The previous research work conducted on PHS of wheat and other cereals is briefly reviewed in this chapter.

2.1 Domestication of cereals

The process of domestication of cereals started about 12,000 years ago, when human civilizations shifted from hunting to agriculture for their continued existence during the Neolithic period (Shewry, 2009; Leigh et al., 2013). The first cultivation of cereals started in Palestine, Jordan, Lebanon, western Syria and south-east Turkey along the Tigris and Euphrates rivers, Iraq as well as western flanks of Iran (Heun et al., 1997; Dubcovsky and Dvorak, 2007).

The family triticeae, which belongs to the grass tribe gramineae includes several of the world’s most essential cereal crops, such as wheat, barley, triticale and rye (Kellogg, 2001; Kawahara, 2009). The most important five genera are Aegilops, Elymus, Triticum, Secale and Hordeum are included in the family triticeae. All the 23 varieties of wheat belong to the genus. Triticum polyplloid series with a basic chromosome number $x = 7$ (Von Braun, 2007). The wild types of Triticum are diploid i.e. $T. \text{ monococcum}$ and $T. \text{ tauschii}$ and $T. \text{ speltoides}$ and their chromosome number is $2n = 2x = 14$. The tetraploid species i.e. $T. \text{ turgidum}$ have chromosome number $2n= 4x= 28$ and the modern bread wheat $T. \text{ aestivum}$ is hexaploid with chromosome number $2n= 6x= 42$ (Shewry et al., 2012). Because of the polyploid nature of wheat, there is great potential for genetic distinction and about 17, 000 wheat cultivars had been produced in the beginning of 1970s (Baden and von Bothmer, 1994; Baik and Ullrich, 2008; Kumar et al., 2011).

An important attribute of wheat is its flexibility to diverse climatic conditions. Although grown mostly in temperate climates, it can be grown from within the arctic circle to higher elevations near the equator, and from sea level to as much as 3000 msl. As such, it is one of the most widely cultivated crops with a short growing
season, and giving good yield per unit area. These attributes make wheat one of the most important commodities in global trade. Wheat continues to be one of the largest food crops in terms of region of crop growing as well as production (FAO, 2011; Shewry et al., 2010).

2.2 Origin of the modern triticaceae

The natural hybridization events had produced the triticaceae grown today, between 7,000 to 10,000 years ago in the fertile crescent area of northern Egypt, through Israel, Palestine, Jordan, western Syria, south east Turkey and northern Iraq (Zhu et al., 2010). Their wild grass forebears still exist in the region and are valuable sources of new genes for wheat breeders. The wild grass species are mostly diploids (Fuwa et al., 2005; Shewry et al., 2012). Tetraploids like wild emmer wheat (*Triticum turgidum*) were the originators of modern durum or pasta wheat (*Triticum turgidum* var. durum) (Ravel et al., 2006; Godfrey et al., 2010). The natural hybridization events between domesticated emmer and the diploid grass (*Aegilops tauschii*) had given rise to wheat variety with 21 pairs of chromosomes (hexaploids), to bread wheat variety (*Triticum aestivum* L.) and spelta (*Triticum aestivum* ssp. *spelta*) (Cane et al., 2004). The wheat grains of different species are shown in figure 2.1.

2.3 Cultivation of wheat in Iran

Wheat is the main grain crop cultivated in Iran in autumn, which matures in late spring to early summer (Mollasadeghi et al., 2011; Ahmadizadeh et al., 2011; Dadbakhsh et al., 2012). The cultivation of wheat in Iran is largely determined by climatic condition (Table 2.1). Because of drought, cold and pre-harvest sprouting in northern Iran wheat production is considerably reduced (Nourinia, 2001; Habibpor et al., 2011). In northern Iran, harvesting generally commence at the end of June and completed by mid of July. Over the last five years (2007 to 2011), wheat production in Iran was 13,500,000 tons, but over the last 20 years, production was only about eight million tons per annum, with the average being 10 million tons annually. Iran is unique in grain-management and maintenance of grain quality (Mousavi, 2012). For the proper assessment of grain quality several methods like test weight and protein content were deployed across the country (Roohani et al., 2012; Peighambardoust
et.al., 2013; Badii et.al., 2012). The climatewise cultivation of wheat in different parts of Iran is shown in table 2.1.

2.4 Importance of wheat in global food security

Wheat is the world’s most important protein source providing about 21% of the daily dietary protein intake (Parry et.al., 2011). It accounts for 41% of the calories and 50% of the proteins, when compared with total cereals’ consumption. Its share increased to 75 - 81% for cereal-based calories and proteins, respectively, in the developed regions and 35 to 44% in the developing regions. According to Shiferaw et.al., (2013) the highest annual per capita food demand of wheat is in central Asia (171 kg), followed by north Africa (165 kg), west Asia (122 kg), eastern Europe and Russia (120 kg). Wheat also remains a major source of dietary calories and proteins in Eastern Europe, Russia and south Asia and in high income countries. It is a critical component of the diet for about 2.5 billion poor people (Abdoli and Saeidi, 2012 b; Ghasemi et.al., 2013; Shiferaw et.al., 2013). In these regions, wheat accounts for over three-quarters of cereal intake and its demand in developing regions has increased by more than five-fold (Duveiller and Sharma, 2009; Shiferaw et.al., 2011; Shiferaw et.al., 2013).

The total global demand for wheat has almost quadrupled since 1960 and doubled over the four decades since 1980 (CIMMYT, 2011). About two third of the demand for wheat comes from the developing regions. Since 1970, the total demand in developing regions was tripled to about 390 million tons (Joshi et.al., 2011; Shiferaw et.al., 2011). The total global annual demand for wheat has grown at an average rate of about 2.24 % per year since 1960 (Dixon et.al., 2009). The economic dependence of developing nations on wheat is indicated by the fact that it is their single largest food import and it is an important share of emergency food aid (Dixon et.al., 2009).

