because of presence of same type of protein and the cross reactivity of those proteins as the soya has. Using the above protocol, major allergenic proteins and their cross reactivity in human can be explored and it could help in prophylaxis. This allergenic information can be incorporated in the labels of commercial food products for safe consumptions, these information can also be used in food processing techniques, and foods can be specially designed for allergic people, and also help in developing genetically modified plants without the presence of allergic proteins.

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