Chapter 1
## Chapter 1
**Introduction & Review of Literature**

1. **Daily protein requirement** ... 1-2
2. **Proteins in vegetarian diet** ... 2-2
3. **Legumes as proteins source** ... 2-4
   - 1.3.1. Production and consumption pattern of legumes globally ... 4-4
4. **Food allergy** ... 5-5
5. **Dietary habits and food allergy** ... 6-15
   - 1.5.1. The big eight ... 7-7
   - 1.5.2. Food allergens and their physico-chemical characteristics ... 7-8
   - 1.5.3. Classification of plant allergens ... 8-9
   - 1.5.4. Clinical manifestation of the allergy to legumes ... 9-11
   - 1.5.5. Allergens of legumes ... 11-12
   - 1.5.6. Cross-reactivity among legumes ... 13-15
6. **Animal models for food allergy** ... 15-15
7. **Food allergy scenario of Indian population** ... 16-17
8. **Genesis of study** ... 18-19
Food is the basic requirement for life, which provides energy and nutrients to sustain. Food is primarily composed of carbohydrates, proteins and fats that can be consumed for nutrition or pleasure. The proportions of proteins, fat and carbohydrate in balanced diet should be approximately 1:1:4, respectively. A balanced diet is one that contains all the food constituents in proper proportions to provide enough energy, build up body tissues, keep the body warm and maintain good health. Proteins are essential for human health and better living. Proteins are the most versatile macromolecules in living systems and serve crucial functions in essentially all biological processes. They act as catalysts, transport and store other molecules such as oxygen, provide mechanical support and immunological protection, generate movement, they transmit nerve impulses and they control growth and differentiation as well. They circulate in our blood and grow in long strands out of our head. Proteins are the key components of biological materials and essential for various functions such as:

1. Growth of body tissue
2. Repair of body tissue
3. Proper functioning of antibodies that resist infection
4. Regulation of enzymes and hormones i.e. proper metabolism.

1.1. Daily protein requirement
The Recommended Daily Allowance (RDA) of protein is 0.8 gram per kilogram (2.2 pounds) of ideal body weight for the adult (Food and Nutrition Board, 2002). The protein requirement for athletes such as runners, cyclists and triathletes ranges from 25 to 50% more than for non-active individuals.
(i) Protein needs of adults
An adult male, who weigh about 154 pounds, or 70 kilograms, requires about 56 grams of protein daily. For an adult female, whose weight is 110 pounds, or 50 kilograms, needs 40 grams a day.

(ii) Protein needs of other people
The RDA increases by 30 grams per day during pregnancy and 20 grams per day during lactation. During body growth, different amounts are needed. For example, 2.2 grams of protein are needed per kilogram of body weight each day in the first six months of life and 2.0 grams per kilogram for the next six months.

1.2. Proteins in vegetarian diet
The vegetarians can meet their requirement of protein easily if they eat a variety of protein rich foods. Almost all vegetables, beans, grains, nuts and seeds contain protein. Protein content in pulses is 25%, in nuts and seeds it is 13% and in cereals it is ranging from 7-13% (Codex Alimentarius, 2007). On the other hand, fruits, sugar, fat and alcohol are low in protein. Therefore, pulses (legumes) are important source of proteins for vegetarian population.

1.3. Legumes as protein source
Legumes are dicotyledonous plants that belong to family Leguminoseae, include variety of beans, peas and pulses. It is member of the botanical order of the Fabales that includes 3 subfamilies:

   i. Mimosaceae,
   ii. Papilionaceae, and
   iii. Cesalpiniaceae
The Papilionaceae family includes the most important allergenic species: *Lens culinaris* (lentil), *Cicer arietinum* (chickpea), *Pisum sativum* (pea), *Arachis hypogea* (peanut), *Phaseolus vulgaris* (bean) and *Glycine max* (soybean).

Legumes are an excellent source of proteins. The high protein content of legumes is correlated with the presence of root nodules which contain nitrogen-fixing bacteria. Legumes are also good source of important minerals such as calcium, zinc, iron and selenium. Legumes are chosen for healthy diet because these are:

- Excellent source of protein
- Contain iron, zinc, calcium, selenium and folate
- Rich in antioxidants
- Good source of fiber
- Low in fat
- Effective in reducing the risk of chronic diseases; such as, heart disease, diabetes mellitus, obesity and cancer.

There are reports suggesting role of certain legumes in preventing cancer of colon, esophagus, oral cavity and larynx (Benito *et al.*, 1991; Le Marchand *et al.*, 1997). Pulses are 20 to 25% protein by weight, which is double the protein content of wheat and three times that of rice. For this reason, pulses are sometimes called "poor man's meat". While pulses are generally high in protein, they often are relatively poor in the essential amino acid: methionine. Grains (which are deficient in lysine but rich in methionine) are commonly consumed along with pulses to form a complete protein diet.