Most of the larger producers such as India and China are self-sufficient (Pal and Byerlee, 2005; Trego, 2011). At the global level wheat is mainly used for food (71%) and feed (20%). About one third of the people living in central Asia and Caucasus depend heavily on wheat for daily calories. Wheat also plays a major role in food security in north Africa (201 kg/capita) and West Asia (155 kg/capita). Over
90% of the wheat is consumed in south Asia especially in Pakistan, Afghanistan and north India. Wheat is also very important in east Asia where 84% of the people depended on wheat (Pingali, 2007; Duveiller et al., 2010; Dixon et al., 2009; Lobell and Burke, 2010). According to Meng et al., (2009) increase in the share of western bread products along with shifts to end-uses associated with convenience and higher quality of wheat.

Breeding wheat with specific quality characteristics has the potential to add economic value (Lantican et al., 2005; Duveiller and Sharma, 2009). The global production showed a dramatic annual growth of about 4.4% during the first decade (1960–70) of the green revolution, about 4% during the following decade, 3.2% during the third decade, stagnated during 1990–2000 and grew by 1.27% per annum during the last decade (2001–10). Steady growth in world wheat production during the 20th century was due to both area expansion and yield increases, (Aquino and Carrion, 2009).

2.5 Grain morphology

The grain morphology assists in understanding the grain processing and flour performance in the production of end products (Monneveux et al., 2005; Rai et al., 2011). Grain components like bran layers and crease material, having nutritional value in human diet have negative impact on flour functionality in bread making (Rai et al., 2011; Tsilo et al., 2010). These components are the primary location of oxidative enzymes like polyphenol oxidase and if not removed effectively during milling can result in poor shelf life of fresh products like noodles and discolouration over time of stored frozen dough (Breseghello and Sorrels, 2006; Wilkinson et al., 2008). The average wheat kernel (Wilkinson et al., 2008) is comprised of more than 80% endosperm, 8% bran and seed coat material, 6% aleurone and 3 to 4% embryonic tissues (Breseghello and Sorrels, 2006).

The kernel size varies with the region of cultivation and type of cultivar (Sun et al., 2009; Hands et al., 2012). The cross section of the wheat kernel shows the major groups of tissues that comprises the kernel. The seed is made up of embryonic axis, scutellum, endosperm, nucellus and testa or seed coat, surrounded by fruit coat or pericarp. The embryonic axis has root primordia and a shoot with leaf initials and
together with scutellum comprises the embryo (Breseghello and Sorrels, 2006; Gegas et.al., 2010). The endosperm is the largest tissue of grain and is mainly made up of starch, surrounding the starchy endosperm is the aleurone, a single layer of cubic-shaped thick walled cells. Aleurone cells do not contain starch but are rich in protein and lipid. The testa is only 1-2 layer thick, but the pericarp is multi-layered (Li et.al.,2010). At maturity, the pericarp consists of dry empty cells. The trichomes exist at the non-embryonic end of the grain, which is known as brush (Zhu et.al.,2009). Endosperm cells are packed with starch granules and remnants of protein bodies and cell membranes (Blakeney et.al., 2009).

During milling, the fracturing of starch granules contributes to raising the water absorption capacity of the flour (Giura et.al.,1996; Yu et.al.,2014). Wheat endosperm is relatively rich in protein and the starchy endosperm is comprised of two distinct types of starch granules (Zhao et.al.,2005; Hogg et.al.,2005; Blakeney et.al.,2009). At the molecular level, starch granules are comprised of amylase and amylopectin (Zhao et.al.,2005; Jerkovic et.al.,2010). Variation in the ratio of amylase to amylopectin has implications for functionality of certain wheat-based products (Zhao et.al.,2005; Jerkovic et.al., 2010; Brouns et.al 2012). The internal structure and types of food storage in wheat grain is shown in figure 2.2 a&b)

2.6 Grain development, maturity and its relation with PHS:

The instance of grain maturity and the extent of its drying are markedly significant in relation the phenomenon of pre-harvest sprouting (Imtiaz et. al.,2008; Sreenivasulu et.al.,2010). A half-mature embryo of wheat grain is approximately 25-days old and showing tendencies of germination when exposed to favorable conditions. The level of maturity of aleurone layer controls the synthesis of α-amylase, which is suppressed until the total grain development and drying (Radchuk et.al.,2011). This shifting from inactive aleurone to active aleurone takes place during pre-harvest sprouting, when the grains germinate in the spike.

In situations of slow grain drying and favorable conditions for germination (such as occurrence of rainfall, optimal humidity, and temperature), the grain germinates on the spike, initiating the enzymatic activity that accompany degradation
of protein and starch in the endosperm. From the studies it is evident that immature seeds are rich in abscisic acid (ABA) which plays an important role in dormancy induction during grain development. PHS is induced by different factors such as temperature, humidity, rainfall and rate of grain desiccation (Paterson et.al., 1989; Nakaune et.al., 2005; Shorter et.al., 2005; Sreenivasulu et.al., 2010; Lohwasser et.al., 2013).

2.7 Structure of Seed:

2.7.1 Seed coat

Seed coat development commences after fertilization and the inner and outer integuments of the ovule progress into seed coat layers, hence, seed coat is of maternal origin. (Gong and Bewley, 2006; Zhou et.al., 2009). It is generally known that the seed coat plays an important role in embryo nutrition during seed development, all sugars, amino acids, and minerals transported to the developing seeds are unloaded in the seed coat and move apoplastically to the embryo and endosperm (Patrick and Offler, 2001).

Several roles of the seed coat in seed dormancy and germination have been demonstrated such as: interference with water uptake, mechanical control for radical overhang, interference with gas exchange, particularly oxygen and carbon dioxide, prevents inhibitor (hormone) leakage from the embryo, supplies inhibitors to the embryo, and involved in light filtration (Bewley and Black, 1994; Nakaune et.al., 2005). Studies have demonstrated that phenolic compounds, particularly flavonoids, contribute to the above-mentioned germination inhibiting effects of the seed coat (Debeaujon et.al., 2007; Gao et.al., 2012).

