1. Introduction
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Currently, the world population is approx. 6.8 billion, with an expected annual growth of 1.4% as per the World Bank data. To maintain this population, approx. 230 million tons of protein is needed per year. Legumes are an important source of proteins which may correspond up to 20-40% of the dry weight of their seeds (Carlini and Grossi-de-Sa´, 2002). The crop yield suffers substantial losses, before or after harvesting, due to the attack of a variety of pests including insects and nematodes, viral, bacterial and fungal diseases, and competition by weeds. These losses may account for about 100 billion dollars annually. The major damage is caused by insects, an amazingly diverse group of animals with a million or more species, cause about 70% crop losses without the use of pesticides and other non-chemical control strategies in the world (Thomas, 1999). The methods available today for protecting plant crops against insect predation are heavily dependent on environmentally aggressive chemicals such as fertilizers and protectants (insecticides, fungicides, herbicides, etc.) and are estimated to reduce the losses by only about 7% (Oerke et al., 1994). Moreover, notwithstanding the huge expenditures (more than 10 billion dollars annually) on production of synthetic chemicals, the annual loss reaches 37% of the total crop with an estimated cost of 300 billion dollars and the insect pests are responsible for 13% of the loss (Dunaevsky et al., 2005). Although more specific and less stable protectants are continuously developed to reduce the ecological hazard created by using them, the consumption of synthetic chemicals has started to decrease (mostly in Europe). Hence, it is necessary to design new technologies in order to reduce the use of synthetic compounds without increasing the loss of crops. This fact justifies the necessity for research and development of alternative approaches to solve this problem.

Among various insects studied, *Helicoverpa armigera* (podborer; Lepidoptera: Noctuidae) is the most devastating polyphagous crop pest of many important crops worldwide leading to heavy losses in the field (Manjunath et al., 1989). It is a major pest of crops such as cotton, pigeonpea, chickpea, tomato, vegetables, and fruits. *H. armigera* larvae have an alkaline gut, which can produce at least six major and several minor serine proteinases that are able to overcome the native protease inhibitors of its host plants (Harsulkar et al., 1999; Patankar et al., 2001). Also, *Spodoptera litura* is an important polyphagous pest in India, China and Japan.
It is a serious pest of various economically important crops such as cotton, groundnut, chilli, tobacco, castor, bendy and pulses etc. (Armes et al., 1997; Niranjankumar and Regupathy, 2001). Beside insects, fungi are also destructive agents causing losses of agricultural commodities in many zones of the world. These losses can occur on growing in-field crops as well as harvested commodities, leading to damage ranging from rancidity, odour, flavor changes, loss of nutrients, and germ layer destruction. Estimates on crop losses that can be directly attributed to fungi may vary. Crop losses due to pathogens are often more severe in developing countries (e.g. cereals, 22%) as compared to those in developed countries (e.g. cereals, 6%) (Oerke et al., 1994). For individual crops, Oecke and Dehne (2004) estimated that worldwide, fungal losses can be 100% if a susceptible cultivar is planted or the climate is favorable in any year, and down to 0%, if resistant varieties are planted and fungicides used, and good husbandry employed. *Aspergillus*, *Alternaria* and *Fusarium* are amongst the most common fungal species associated with growth and damage to food crops in the field.

As a consequence of evolution, plants improvised protective mechanisms against insect pests and phytopathogens. Plants produce several kinds of defensive chemical compounds including enzymes (e.g. chitinase, lipoxygenase, acylhydrolase, and glycohydrolases), protease and amylase inhibitors, and lectins (Ryan, 1990; Peumans and Van Damme, 1995; Koiwa et al., 1997) etc., which exhibit toxic or antimetabolic activities towards insect pests and phytopathogens. Among these chemical compounds protease inhibitors (PIs) are known to play an important role in plant defense against insects and pathogens which are major constraints to plant growth and development. Since insect pests and phytopathogens use proteolytic enzymes for penetration into the host plant tissues, plants counteract their damaging advances by synthesizing constitutive and induced PIs (Jamal et al., 2012).

Plant PIs are ubiquitous, small regulatory proteins generally present at high concentration in storage tissues (5–15% of total protein) (Garcia-Oimedeo et al., 1987). These are well spread in the seeds of plants belonging to family Fabaceae, Brassicaceae and Poaceae as well as in tubers of Solanaceae (Ryan, 1981; Bolter and Jongsma, 1997; Ascenzi et al., 1999; Oliva et al., 2000). Seeds usually accumulate PIs during maturation and the concentration of these inhibitors varies between 1 and 10% of total seed proteins (Ryan, 1981). Among the
cereals such as corn, barley, wheat and rye, they are primarily present in the endosperm. PIs are also located in aerial parts of plants (Jamal et al., 2012).

Though PIs show specificity for serine, cysteine, aspartic, and metallo-proteinases (Bode and Huber, 2000) yet serine and cysteine PIs have been studied the most. Plant PIs differ in their specificity and the ability to inhibit one or more proteases at the same time. Majority of them inhibit trypsin and many inhibit chymotrypsin. Nine families of plant PIs have been identified including Kunitz and Bowman-Birk inhibitor (BBI) families, which are abundant in various leguminosae seeds. Kunitz-type inhibitors are approximately 18-26 kDa proteins (Calderon et al., 2010) with four cysteine residues forming two disulfide bridges and possess a single reactive site (mostly against trypsin) (Richardson, 1991) whereas BBIs are generally 6-15 kDa proteins (Lawrence and Koundal, 2002; Calderon et al., 2010) with 14 cysteine residues forming seven disulfide bridges and possess two reactive sites (Qi et al., 2005; Clemente and Domoney, 2006). These proteins are fundamental in the control and/or the protection against proteolytic action of the digestive enzymes of seed predators (Batista et al., 1996; Shewry and Lucas, 1997). The inhibitory activity of PIs is largely brought about by intramolecular interactions viz., disulfide bond, hydrogen bond and hydrophobic interaction which are involved in stabilization of the primary binding loop (reactive site loop) structure enabling a stable complex with a cognate protease (Bode and Huber, 1992; Iwanaga et al., 2005).