Not only do the grain legumes provide variety to the human diet but they are primary source of dietary protein to vegetarian populations.
Legumes are used in different food recipes in different forms like sprouted form, roasted form, in dal moth, khichadi, dhokla, soups, flour, cakes and several other food items. The grain legumes, especially soybeans and peanuts are excellent sources of vegetable oils used in the production of cooking oil, margarine, mayonnaise and salad dressings.

1.3.1. Production and consumption pattern of legumes globally

Globally, legumes provide a major source of affordable protein. Legumes are cultivated throughout the world and account for about 25% of the total dietary protein. The share of cereals is 50% and that of animal product is 25% in terms of protein resources. Legumes are an important source of food protein in the Indian diet as well. In 2004-05, India produced 30.91 million tonnes of legumes, out of which quantity of pulses was 15.01 million tonnes. This accounts for 26 per cent of total production of legumes in the world (MOIB, GOI, 2006). At present India’s production for pulses is 15.8 million tonnes and occupies first rank in world for pulses production. Since Indian population is 1/6 of the total world production, it is clear that we are the major consumer of pulses/legumes. India accounts for 33 per cent of the world’s area under pulses and 22 per cent of production. About 90 per cent of the total global area under pigeon pea, 65 per cent under chickpea and 37 per cent under lentil fall with in India, with a corresponding share of production of 93, 68 and 32 per cent, respectively (Reddy, 2004). Despite the large area under pulses production but poor yield and increase in population, the net availability of pulses came down from 60.7 gm/day/person in 1951 to 31.9 gm/day/person in 2000 (Joshi and Saxena, 2002) that is less than the ICMR recommended intake of 65 gm/day. Similarly, consumption of pulses also declined from 63.01 gm/capita/day in 1961 to 30.14 gm/capita/day in 2003 (http://faostat.fao.org).
1.4. Food allergy

Food allergies are adverse reactions to an otherwise harmless food or food component that involves an abnormal response of body’s immune system to specific protein(s) present in food. Allergic reactions to food are either Immunoglobulin E (IgE) mediated or non-IgE mediated. In IgE mediated food allergy, on first exposure the allergens may be cleaved to some degree by digestive enzymes, absorbed by the gut mucosa, processed in immunopotent cells and then presented to the immune system, resulting in the production of allergen-specific IgE (Fig. 1.1). The allergen-specific IgE antibodies circulate in the body and will bind to mast cells throughout the body tissues and basophils in the blood. On renewed contact with some food allergen, the allergens bind to the IgE antibodies, which triggers the degranulation of the mast cells and basophils and production of mediators following cross-linking of cell-bound allergen specific IgE. These mediators such as histamine and leukotrienes, induce various clinical symptoms. IgE-mediated reactions are typically rapid in onset, mostly involve the skin and may lead to anaphylaxis; whereas non-IgE-mediated disorders become evident hours to days after allergen ingestion, and mostly manifest in the gastrointestinal tract. Non-IgE-mediated reactions involve tissue bound lymphocytes rather than antibodies and mainly T cells and macrophages most likely play a role. Activation of lymphocytes and the recruitment of eosinophils and mast cells are cardinal features of many of these diseases. Non-IgE-mediated gastrointestinal allergic disorders include food protein-induced enterocolitis, proctocolitis and enteropathy. Some gastrointestinal diseases, such as eosinophilic esophagitis and gastroenteritis, involve both IgE and non-IgE-mediated mechanisms (Table 1.1).
Figure 1.1. MECHANISM OF ALLERGIC REACTIONS
Table 1.1: Food Allergy: Target Organs and Disorders

<table>
<thead>
<tr>
<th>Target organ</th>
<th>IgE-mediated disorder</th>
<th>Non IgE-mediated disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Urticaria and angioedema, atopic dermatitis</td>
<td>Atopic dermatitis, dermatitis herpetiformis</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>Oral allergy syndrome, gastrointestinal &quot;anaphylaxis&quot;</td>
<td>Proctocolitis, Enterocolitis, allergic eosinophilic gastroenteritis, enteropathy syndrome, celiac disease</td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>Asthma, allergic rhinitis</td>
<td>Heiner syndrome (delayed hypersensitivity to proteins from cow's milk)</td>
</tr>
<tr>
<td>Multisystem</td>
<td>Food-induced anaphylaxis, Food-associated exercise-induced anaphylaxis</td>
<td>...</td>
</tr>
</tbody>
</table>
1.5. Dietary habits and food allergy