2.7.2 Embryo

The embryo of seed gives rise to new plant which include embryo radicle (axis), scutellum and suspensor. Based on morphological criteria, the development of the embryo in cereals can be divided into several stages (Radchuk, et.al., 2011). The initial stage of embryo development is the creation of the terminal cells leading to the formation of the globular-shaped embryo which then lengthen to a club-like shape showing radial symmetry (proembryo stage). This is followed by the coleoptile stage.
where the developing embryo becomes bilaterally-symmetrical through differentiation of the scutellum and coleoptiles, indicating the establishment of the embryo axis (Black et al., 1996; Radchuk et al., 2011; Etienne, 2013). This is followed by the leaf stage where shoot and root meristems become visible and seed storage products, such as oil bodies, are accumulated. The embryo is fully developed to become quiescent. The length of time to complete embryogenesis varies among different species. For instance, under normal growing conditions a typical wheat embryo completes development to the final three leaf stage in more than 20 days after fertilization (Smart and Obrien, 1983; Hong et al., 1995; Etienne et al., 2013).

2.7.3 Endosperm

In cereals, the fully developed endosperm constitutes the major portion of the grain which consists of mainly four major parts: starchy endosperm, aleurone layer, transfer cells and the cells surrounding embryo. The endosperm is basically the source for human food, animal feed and industrial raw materials (Radchuk et al., 2011; Popielarska-Konieczna et al., 2013). The grain filling period is normally 25-60 days after pollination. For example, in spring wheat, grain filling period is between 25-30 days, during this period, the endosperm grows fast and is filled with starch granules and prolamin, the storage proteins (Bozhkov et al., 2005; Popielarska-Konieczna et al., 2013).

The cells of starchy endosperm desiccate and die towards the end of the seed maturation period. In contrast, the cells of the aleurone layer are desiccation tolerant, and are alive in the mature, dry grain. These cells remain quiescent until imbibitions, causing the embryo to produce gibberellins (GA), which diffuse into the endosperm. This hormonal activity induces the expression of glucanases, amylases and proteases that break down cell walls and starchy endosperm (Sun and Gubler, 2004; Thiel et al., 2008).

The mobilization of the degraded endosperm as a nutrient source contributes to embryo growth after germination. The degradation of the cereal endosperm has been studied intensively, especially the synthesis and secretion of amylases and proteases from the aleurone layer and regulation of their synthesis by gibberellins and
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abscisic acid (Gubler et.al.,2005; Sreenivasulu et.al.,2006; Verdier et.al.,2008; Sreenivasulu et.al.,2010).

2.8  Grain quality influenced by heredity, environment and GxE

Grain protein in wheat is largely determined by environmental factors, farm management and genetics (Altenbach et.al.,2002; Quinde-Axtell et.al.,2005). By contrast, protein quality is determined by the genetic composition of the wheat variety and also how the environment influences genetic expression (Dupont and Altenbach, 2003; Snape et.al.,2007;Hossain et.al.,2013 ). A suitable balance of acid insoluble glutenin and gliadin, in combination with a particular level of total protein, is needed for most flour end-uses (Chen et.al.,2010; Chen et.al., 2013). One of the aspects contributing to the complexity of wheat quality is that its expression as measured through a range of analytical tests is the result of genetics, the growing season, environment and the interaction between the two, known as genotype x environment (G x E) interaction. These effects on wheat quality are summarized in table 2.2.

2.8.1  Wheat proteins

Wheat bran contains the majority of protein found in the kernel (Pedersen and Lindberg, 2010; Jerkovic et.al.,2010). All proteins found in the outer portion (epidermis and hypodermis) function to protect the kernel from degradation (Hurkman et.al.,2007). Proteins in wheat are of four types, namely albumins, globulins, prolamins and glutelins (Wang et.al.,2006). Wheat grain proteins are also classified into structural/metabolic (non-gluten) and storage (gluten) proteins (Shewry, 2003; Yahata et.al.,2005). The non-storage proteins, albumins and globulins constitute 15-20% of the total wheat grain proteins and are accountable for enzymatic activity and starch breakdown (Singh and Skerritt, 2001; Hurkman et.al.,2007). The storage proteins (gluten) include up to 80-85% of the total wheat grain proteins (Shewry et.al.,1995; Shewry et.al.,2012). Wheat storage proteins are situated in the starchy endosperm of the developing wheat kernel. (Shewry et.al.,2002; Hurkman et.al.,2007; Ross et.al.,2012).
Gluten plays an important role in bread-making quality of wheat because of its visco-elastic properties (Nishimura et al., 2007). The major components of the gluten proteins are gliadins and glutenins in wheat (Wang et al., 2007; Pandey and Budhathoki, 2007). Gliadins constitute 40-45% of the total wheat grain proteins and are monomer. On the basis of molecular mobility at low pH, gliadins are classified into four groups, $\alpha$, $\beta$, $\gamma$ and $\omega$ (Wieser, 2007; Saint-Pierre et al., 2008). Glutenins comprise around 40-45% of the total wheat grain proteins which are polymeric (Field et al., 1983; Dupeux et al., 2011).

2.8.2 Effect of biotic and abiotic factors on wheat growth and development

Wheat is grown both in irrigated and rainfed conditions, where annual rainfall ranges from 350-700 mm (Ahmadi et al., 2009; Fig 2.3). Wheat is cultivated on 2.2 million hectares as irrigated and on 4 million hectares as rainfed, where the crop often suffers from severe moisture stress (Galeshi and Bayat Tork, 2005; Tavakoli et al., 2009; Mousavi, 2012; Ehdaie et al., 2011; Gravandi et al., 2011). After completion of tillering, plant starts quick growth of leaves and internodes and this stage is called jointing (Koocheki et al., 2006; Kadioglu et al., 2012). During this stage, size of leaves and internodes increases starting from the ground side (Saeidi et al., 2007). Leaf and internode once complete their growth, limitation in supply of water at this stage affects the growth of leaves and internodes, causing decrease in leaf area and plant height. At booting stage, the flag leaf encloses developing spike and appear as dumble shaped (Saeidi et al., 2010).