Naturally occurring PIs are essential for regulating the activity of their corresponding endogenous proteases and play key regulatory roles in many biological processes by exhibiting antifeedant, antifungal, antitumor and cytokine inducing activities (Kuhar et al., 2014). In addition, a few PIs may have growth factor activities and may be involved in receptor clearance signaling or carcinogenesis (Qi et al., 2005). The presence of PIs in seeds is essential for maintaining the physiological processes such as storage proteins (Mandal et al., 2002) and stabilization of the enzymes during desiccation phase (Lam et al., 1999). In plants, PIs play a role in controlling programmed cell death (Solomon et al., 1999) and their expression is known to increase in response to various abiotic (Casaretto et al., 2004) and biotic stresses, particularly while defending against insect pests (De Vos et al., 2006).
Proteinases play an active role in the development of diseases (Sara and Heale, 1990). Several phytopathogenic fungi are known to produce extracellular proteinases (Kalashnikova et al., 2003). Inhibitory polypeptides synthesized by plants suppress the enzyme activities in response to the attack of proteinases produced by phytopathogens (Ryan, 1990). This phenomenon was first recorded in tomatoes infected with Phytophthora infestans (Woloshuk et al., 1991) in which increased levels of trypsin and chymotrypsin inhibitors were found to be correlated with the plants resistant to the pathogen. The role of plant PIs in plant defense against insect pests is also well known (Lawrence and Koundal, 2002; Haq et al., 2004). PIs are known to affect the insect growth and development either by inhibition of gut proteinases or due to overproduction of digestive enzymes thereby reducing the availability of essential amino acids for the production of other proteins (Pompermayer et al., 2001). Soybean inhibitor was the first isolated PI which was inhibitory against the red flour beetle digestive proteolytic activity (Lipke et al., 1954). Since then many PIs have been isolated and characterized from several plant species including legumes (Kuhar et al., 2010). Genes encoding plant PIs have been isolated from various plants (Marchetti et al., 2000; Haruta et al., 2001; Lawrence et al., 2001). The potential of PIs has been demonstrated by the transfer of PI genes from different sources to several plants of economic interest, resulting in transgenic plants more resistant to predation (Lawrence & Koundal, 2002) and fungal pathogens (Dunaevsky et al., 2005; Kuhar et al., 2010). It was suggested that the presence of antifeedant and antifungal activity on a single protein opens up a possibility of raising a transgenic plant resistant to pathogens as well as pests, by transfer of a single protein (Joshi et al., 1998). Accordingly, studies on purification and characterization of PIs having more efficient biological activities such as antifeedant and antifungal, and isolation of their genes from crop plants will be useful in producing transgenic plants resistant to the attack of insect(s) and fungal pathogen(s). Even though extensive work is available in the literature on the scope for utilizing plants as valuable source for deriving PIs, some of the commonly available plant sources are still to be explored completely to obtain a more effective TI. Further, the availability of information on different PIs with varying effectiveness and biological activities might enable the development of combination cassettes for plant transformation. With this anticipation, an effort has been made to isolate, purify and characterize a trypsin inhibitor from Phaseolus vulgaris for possible applications.
Kidney beans (*P. vulgaris*), commonly known as Red kidney bean, Green beans, Large white beans, Flageolet, Black bean, Borlottino bean, Cannellino bean, and Sugar bean, is a herbaceous annual plant which is grown worldwide for its edible bean, popular both as dry and as a green bean. Kidney beans are high in proteins and dietary fibers and are very useful in lowering cardiovascular health risks. They are excellent foods to be included in weight loss diets because of very low calories and lack of cholesterol. As per USDA Nutrient Database, the raw kidney beans contain (per 100 g) energy 1,393 kJ, carbohydrates 60 g, sugars 2 g, dietary fiber 15 g, fat 1 g, protein 24 g, water 12 g, pantothenic acid 0.8 mg, folate 0.39 mg, calcium 143 mg, iron 8 mg, magnesium 140 mg, and zinc 3 mg. These are very high in molybdenum, a half cup providing nearly half of the daily requirement which help in reducing the effect of sulfites in the body and also provides large amounts of folate and tryptophan. The soluble fiber in kidney beans binds the bile attached with cholesterol in the digestive track and transports it out of the body thereby reducing the cholesterol level in the body (Vohra, 2007).

Therefore, the present investigation was planned with the following objectives:

1. Screening of kidney bean (*Phaseolus vulgaris*) varieties for the presence of trypsin inhibitor in seeds
2. Purification of trypsin inhibitor from the seeds of kidney bean
3. Physico-chemical characterization of the isolated trypsin inhibitor
4. Isolation and characterization of the gene encoding trypsin inhibitor
5. To investigate potential of the isolated protein as insecticidal and antifungal agents
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