Some individuals can undergo unfavorable physiological and neurological reactions or allergy after ingestion of foods that are known to be safe for consumption by the vast majority of the population. Allergy can develop to almost any food, but some foods are more allergenic than others. Theoretically, any food/food products that contain protein may cause allergic reactions. These vary among the region depending upon the availability of the food item, processing and eating habits. Generally, dietary habits of the major population of a region plays an important role in food allergy development i.e. increased consumption of a particular food may lead to its increased sensitization. Allergy to sea food (shrimp, lobster, prawn, crab and mollusks) and fish are seen in coastal areas where sea foods and fish are freely available. In Japan, rice has been shown to aggravate atopic dermatitis through IgE mechanism (Shibasaki et al., 1979). Peanut allergy in the UK, France and North America and sesame allergy in Israel is common (Dalal et al., 2002). Instances of IgE-mediated allergic reactions induced by legumes in Mediterranean and Asian countries have been reported as legumes are an important source of dietary protein in these countries (Sandin et al., 1999; Pascual et al., 1999; Sanchez-Monge et al., 2000). Previous reports show cases of bronchial asthma and/or anaphylaxis induced by inhaling vapours from cooking, eating lentil and/or chickpea belonging to the legume group (Martin et al., 1992; Kalogeromitros et al., 1996; Niphadkar et al., 1997). Legumes are an important source of food protein in the Indian diet as well and therefore chances of finding majority of population sensitized or allergic to legumes are much more in our country.
1.5.1. The big eight

The prevalence of food allergies is 3% in adults and 8% in children population (Sicherer and Sampson, 2006). The incidence of perceived adult food hypersensitivity varies (1.4-19.1%) largely across different countries (Crespo and Rodriguez, 2003). Overall, approximately 90% of all food allergies are associated with a small number of specific proteins represented by eight major food groups: peanuts, tree nuts, cow's milk, hen's eggs, fish, crustaceans (e.g., shrimp, crab, etc), wheat and soybeans (Metcalfe et al., 1996).

1.5.2. Food allergens and their physico-chemical characteristics

All known food allergens are proteins. Foods contain many proteins, but only a small number of them are known to be allergens. Based on patient's reactivity studies, several hundred food allergens have been identified and classified into major allergens and minor allergens. Allergens to which the majority of patients react (more than 50% of individuals sensitive to the allergen react in IgE-specific immunoassays) are known as “major allergens”, whereas allergen to which a minority of patients reacts are called “minor allergens”. Generally major allergens tend to be one of the predominant proteins in the allergenic food sources (Taylor and Lehrer, 1996).

Most food allergens have molecular weights (mol wts) between 10 and 70 kDa. The upper limit is probably a result of restricted mucosal absorption of larger molecules. However, some allergens, such as peanut allergens Ara h1 (63.5 kDa) and Ara h2 (17 kDa), exist in native form as large protein multimers that are 200 to 300 kDa size (Burk et al., 1991; 1992). Many food allergens are glycosylated and the carbohydrate structures of plant food allergens are known to represent important IgE epitopes (Garcia et al., 1996; Wilson et al., 1998). Stability to digestion, proteolysis, hydrolysis, heat and...
changes in pH has been found to distinguish food allergens from other plant proteins (Taylor and Lehrer, 1996). The resistance of food allergens to heat suggests the presence of linear epitopes in the protein molecules, e.g. the IgE-binding capabilities of a crude peanut extract and two major peanut allergens, Ara h1 and Arah2 were found to be unaffected by heating at 100°C for up to 60 minutes (Burks et al., 1992). The IgE-binding ability of another major peanut allergen, the concanavalin A-reactive glycoprotein, is stable to temperatures up to 100 °C over a wide pH range of 2.8 to 10.00 (Barnett and Howden, 1986).

1.5.3. Classification of plant allergens

Plant food allergens can be classified into families and superfamilies on the basis of their structural and functional properties. Many allergens belong to the cupin and prolamin super family. Plant food allergens are also found among the various groups of defense proteins known as pathogenesis-related proteins (PRs) represent a heterogeneous collection of 14 plant protein families that are involved in plant resistance to pathogens or adverse environmental conditions (Van Loon et al., 1999). In addition, there are some unrelated families of structural and metabolic plant proteins that harbor allergenic proteins, such as the profilins. Details of different classes of plant allergens are given in Fig. 1.2.

The number of allergens with known sequences is continuously increasing. New families of storage and structural proteins and metabolic enzymes are added to the firmly established protein families that contain allergens. Solut 1, a patatin storage protein, was described as a novel allergen of potato tuber (Seppala et al., 1999). A peanut oleosin was suggested as a new allergen (Pons et al., 2002). Oleosins are proteins of 16 to 24 kDa that represent the protein components of plant lipid storage bodies.
Fig. 1.2. Classifications of Plant Allergens

**Plant Allergens**

- **Cupin super family**
  - Shared 2 conserved consensus sequence motifs and a β-barrel structural core domain

- **Prolamin super family**
  - Characterized by conserved skeleton of 8 cysteine residues unite in the protein's sequence

- **Pathogenesis-related proteins**
  - Induced in response to infection by pathogens, proteins function in plant defence system

- **Profilin**
  - 12 to 15 kDa, specific protein found in autophagic cells, having highly conserved sequences
  - E.g. Pyc c4 from pear, Pru a4 from cherry, Cor a1 from hazelnut

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(i) **Vicilins/7S globulins**
- Trimeric proteins with mol wt ~150-190 kDa; subunits mol wt ~40-80 kDa
  - E.g. Canadin from jack bean, phosacin from French bean, β subunits of β-conglycin from Soybean, Ari a1 from Peanut