The spike emerges out from this and pollination is completed (Sadat noori et al., 2007). The effect of inadequate availability of water at this stage decreases number of spikelets and flowers. Filling is the last stage, when photoassimilates of the plant move towards the spike and take part in the filling of grain (Sapone et al., 2012). The size and weight of the grain is decreased due to the effect of water stress on photosynthesis and movement of assimilates towards the developing grain (Leigh et al., 2013).

Under water stress condition, the plant makes an effort to maintain its turgor through decrease in evapotranspiration by closing its stomata (Sapone et al., 2012; Leigh et al., 2013; Thanh et al., 2013). Low stomatal frequency along with small size
of stomata, help in the reduction of evapotranspiration and enable the plant to withstand the water shortage situation. Development of suitable varieties for variable moisture stressed environments has been a difficult task of wheat breeding programmes (Nicky et.al., 2012; Baloglu et.al., 2012). Some traits which help in improving the performance of a plant under stress conditions are constitutive while others are adaptive (Donald et.al., 2013; Chen et.al., 2013). Plant performance is the result of combination of constitutive and adaptive traits. Wheat is responding to environmental conditions by making changes in morphological, physiological and metabolic activities (Thanh et.al., 2013; Baloglu et.al., 2012; Moheb et.al., 2013). The gene action and combining ability help in choosing the parents for hybridization programme (Sapone et.al., 2012; Leigh et.al., 2013). Wheat in all stages of growth is subjected to various abiotic and biotic stress conditions which interfere with its normal growth, development and yield. Among them, Pre-harvest sprouting is a serious problem especially in humid regions (Derera, 2004; Fanner, 2006; Rajaram et.al., 2006).

2.8.3 Weather conditions and pre-harvest sprouting

The pre-harvest sprouting in wheat occurs when rainfall is 10 to 20 mm over a period of 30 minutes, followed by grain moisture reaching to 30%, or higher, within 12 to 24 hours, over two to three days (Kamal et.al., 2009; Bykova et.al., 2011; Oberforster et.al., 2012). The detailed study of interaction between genotypes and environment is necessary to understand the phenomenon of PHS in wheat. The different wheat genotypes respond differently to pre-harvest sprouting and to a given set of weather conditions, making it difficult to predict the sensitivity or tolerance to PHS (Mares, 1993; Miao et.al., 2013). The weather conditions during the year 2000 to 2013 are shown in figure 2.3.

Several researchers have attempted the interaction between genotypes and environment (Kamal et.al., 2009; Oberforster et.al., 2012). They reported conflicting results over how much sprouting is likely to occur in a given set of moisture and temperature conditions during grain-filling and maturation. Mares (1993) found that the level of resistance to pre-harvest sprouting in wheat was closely related to the amount of rainfall in 20 days prior to harvest maturity. Greater the amount of rainfall
during harvesting period, lower will be the level of resistance to PHS. There was no significant relationship between either the maximum or minimum daily temperature during harvesting period and the level of PHST (Masojc and Milczarski, 2009; Simsek et al., 2013).

According to Belderok (1968) average daily temperatures above 12.5ºC during the stage of dough ripeness reduce the dormancy period of mature wheat kernels. This was complicated by the fact that the stage of dough ripeness varied in length from 10 to 23 days in different seasons, depending on the amount of rainfall during the period concerned (Belderok, 1968; Jacobsen et al., 2013). Similarly they found that dormancy could be induced in previously non-dormant wheat grains with a moisture content of 16% or higher, after harvest maturity, when exposed to temperature range of 4-8 ºC, which is known as secondary dormancy (Belderok, 1980; Jacobsen et al., 2013). Some researchers found that diurnal temperature fluctuations, accumulated mean daily temperatures above 12.5ºC and rainfall in the two weeks before, as well as the week after, physiological maturity were correlated with α-amylase activity causing PHS (Kamal et al., 2009; Masojc et al., 2013). Large diurnal temperature differences in the order of 10-16ºC, during the two weeks prior to grain maturation, reduced the possibility of pre-harvest sprouting via reduced α-amylase activity in the mature grain (Penfield et al., 2006; Masojc and Milczarski, 2005).

In contrast, high levels of mean daily temperatures, in the order of 20-25ºC during the same period, caused the mature grain more susceptible to pre-harvest sprouting, but in the seven days after physiological maturity, such levels were negatively correlated with sprouting susceptibility. Rainfall at any time during the period of physiological maturity of grain was associated with increased α-amylase activity and, hence, susceptibility to pre-harvest sprouting was increased (Penfield et al., 2006; Masojc and Milczarski 2009). Environmental effects on grain dormancy in wheat was genotype-specific (Masojc and Milczarski 2009; Masojc and Kosmala 2012).

Kettlewell et al. (1996) proposed that low temperature and rainfall during maturation phase are the important factors in predicting the pre-harvest sprouting which cause germination of grains and decline the quality of grains due to increase in
α-amylase activity, which predispose grains to either germinate more quickly or commence the process of enzymatic degradation of the endosperm. The other method to measure PHS is Falling Number (Oracz et.al.,2009; Qin et al.,2010; Rehman Arif et al.,2012). The dormant wheat cultivar, grown at different location under different climatic conditions, exhibited an exponential relationship between mean daily temperature and the duration of dormancy, suggesting that environmental factors other than temperature are influencing the PHS (Lunn et.al.,2002; Masojc et.al.,2013).

A study conducted in Japan revealed that fluctuating temperatures both during grain development and the period of imbibitions were able to either partially, or totally, break the dormancy of wheat grains, depending on the duration of temperature fluctuation cycle applied. The late-maturity α-amylase activity is controlled by a gene on the long arm of chromosome 7B, but its expression can be controlled by a second gene on chromosome 3B resulting in considerable variation between genotypes (Mrva and Mares, 2001; Qin et.al., 2010; Oracz et.al.,2009).