(ii) **Legumins**
- Hexameric proteins
  - E.g. Ari a3 from Peanut, Cor a3 from hazelnut, Ari a2 from cashewnut

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(i) **Proteases**
- (ii) **Kunitz-type Protease inhibitors**
  - Grouped on the basis of detectable sequence similarity
  - Two subfamilies contain allergenic proteins:
    - (i) Papain-like cysteine proteases
      - E.g. Gay m2 from soybeans
    - (ii) Subtilisin-like serine proteases
      - E.g. Cor m1 from melon

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(i) **2S albumins**
- Major seed storage proteins, heterodimeric proteins of 4 and 9 kDa
  - E.g. Ber c1 from Brazil nut, 2S albumin from cascaro

(ii) **Non-specific lipid transfer protein (nsLTP)**
- 7 to 9 kDa monomeric protein, resistant to proteolysis, pH changes, or thermal treatments
  - E.g. Pru P3 from peach apricot, Med a2 from apple, Pru a1d from sweet cherry

(iii) **Cereal α-amylase and protease inhibitors**
- Plant proteins with inhibitory activity against various proteases, present in a range of legumes species
  - E.g. Sol 1, 2, 3, and 4 from Potato

(iv) **Cereal prolams**
- Group of plant storage proteins having a high prolamine content
  - E.g. Glutelin from wheat, Hordulin from barley
called oil bodies (Murphy et al., 1991). Lyc e 2, a glycosylated allergen from tomato, was characterized as a β-fructofuranosidase (Westphal et al., 2003). Api g 5, a glycoprotein allergen from celery with homology to flavin adenine dinucleotide-containing oxidases, was used to show that cross-reactive carbohydrates were capable of eliciting allergic reactions in vivo (Bublin et al., 2003).

1.5.4. Clinical manifestation of the allergy to legumes

Food allergic reactions occur typically in individuals either previously sensitized to the allergen or in genetically pre-disposed condition (Sicherer, 2000). Individuals with food allergy are at risk of allergic reactions, if they ingest food to which they are sensitive. These allergic reactions range from mild symptoms such as skin rashes to life threatening anaphylactic shock. These reactions may affect various organs including: gastro-intestinal tract (nausea, vomiting and diarrhea), skin (urticaria, dermatitis, angioedema) and respiratory tract (rhinitis, asthma, bronchospasm). Minute quantities of a food have been known to trigger allergic reactions in sensitized individuals (Yunginger, 1993). The potential consequences of food allergy may be serious in few subjects depending on the severity of reaction, e.g., occurrence of anaphylaxis has been reported in approximately 3 individuals per 100,000 a year (Burks and Sampson, 1997). Food allergy is the most frequent single cause of emergency room visits for anaphylaxis (Plaut, 1997).

The clinical manifestations of legume allergy include oral allergy syndrome (OAS), urticaria, angioedema, bronchial asthma (BA), allergic rhinitis (AR), abdominal cramps and even anaphylaxis (Martin et al., 1992; Garcia et al., 1995; Kalogeromitros et al., 1996; Niphadkar et al., 1997; Kumari et al., 2006; Martinez et al., 2008). Ingestion is the principal route for food allergens, yet some highly sensitive patients may develop severe symptoms
upon skin contact. Cases of urticaria and angioedema have been reported after ingestion and/or contact with chickpea, beans, lentils, peanut, soybean, flour of lupin (Hetle et al., 1994; Igea et al., 1994; Gutierrez et al., 1997; Patil et al., 2001).

Although the vast majority of IgE-mediated allergic reactions to food occur through ingestion, a few cases of unexpected allergic reactions to foods may occur through the exposure to airborne food allergen particles. Food allergy may present with a variety of respiratory tract symptoms that generally involve IgE antibody-mediated responses. Upper and lower respiratory tract reactions are often a significant component of multisystem anaphylactic reactions. Foods are known to cause bronchospasm in the context of occupational exposure. Examples include baker's asthma (Baur et al., 1998), buckwheat (Choudat et al., 1997), cow's milk (Rossi et al., 1994) or egg (Blanco et al., 1992) in the food industry.

Several authors reported that inhalation of allergens is also one of the significant route of sensitization in some food allergies (Breiteneder and Ebner, 2000). Asthma is reported by soybean dust inhalation (Bush et al., 1977; Falleroni and Zeiss, 1996), also by steam inhalation of baking of lentil (Martin et al., 1992; Garcia et al., 1995), chickpea (Garcia et al., 1995; Niphadkar et al., 1997), lupin flour (Crespo et al., 2001; Parisot et al., 2001), pea (Geraldes et al., 2007) and bean (Igea et al., 1994; Daroca et al., 2000). In Spain, two epidemics of asthma by soybean dust inhalation have been described (Hendrick, 1989; Navarro et al., 1993). A 20-year-women experienced contact urticaria and OAS during handling of several kinds of raw fish in shop, where she had worked (Yamaguchi et al., 2007). Kalogeromitros et al. (1996) described a child with repeated anaphylactic reactions related to lentils after ingestion of cooked lentils and one episode
with inhalation exposure to cooking lentil soup. Garcia et al. (1995) reported BA induced by hypersensitivity to legume when exposed to vapours from cooking some kinds of legume (peas, chickpeas, beans, lentils etc.) and OAS with peanuts. They also found positive skin prick test (SPT) for the same legumes.

In a Spanish study, 10.1% of 355 pediatric patients with food allergy had a convincing history of allergy to lentil. Urticaria and OAS were the most common symptoms (Garcia et al., 1995). The symptoms may develop after ingestion of cooked lentil and/ or after inhalation of steam from boiled lentil (Pascual et al., 2001).

In another report, urticaria, angioedema, abdominal symptoms, rhinoconjunctivitis and asthma following ingestion or inhalation of vapours from cooked legumes (lentil, chickpea and bean) have been reported, but lentil was found to induce the most severe reactions (Carillo et al., 1986). The anaphylactic shock frequently has been described after ingestion of peanut, lentil, chickpea, lupin and other legumes (Sampson et al., 1992; Kalogeromitros et al., 1996; Niphadkar et al., 1997; Matheu et al., 1999; Wensing et al., 2003). The death by anaphylaxis is the most serious manifestation of the allergy to legumes (Sampson et al., 1992). These studies indicate that legumes are an important source of IgE-mediated reaction in Mediterranean and Asian countries. Therefore, in these subjects avoidance of allergens should include not only for prevention of ingestion but also the prevention of exposure to aerosolized particles or vapours through inhalation.

1.5.5. Allergens of legumes

Soybean and peanut are the major species involved in IgE-mediated hypersensitivity responses in countries such as the United States of America,
United Kingdom and Japan (Skolnick et al., 2001). In fact, peanut is the food most frequently associated with fatal food anaphylaxis (Yunginger and Jones, 1987). The major allergens from soybean and peanut have been extensively studied and a number of allergens from these two crops have been identified and characterized (Table 1.2)

Lentil, chickpea and pea are the main offending legumes in the Mediterranean area and several Asian countries, like India (Niphadker et al., 1997; Martinez et al., 2000; Patil et al., 2001). Lentils seem to be the most common legume implicated in pediatric allergic patients in the Mediterranean area and in many Asian communities (Pascual et al., 1999). However, very few reports have been devoted in identifying lentil allergens from Mediterranean population (Sanchez-Monge et al., 2000). Two different types of allergens have been identified in boiled lentils, called Len C1 and Len C2 of 16 kDa and 66 kDa molecular weights (mol wts), respectively (Sanchez-Monge et al., 2000). The major potential allergens from pea have been identified using sera from a group of patients with pea allergy in Spanish patients, namely Pis s 1 (47 kDa, vicilin chain protein) and Pis s 2 (63 kDa, convicilin) (Sanchez-Monge et al., 2004).

Chickpea has been reported as an important source of allergens that caused IgE mediated hypersensitivity reactions ranging from rhinitis to anaphylaxis in India and Spain (Martin et al., 1992; Niphadkar et al., 1997; Patil et al., 2001). Allergenic proteins ranging from 10 to 106 kDa has been identified from crude as well as boiled chickpea (Niphadkar et al., 1997, Patil et al., 2001). However, no allergen has been fully characterized from chickpea till date. Kumari et al. (2006) reported that black gram induces IgE mediated reactions in asthma and allergic rhinitis patients.
Table 1.2: Allergens of different legumes