In the Australian cultivar Spica, grown at Narrabri, NSW, LMA was produced under all environmental conditions during grain maturation, but the level of activity was markedly increased when maturation occurred under predominantly cooler temperatures (10-14°C at night and 18-23°C during the day) (Mrva and Mares, 2001; Oracz et.al., 2009; Qin et.al., 2010; Rehman Arif et.al., 2012). The above research suggests that level of rainfall and temperature, during grain development and physiological maturity affect the susceptibility of wheat to pre-harvest sprouting. However, dormancy is expressed via physiological mechanism, and these two environmental factors probably act differentially on this mechanism in different genotypes. At present, with the exception of environmental influences on LMA, it is unclear that which other factors are acting upon grain development to influence dormancy (Masojc et.al., 2013). Molecular-marker techniques have been used to map quantitative trait loci (QTLs) associated with dormancy (Flintham et.al.,2002). Because of genotype x environment interaction in the expression of dormancy, such studies must be conducted on a range of cultivars, over a number of environmental factors and years, to provide a reliable basis in breeding program for a particular
dormancy level in new cultivars (Oracz et al., 2009; Qin et al., 2010; Masojc et al., 2013).

The high rate of water uptake by kernels and long exposure to moisture/humidity increases the sprouting susceptibility of wheat genotypes. The morphology of inflorescence and seed coat influence the absorption of water. In wheat and barley, imbibitions of water is increased by specific type of awns, waxiness, pubescence, and angle of inflorescence (King and Von Wettstein-Knowles, 2000; Saba, 2013), which control the rate of water absorption, grain hardness, colour, restriction by the seed coat, thickness of the testa, grain size and surface-to-volume relation of the grain (King, 1984; Paulsen and Auld, 2004).

The sensitivity of embryo to ABA is also the primary cause for pre-harvest sprouting susceptibility (Gubler et al., 2008). The absence of sensitivity to ABA during seed development results in the isolation of viviparous mutants that are deficient in ABA (Koornneef et al., 2002). The sensitivity to ABA during seed development can also be modulated by the environment (Garello and LePage-Degivry, 1999; Saba et al., 2013). The endogenous and exogenous ABA have different modes of action, preventing sprouting or germination (Chono et al., 2006). Temperature during seed development is main factor controlling grain dormancy of a particular genotype. Positive role of temperature and seed dormancy in DHS is well established (Biddulph et al., 2005).

Many researchers reported that low temperature affect ABA content and sensitivity, that in turn influence the degree of dormancy during seed development and grain maturity.

Pre-harvest sprouting is decided by the interaction between genotype and environment (Xiao et al., 2004; Biddulph et al., 2005; Munkvold et al., 2013). The different wheat genotypes respond differently to a given set of weather conditions and hence it is difficult to predict the pre-harvest sprouting in particular wheat cultivar (Lillemo et al., 2005; Farrell and Kettlewell, 2008; Lan et al., 2012). The different environmental variables that affect germination, either individually or in combination are light, temperature, rainfall, humidity, and nutrition (Mori et al., 2010; Lobell et al., 2011; Semenov and Shewry, 2011). The seed dormancy is light dependent.
and hence it is one of the environmental factors which promote germination (Bewley and Black, 1994; Lopes et al., 2010). It involves the presence of far-red phytochrome in the seed coat (Humphreys and Noll, 2002; Barrero et al., 2009). Temperature is one of the key factors that plays an important role in the induction of seed dormancy during grain development and adds in expression of dormancy after maturity (Ogbonnaya et al., 2007; Rasul et al., 2005). The high as well as low temperatures increase rate of germination (Matus-Cadiz and Hucl, 2003; McCaig et al., 2006). Low temperature during grain development induce dormancy but during germination it breaks the dormancy (Fox et al., 2003; Brule-Babel et al., 2009). While high temperatures during grain development hastens grain growth but decreases the dormancy induction (Bahin et al., 2011). If high temperatures are followed by low temperatures germination is promoted (Robertson et al., 2003). Low temperatures and rainfall with high humidity at grain maturation stage induce pre-harvest sprouting (Gubler et al., 2008; Footitt et al., 2011).

Grain nutrition plays a key role in sprouting and presence of high grain protein favors germination. The resistance to pre-harvest sprouting depends on factors influencing water uptake and drying rate of the grain and its dormancy as well as remobilization of nutrients to support germination. These factors are controlled by a number of genes that powerfully interact with environmental factors (Morris et al., 1991; Millar et al., 2006 a; Mendiondo et al., 2010). Environmental factors both before and after seed maturity influences the rate of PHS. The dry conditions usually produce seed with lower sprouting tendency and cooler temperatures just previous to maturity also affect on seed with higher dormancy and less sprouting. The cool and humid conditions after maturity lead to increased PHS (Millar et al., 2006 b; Mendiondo et al., 2010; Munkvold et al., 2013). Quantitative phenotypic traits are influenced by genetic and environmental variables as well as the interaction between the two (Annelie and Marie, 2008; Gubpta, 2009; Yang and Ham, 2011).

2.9 Worldwide occurrence of pre-harvest sprouting

Pre-harvest sprouting is a widespread phenomenon which affects the production of cereal grains in many parts of the world. The cereal growers throughout the world suffer from significant losses, which are threatening the viability of
agriculture industry. The effect of pre-harvest sprouting on cereal crops, i.e., wheat, barley, rye and triticale is common. (Derera, 1989; Derera, 1990; Table 2.3; 2.4). Pre-harvest sprouting affects many wheat producing regions of the world including Canada, Australia, South Africa, USA, Central Asia and Europe as well as northern Iran. Since long time, very little information has been published regarding the occurrence and economic impact of PHS. In China PHS is very common in North Eastern and Northern part where spring wheat and winter wheat cultivated (Xiao et al., 2002). In the Western Australian wheat belt, PHS occurs once in a four years time (Biddulph et al., 2007). In year 2004 to 2005 farmers in Australia lost about 22% of their grain income due to sprouting (Biddulph et al., 2007). In Canada, during 1978-1988 damage due to PHS was about $100 million, the PHS was induced by elevated $\alpha$-amylase activity and unacceptable Falling Number values (Derera, 1990; DePauw et al., 2012; Clarke et al., 2005).