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Allergen Scientific name</th>
<th>Species Scientific Name</th>
<th>Allergen Family</th>
<th>Molecular Weight (kDa)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ara h 1</td>
<td>Peanut (Arachis hypogaea)</td>
<td>Cupin (Vicillin-type, 7S globulin)</td>
<td>64</td>
<td>Burks et al., 1991</td>
</tr>
<tr>
<td>2.</td>
<td>Ara h 2</td>
<td></td>
<td>Conglutin (2S albumin)</td>
<td>17</td>
<td>Burks et al., 1992</td>
</tr>
<tr>
<td>3.</td>
<td>Ara h 3</td>
<td></td>
<td>Cupin (Legumin-type, 11S, Glycinin)</td>
<td>60</td>
<td>Eigenmann et al., 1996</td>
</tr>
<tr>
<td>4.</td>
<td>Ara h 4</td>
<td></td>
<td></td>
<td>37</td>
<td>Kleber-Janke et al., 1999</td>
</tr>
<tr>
<td>5.</td>
<td>Ara h 5</td>
<td></td>
<td>Profilin</td>
<td>15</td>
<td>Kleber-Janke et al., 2001</td>
</tr>
<tr>
<td>6.</td>
<td>Ara h 6</td>
<td></td>
<td>Conglutin (2S albumin)</td>
<td>15</td>
<td>van Wijk et al., 2004</td>
</tr>
<tr>
<td>7.</td>
<td>Ara h 7</td>
<td></td>
<td>Pathogenesis-related protein, PR-10</td>
<td>17</td>
<td>Sicherer and Sampson, 2007</td>
</tr>
<tr>
<td>8.</td>
<td>Ara h 8</td>
<td></td>
<td>Nonspecific lipid-transfer protein 1</td>
<td>9.8</td>
<td>IUUS, 2007*</td>
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<tr>
<td>9.</td>
<td>Ara h 9</td>
<td></td>
<td>16 kDa oleosin</td>
<td>16</td>
<td>Pons et al., 2002</td>
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<td>10.</td>
<td>Ara h 10</td>
<td></td>
<td>P34, 34 kDa maturing seed vacuolar thiol protease</td>
<td>7</td>
<td>Helm et al., 1998</td>
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<tr>
<td>11.</td>
<td>Gly m 1</td>
<td>Soybean (Glycine max)</td>
<td>Defensin</td>
<td>8</td>
<td>Wilson et al., 2005</td>
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<tr>
<td>12.</td>
<td>Gly m 2</td>
<td></td>
<td>Profilin</td>
<td>14</td>
<td>Ogawa et al., 2000</td>
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<td>13.</td>
<td>Gly m 3</td>
<td></td>
<td>Pathogenesis-related protein, PR-10</td>
<td>17</td>
<td>Renaud et al., 1996</td>
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<tr>
<td>14.</td>
<td>Gly m 4</td>
<td></td>
<td>7S Vicilin-like globulin</td>
<td>28</td>
<td>Gonzalez et al., 1995</td>
</tr>
<tr>
<td>15.</td>
<td>Gly m Bd28K</td>
<td></td>
<td>Thiol protease of the papain superfamily</td>
<td>34</td>
<td>Gonzalez et al., 1992</td>
</tr>
<tr>
<td>16.</td>
<td>Gly m Bd30K</td>
<td></td>
<td>Lectin, SBA (agglutinin)</td>
<td>14.5</td>
<td>Mittag et al., 2006</td>
</tr>
<tr>
<td>No.</td>
<td>Protein Name</td>
<td>Species</td>
<td>Description</td>
<td>Molecular Weight</td>
<td>References</td>
</tr>
<tr>
<td>-----</td>
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</tr>
<tr>
<td>18.</td>
<td>Gly m Bd 60K</td>
<td>Glycinin</td>
<td>Cupin (α subunit of β conglycinin)</td>
<td>63-67</td>
<td>Codina et al., 1999</td>
</tr>
<tr>
<td>19.</td>
<td>Gly m glyciniin G1</td>
<td>Glycinin, 11S seed storage protein</td>
<td>40</td>
<td>Natarajan et al., 2006</td>
<td></td>
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<tr>
<td>20.</td>
<td>Gly m glyciniin G2</td>
<td>Glycinin, 11S seed storage protein</td>
<td>22</td>
<td>Natarajan et al., 2006</td>
<td></td>
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<tr>
<td>21.</td>
<td>Gly m glyciniin G4</td>
<td>Glycinin, 11S seed storage protein</td>
<td>61.2</td>
<td>Natarajan et al., 2006</td>
<td></td>
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<tr>
<td>22.</td>
<td>Gly m TI</td>
<td>Kunitz trypsin inhibitor</td>
<td>20</td>
<td>Gu et al, 2001</td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td>Len c 1</td>
<td>Lentil (Lens culinaris)</td>
<td>Vicilin</td>
<td>47</td>
<td>Sanchez-Monge et al., 2000</td>
</tr>
<tr>
<td>24.</td>
<td>Len c 2</td>
<td>Lentil (Lens culinaris)</td>
<td>Seed biotinylated protein</td>
<td>66</td>
<td>Sanchez-Monge et al., 2000</td>
</tr>
<tr>
<td>25.</td>
<td>Lup an 1</td>
<td>Lupin (Lupinus angustifolius)</td>
<td>Conglutin beta (7S seed storage globulin, vicilin)</td>
<td>55-61</td>
<td>Goggin et al., 2008</td>
</tr>
<tr>
<td>26.</td>
<td>Pis s 1</td>
<td>Pea (Pisum sativum)</td>
<td>Vicilin</td>
<td>44</td>
<td>Sanchez-Monge et al., 2004</td>
</tr>
<tr>
<td>27.</td>
<td>Pis s 2</td>
<td>Pea (Pisum sativum)</td>
<td>Convicilin</td>
<td>63</td>
<td>Sanchez-Monge et al., 2004</td>
</tr>
<tr>
<td>28.</td>
<td>Vig r 1</td>
<td>Mung (Vigna radiate)</td>
<td>Pathogenesis related (PR-10) protein</td>
<td>16</td>
<td>Mittag et al., 2005</td>
</tr>
</tbody>
</table>

1.5.6. Cross-reactivity among legumes

Cross-reactivity is the occurrence of allergic reactions to foods that are related to foods known to cause allergy in an individual. If someone has a life-threatening reaction to a certain food, it is advisable to avoid related foods which also might induce the same reaction on exposure. Allergens belonging to crops of common botanical families are known to share antigens or epitopes (Ibáñez et al., 2003; Ireno et al., 2008). For example, if a person has a history of a severe allergy to peanut, he or she can also possibly be allergic to pea, soybean, green gram and other legumes. Legumes being from the same family (leguminosae) have a high degree of immunological cross-reactivity, as shown in in vitro studies (Barnett et al., 1987; Ireno et al., 2008). The allergenicity of legumes is mainly related to allergens from the storage proteins of seeds. Vicilins from this group of proteins could be an important common allergen in clinical allergy to legumes. Using RAST, Barnet et al. (1987) screened sera from 40 patients with peanut allergy against 10 other legumes and demonstrated IgE binding to multiple legumes for 38% of patients. Most of the allergens responsible for cross-reactivity are related to the super family of storage proteins 2S (Breiteneder and Ebner, 2000). In vitro studies showed stronger cross-reactivity between peanut, pea and soybean (Barnett et al., 1987). It has been observed that patients with immediate hypersensitivity to lentil were sensitized to 54% to pea and 80% to chickpea (Ibáñez et al., 1999).

The clinical cross-reactivity among legumes is rare in children according to a pioneering study of Bernhisel-Broadbent and Sampson (1989) that includes 69 children with positive cutaneous test to one or more legumes, in which 41 patients had positive provocations and only 2 patients had symptomatic hypersensitivity to two legumes. Nevertheless, 79% of the
patients showed immunological cross-reactivity to two or more legumes. Extensive cross-reactivity between soybean and peanut allergens has been reported by Eigenmann et al. (1996). Moreover, major soybean allergen glycinin G1 shared epitope with peanut Ara h3. Cross-reactivity among pea and lentil is noted as Len c1 and pis s1 show a high degree of sequence identity as evident by immune blot inhibition assays also. Lentils appear to be cross-reactive with chickpea and beans (Martin et al., 1992; Sanchez-Monge et al., 2000). In a Spanish study, aimed at determining the prevalence of lupin sensitisation in 1,160 subjects consulting allergologists, 4.1% sensitization rate to lupin (28 patients) was found, with 75% co-sensitisation between lupin and other legumes. Of 28 patients, 13 were shown to have skin reactivity to chickpea, 8 to pea, 12 to peanut, 13 to bean and 7 to lentil (Reis et al., 2007). Cross-reactivity among lentil, chickpea, and pea has been detected (Pascual et al., 1999; Martinez et al., 2000; Patil et al., 2001). Hefle et al. (1994) reported that 5/7 peanut allergic patients had positive SPT to lupin after one reported symptoms and these individuals also reported reactions against green pea.

Clear IgE cross-reactivity was demonstrated by Moneret-Vautrin et al. (1999) between peanut and lupin; although lupin was unable to inhibit peanut binding to IgE; it may be that peanut was the initial sensitizer. Matheu et al. (1999) reported about allergic to several legumes, where an open challenge showed that patient could tolerate 8 gm of peanut or green bean but gave itching and watering eyes, rhinorhea, sneezing and itching palms with pea. IgE cross-reactivity was also observed by immunoblotting inhibition. Faeste et al. (2004) reported that a peanut-allergic patient reacted against hidden lupin protein in hot dog bread. In summary, allergy to lupin seems to be associated with multiple sensitisations and IgE cross-reactivity to
other legumes. Cross-reactivity of black gram with lentil, limabean and pea was reported by ELISA inhibition and immunoblot inhibition (Kumari et al., 2006).

1.6. Animal models for food allergy
Currently, no ideal animal model exists for food allergy that can correctly predict the allergenic potential of food proteins. Different animal models have been tried like BALB/c (Dearman and Kimber, 2001), C3H/Hej mice (Li et al., 2000), C57BL/6 (Cardoso et al., 2008), Brown Norway rat (Atkinson et al., 1994), atopic dog model (Ermel et al., 1997) and guinea pig model (Piacentinin et al., 1994). These models are being assessed for production of IgE, clinical response to re-exposure and a ranking of food allergens based on potency.

Guinea pigs have frequently been used for oral sensitization studies to food proteins (Piacentinin et al., 1994). Studies in mice on oral protein administration without adjuvant resulted in tolerance induction (Mowat, 1987). However, repeated oral protein administration in combination with adjuvant showed that immune sensitization can be achieved (Li et al., 1999, 2000). For the assessment of potential allergenicity, intra peritoneal route of exposure is also studied in mice (Dearman et al., 2000) focusing on antigenicity and allergenicity of proteins. For the rat, oral sensitization to food protein administered through diet or by gavage-dosing in the presence (Atkinson et al., 1996) or absence of adjuvant (Knippels and Penninks, 2002) has also been reported. BALB/c and C3H/Hej mice models were found to be equally good for evaluation of allergenic potential of food (Dearman and Kimber, 2001; Morafo et al., 2003).
1.7. Food allergy scenario of Indian Population

In India, the prevalence of food allergy has not been systematically studied, and unfortunately, there is not much awareness about food hypersensitivity reactions in Indian population. Many asthma and rhinitis patients may be suffering from food allergy but our knowledge about food hypersensitivity is limited to few studies carried out in the country (Patil et al., 2001; Parihar et al., 1984; Sharman et al., 2000). Sharman et al. (2000) undertook food challenge studies by SPT on children with respiratory allergy.