The incidence and economic losses due to PHS in wheat, rye and other cereals in different countries during the period of 1978-1988 are given in Table 2.3. The table indicate that PHS is very common in Japan, Poland, United Kingdom, Brazil and Canada, while the economic losses were up to 100 $ million. Table 2.4 explains the production of wheat, total value and losses due to PHS in spring and winter wheat for different countries.

2.10 Pre-harvest sprouting of wheat in Iran:

Damages due to pre-harvest sprouting in cereals in moist zones are relatively common and occur three or four times per 10 years of span. In Iran, pre-harvest sprouting occurs mostly in northern regions during harvest season, because of rainfall and high humidity during that period. e.g. raining in May and June during 2003-2012 caused pre-harvest sprouting in more than 11092 to 22000 ha of wheat farms in Iran due to late planting, use of late maturity and non PHS tolerant cultivars. The different weather conditions in different zones of Iran had different types and levels of PHS. The average losses per hectare as well as percent loss due to PHS in Neka and Behshar areas for different wheat varieties such as Morvarid, N-80-19, Milan and Tajan are shown in Table 2.5 and Fig 2.4 a, b & c. The average rainfall, humidity and
temperature favourable to induce PHS in wheat at Mazandaran province of Iran is given in table 2.6.

### 2.11 Pre-harvest sprouting and seed coat color

The seed coat color plays important role in seed dormancy and germination (Su et al., 2011). The phenolic compounds, particularly flavonoids, present in seed coat contribute to the seed dormancy and inhibit seed germination (Debeaujon et al., 2007). Genotypes with white seed-coat are commonly more susceptible to PHS than those with red seed coats, which are controlled by three independent genes (Bassoì and Flintham, 2005). The genes that control PHS are associated with or close by on the chromosome (Bassoì and Flintham, 2005). Red seed coat color is dominant over white and it is additive, meaning that wheat genotypes with three red seed coat genes produce the darkest red seed coat colour (Etezadi et al., 2005). Usually, dormancy is superior in these genotypes with higher number of red seed-coat genes (Himi and Noda, 2005; Rajender et al., 2010; Yang and Ham, 2011). The variation in seed coat colour among different wheat lies is shown in figure 2.5.

### 2.12 Effect of PHS on end-product quality

PHS causes loss in grain and end product quality due to degradation of native starch granules, which negatively affect the quality of breads, cookies and noodles. Wheat flour from sprouted grains exhibits a lower swelling power and gelatinize at a lower temperature (Afshari and Yazdi Samadi, 1995; Corcuera et al., 2007). Breads baked from hard wheat are affected more than other wheat products by PHS. Bread production is complicated by increased stickiness of the dough, which necessitates special handling in small bakeries and can disrupt operations of large bakeries. Even minor sprout damage can cause significant reductions in gluten strength of wheat flour making it unsuitable for bread making (Noda et al., 2004; Etezadi et al., 2005).

Bread is one of the oldest and most popular diet all over the world including Iran, where the consumers demand for high quality fresh bread (Koocheki et al., 2009; Fathi et al., 2009; Azizi and Rao, 2005; Mahdi Karimi et al., 2012). However it has very short shelf life (Moayedallaie et al., 2010; Chin et al., 2009). Therefore, application of some additives and process modifications are necessary to overcome
this limitation and improve the quality parameters of bread such as sensory and rheological features (Qiao et.al.,2007; Mahdi Karimi et.al.,2012). The main mechanism by which the emulsifiers delay the firming or retrogradating of crumb is based on their capability to form addition complexes with amylase, part of starch during the baking process (Najafi et.al.,2012; Peighambardoust et.al., 2013; Rohani et.al.,2012; Khoshgozaran-Abras et.al.,2012; Shockravi et.al.,2012; Ghasemi et.al.,2013; Hejri-Zarifi et.al.,2013).

PHS affects both the processing and quality of different kinds of noodles. High α-amylase activity in dry noodles weakens the dough so that noodles can not support their own weight and break during the dehydration process (Mares et.al.,2009). As noodle appearance is the first critical judgement made by consumers when evaluating noodle quality, any change to noodle colour, brightness or appearance of undesirable discolorations (spots) will render the noodles less attractive. The noodles made from severely sprouted wheat flour may show up to a five-fold higher number of spots as compared to products made from sound flour (Hatcher and Symons, 2000). Sprout damage also affect the products made from soft white wheat. A reduced thickening power of sprout damaged soft wheat flour results in poor cake baking quality, resulting in cakes with low volume and a deep in the centre (Hatcher, 2011; DeLaethauwer et.al.2013).

2.13 Influence of PGRS on pre-harvest sprouting

PGRS like, GA3, ABA and IAA, influence the tolerance of wheat varieties to PHS as they can induce delay in seed germination and dormancy (Weidner et.al.,2002; Nambara and Marion-Poll 2005; Cai and Shi, 2008; Thiel et.al.,2008). GA3 is involved in promoting the seed sprouting and its levels are usually high during embryo development, which is the first sign of its accumulation. During seed maturity the most active GA3 becomes inactive (Yamaguchi et.al.,2007). The GA3 mutants failed to germinate forming physically abnormal seeds ( Steber et.al.,2006; Gutierrez-Alamo et.al.,2008). The level of GA3 has been identified as a key determinant of seed germination, which promotes the embryo development by breaking the limitation of glume tenacity (Gerjets et.al.,2010). It can break seed dormancy by counterbalancing endogenous inhibitors and promote seed germination as well as induce the α-amylase activity hydrolyzing starch in endosperm, inducing
seed germination through regulating the expression of α-amylase related genes (Gashi et.al., 2012). It has been confirmed that there is completely opposite relationship between the expression of GA and ABA. Various mutants have been used to analyze the regulation process of GA3, ABA and IAA in seed dormancy and germination. Many genes have been identified in regulating the expression of GA3, ABA and IAA, which have been found to participate in regulation of seed dormancy and germination (Kondhare et.al., 2011).

ABA is one of the most important hormones regulating the development of plants and regulate seed sprouting (Xia et.al., 2000; Nambara et.al., 2010). Its level can increase quickly by 2-5 fold in dormant seeds (Gao et.al., 2012). The expression of ABA-response genes represents a long period delay in the hydrated dormant seeds (Piekoszewska et.al., 2008). Some dwarf genes participate in regulating seed dormancy and germination in GA3 insensitive seeds (Curaba et.al., 2004; Barrero et.al., 2010; Monke et.al., 2004). ABA mainly regulates the germination pathways but not responsible for the loss of seed dormancy (Jacobsen et.al., 2002). However, the level of ABA was significantly different in PHS resistant varieties as compared to sensitive one (Gerjets et.al., 2010; Masojc and Kosmala, 2012).

2.14 Influence of carbohydrates on PHS

The accumulation, translocation and distribution of carbohydrates in wheat grain has great effect on harvest index (HI) and grain yield (Kumar et.al., 2011; Zhang et.al., 2013). During the vegetative and early reproductive phases of cereal development, assimilated carbon is temporarily stored as carbohydrate in vegetative sink tissues such as stem and leaf sheaths. The temporary carbohydrate reserves are subsequently remobilized for transport to reproductive sink tissue for filling grain, during later stages of the plant development. In the temperate cereals like wheat and barley, water soluble carbohydrates, consisting principally of fructan and sucrose, are the major form of storage carbohydrates in stem. In tropical cereals species like rice there is lack of fructan accumulating enzymes and carbohydrates accumulate in stems as insoluble starch grains (Ji et al., 2007; Zhang et.al., 2011). In wheat it has been reported that pre-anthesis reserves contribute between 8 - 27 % of the carbon in carbohydrates and 30 to 47 % of the carbon in proteins of the grain. Fructan is the
main form of carbohydrate stored in wheat stems and trace amount of starch (Arduini et al., 2006; Xue et al., 2006; Aller et al., 2011).

Recently, transcripts for granule-bound starch synthases have been reported to be expressed in wheat stem (Peng et al., 2001). Grain filling in wheat depends on two major sources of carbon, namely, current photosynthesis in leaves and mobilization of stored water-soluble carbohydrates (WSCs) from the stem internodes into the growing grains (Ehdaie et al., 2006). Stem WSCs in wheat consist mainly of fructans, followed by sucrose and usually lesser amounts of glucose and fructose (Virgona and Barlow, 1991; Hiltbrunner et al., 2007; Oszvald et al., 2014). In contrast to other cereals, such as maize and rice, wheat stores very little starch in the stems. The contribution of remobilized stem WSC reserves to grain filling becomes more important when current photosynthesis is reduced by partial defoliation (Guitman et al., 1991; Borra et al., 2004; Maydup et al., 2010). Wheat is the main cereal crop in Iran and in many other regions with a Mediterranean climate, which have mild wet winters (when the wheat is sown) and hot and high humidity in summer (Mishra and Dubey, 2013; Masoud and Nesa, 2013).

Despite the high irradiance, photosynthesis during the critical grain filling stage is often limited by water availability as conditions become hotter and drier, which could lead to a substantial reduction in grain yield. However, flag leaf is the most important photo assimilate supplier for growing grains (Zhang et al., 2006) and it is interesting to evaluate changes in grain weight of different wheat cultivars when just the flag leaf is attached on the stem. Carbohydrate is stored in the wheat seed mainly in the form of fructans, which are water soluble polymers of fructose synthesized from sucrose by fructosyltransferases (Lunn, 2008; Fu et al., 2014).

The nitrogen availability strongly affects carbon metabolism in wheat. Its deficiency cause accumulation of carbon in plants, usually in the form of starch in leaves and of wheat barley (Semenov et al., 2011; Hiltbrunner et al., 2007; Hu et al., 2012). N-deficient tissues have been shown to accumulate high amounts of fructan and other soluble carbohydrates (Wang et al., 2000; He et al., 2012; Drecceer et al., 2014). The effects of long-term N limitation on wheat have been examined
recently (Ruuska et al., 2008), it was shown that high amounts of WSC already accumulated in most vegetative tissues by the time of anthesis, i.e. prior to the grain-filling phase (Ruuska et al., 2008; Awole et al., 2011). The main carbohydrate transported in higher plants is sucrose. Wheat grains possess a furrow running along the length of the kernel with a vascular bundle embedded at the bottom. Nutrient unloading occurs along the length of the bundle and has to pass through three distinct layers before reaching the inside of the grain (Dreccer et al., 2014).

The pathway of starch synthesis in non-photosynthetic storage tissue involves the conversion of sucrose into ADPglucose (ADPG) and the subsequent conversion of this soluble precursor into insoluble polyglucan. The enzymes involved in the synthesis of starch from ADPG are the starch synthases, branching enzymes and debranching enzymes are located exclusively within plastids (Smith, 1999; Smith et al., 2005; Dreccer et al., 2014). Sucrose synthase (SuSy) is generally considered to catalyse the first step in the conversion of sucrose to starch in the endosperm of the grain. In wheat grains, SuSy activity is primarily associated with the endosperm, with the highest activities occurring during periods of peak starch synthesis (Dale and Housley, 1986). SuSy exists in both cytosolic and membrane-bound forms. Activity of the cytosolic form is correlated with the production of storage products, such as starch, whereas the membrane-bound form is believed to be involved in cellulose and callose synthesis (Amor et al., 1995; De Bruin and Pedersen, 2008; Ji et al., 2007; Zhang et al., 2011).