Somani (2008) reported that apple and hazelnut were the two most common food allergens in the atopic dermatitis patients and concluded that apple allergy may play a role in both children and young adults. Saraswat et al. (2005) reported a case of 33 year old non-atopic woman with multiple episodes of anaphylaxis after ingestion of different fruits namely apple, banana and lychee. Gaur et al. (2008) purified a 360 kDa hexameric protein Pru du amandin, a known allergen, from almonds (Prunus dulcis). This allergen belongs to the IIS legumin family of seed storage proteins, characterized by the presence of a cupin motif. Qureshi et al. (2006) isolated, purified and crystallized a 24 kDa allergenic protein from Lathyrus sativus. Pramod et al. (2006) reported for the first time food allergy to horse gram, and identified its component Dolichos biflorus agglutinin (DBA), an important dietary lectin as an allergen.

Agarkhedkar et al. (2005) conducted study on children with asthma having seasonal exacerbation and hypersensitivity to a wide variety of foods like rice, lentil, black gram, bengal gram, green gram, brinjal, chicken, egg and melon. Elimination of specific diet, prevent the season exacerbation, suggesting that food avoidance may help in asthma control in children.
Patil et al. (2001) reported that chickpea is an important source of allergen that can cause IgE mediated hypersensitivity reactions, ranging from rhinitis to anaphylaxis. Of 1,400 patients screened for chickpea allergy, 142 were food allergy positive on history, of which 59 implicated chickpeas. Subsequent challenges demonstrated that 31 were Double-blind, placebo-controlled food challenge (DBPCFC) positive for chickpea (Niphadkar et al., 1992). No allergens from chickpea have been fully characterized, but immunoblot analysis has demonstrated that at least four proteins of 70, 64, 35 and 26 kDa are major allergens (Patil et al., 2001). Kumari et al. (2006) reported eight allergens of 16–78 kDa from black gram and concluded that black gram is a potent sensitizing agent in allergic asthma and rhinitis patients. The major pulses grown in India are chickpea, pigeon pea, black gram, green gram, kidney bean, bengal gram and peas. Therefore, chances of Indians showing sensitization towards these legumes are more. However, our knowledge is inadequate regarding allergenic proteins found in these legumes.
1.8. Genesis of study

Food allergy is an emerging problem worldwide and incidence of allergic diseases is on the increase including Asian countries. The prevalence of food allergies in adults is about 3%, while in children it is 8%. Minute amount of offending food can lead from mild discomfort to anaphylactic shock on exposure. After extensive survey of literature, it has been found that eight foods that include peanuts, tree nuts, cow's milk, hen's eggs, fish, crustaceans, wheat and soybeans can cause life-threatening reactions in sensitized individuals. Among the food allergens egg, cow's milk and fish occupies first, second and third places respectively, followed by legume which occupies the fourth place in importance among the food allergens in Spanish children (Carillo et al., 1986).

Leguminosae is a large food family that includes variety of beans, peas and pulses. Since legumes are extremely important source of food protein in the Indian diet, therefore, chances of Indians showing sensitization towards legumes are more. There are some reports, which show evidence of food allergy in asthma and rhinitis patients. The culprits in these cases were found to be different legumes. Asthma and anaphylactic shock is noticed even after inhalation of vapors of cooked legumes. It appears worthwhile to assess the allergenic potential of some of the commonly consumed legumes in animal model as well as identification and characterization of allergenic proteins using allergic patients' sera. Hardly any attempts have been made in India for identification and characterization of different legume allergens. It may help to understand, what characteristics of proteins like it's stability to proteolysis etc. make it allergenic. The recent progress in our understanding of the mechanism of initiation and control of Th2 cell responses will eventually lead to new therapeutic strategies. In India, among legumes
consumption of red gram is maximum, followed by green gram and lentil. Contrary to soybean and peanut (Helm et al., 1998; 2000; Koppelman et al., 2003), the research on other legumes like red gram and green gram is surprisingly rare. Hence, red gram and green gram were chosen for evaluation of their allergenic potential in this study.

It is expected that the data generated may lead to a better understanding of the magnitude of this problem and offer some insights. That will help in identification and physico-chemical characterization of the new allergens from these food crops which may provide the better knowledge in understanding the mechanism and cause of food allergy. In light of above backgrounds and to fill the knowledge gaps, the following studies were proposed as part of this dissertation:

1. Physico-chemical characterization (molecular weight, digestive stability) of different leguminous crops proteins.
2. Screening of legume allergic patients and immunological cross-reactivity determination among allergenic proteins of different legumes.
3. Estimation of histamine, IgG1, IgG2a and IgE levels, Th1/Th2 cytokine profile in animals treated with red gram and green gram crude protein extract (CPE).
4. Identification and characterization of allergenic proteins of red gram and green gram.