2.15 Influence of starch hydrolysis on PHS

Starch is composed of two separate glucan polymers; amylose and amylopectin. The hydrolytic enzymes become active during starch breakdown and α-amylases plays a main role in reducing native starch. Up to 70% of recently synthesized and hidden enzymes in the aleurone layer are α-amylases (Ritchie et al., 2000; Adom et al., 2005). The action of α-amylase, β-amylase, disbranching enzyme and α-glucosidase completely hydrolyze the starch (Anson et al., 2011; Garopalo et al., 2011). The α-amylase an endo amylase are involved in the first step of starch hydrolysis which cleavage the α, 1-4 glucosidic linkages of amylose and amylopectin chains (Howard et al., 2012).
β-amylase and exoamylase, exclusively cleaves α, 1-4 glycosidic bonds at the non-reducing ends of starch-glucan polymers. β-amylase accumulates during grain development in two forms, soluble and bound (Forsyth and Koebner 1992; Hemery et al., 2011). A great section of β-amylase in starchy endosperm is in the bound form, where the enzyme forms disulphide linkages to protein such as glutenins. Bound β-amylase is non-moving and deposit on the margin of the starch granules during grain expansion and is probably synthesized as a mature protein. The enzyme is a component of the protein matrix which covers the starch and might protect starch from premature attack by α-amylase (Zhang et al., 2005; Rose et al., 2008; Lv et al., 2012).

The third group of starch-transforming enzymes is the debranching enzymes represented by isoamylases and pullulanases that exclusively hydrolyze α, 1-6 glycosidic bonds. This suggests that the endosperm communicate directly with the embryo and the aleurone respectively, to accelerate germination and the accumulation of α-amylase activity (Van der Maarel et al., 2002; Haub et al., 2010; Fassler et al., 2006; Cai and Shi, 2010).

The enzyme alpha-amylase is synthesized in the aleurone layer and scutellum and released in the endosperm to decompose the starch into sugars available for germination (Wong et al., 2002; Brewer et al., 2012). The α-glucosidase catalyzes the hydrolysis of α-1,4 glucan bonds at the non-reducing ends of dextrins and maltose to produce glucose (Sharma and Yadav, 2008; Yamamori and Yamamori, 2011). Starch and maltose hydrolysis in germinating wheat seeds is compact in the presence of the α-glucosidase inhibitor which causes a decrease in glucose levels and inhibits plant growth (Robertson et al., 2003; Al-Tamimi et al., 2010; Howard et al., 2012).

2.16 Influence of α-amylase activity on PHS

The α-amylase widely exists and participates in many physiological processes in plants, which can hydrolyze the α-1,4-glycosidic bond in the saccharine. The expression of α-amylase is involved in plant metabolism and can affect the germination rate, cold tolerance and production of seed (Masoje and Mileczarski, 2009; Mrva et al., 2009; Every et al., 2009). The relationship between α-amylase activity and PHS resistance was deemed to be very remarkable (Wu et al., 2002). This
may be due to activity of α-amylase that would increase quickly once absorbed enough water and then promoted the seed sprouting. The activity of α-amylase was also found to have a significant difference between the resistant and sensitive varieties to PHS in wheat (Yang et al., 2010). Three isozymes of α-amylase in wheat have been identified affecting PHS, namely malt-α-amylase (α-amylase-1) located on homologous chromosomes 6, green-α-amylase (α-amylase-2) located on homologous chromosome 7 and α-amylase-3. The expression level of α-amylase-1 and α-amylase-2 is regulated by GA3 (Appleford et al., 2006; Gale, 1983; Mares and Mrva, 2008).

The activity of α-amylase-1 is correlated with the degree of seed dormancy, which accounted for 84% of seed germination (Wolbang et al., 2007). Besides the variation of α-amylase, the α-amylase/subtilisin inhibitors (ASI) in wheat, barley, rice and rye were identified via restraining the activity of α-amylase to restrain the seed germination (Masojc and Milczarski, 2005). The activity of α-amylase could be reduced by combining complex of ASI and α-amylase-1 to increase the variety’s tolerance to PHS (Yuan et al., 2005; Appleford et al., 2006). However, the mechanism of α-amylase regulating varieties tolerance to PHS still needs to be discussed, since the activity and quantity of α-amylase certainly increased after seed sprouting and very low in the dormant seed (Navabi et al., 2009; Masojc and Milczarski, 2009; Masojc et al., 2011).

2.17 Influence of phenols on PHS

A positive correlation between length of dormancy and the content of phenolic compounds was found for developing and ripening of barley caryopses (Weidner et al. 1993). In wheat, rye and triticales the genotypes having very high dormancy usually containing high phenolic acids in the form of soluble esters. Phenolics are of two types namely simple phenols and polyphenols (Anson et al., 2011). The inverse correlation between the contents of free phenolic acids in developing embryos and intensity of precocious germination indicate their role in preventing pre-harvest sprouting in cereals. The genotypes of wheat like Elena and Alba showing very high and medium dormancy contain high level of phenolic acids. (Weidner et al. 1999; Khoddami et al., 2013).
The phenolic acids in the form of esters most probably govern dormancy, which is progressively lost by the process of after-ripening (Weidner et al., 1993; Szopa et al., 2013). A high level of phenolic acids liberated from soluble glycosides also correlates with dormancy in wheat. However, the differences in the concentration of free phenolic acids are less marked for cultivars with different levels of dormancy (Weidner et al. 1999; Weidner et al., 2001).

The role of phenolic compounds have been attributed to their antioxidant activity (Ekiert et al., 2009; Amarowicz and Weidner, 2001). Inverse correlation between the contents of free phenolic acids in developing embryos and intensity of precocious germination indicate the role of phenolic acids in preventing pre-harvest sprouting of cereals. The role of phenolics in preventing pre-harvest sprouting and acclimation to dehydration in cereals is important in PHS (Weidner et al., 2002; Chapleure et al., 2011).

2.18 Influence of Proline on PHS

Proline is a multifunctional amino acid, accumulating during stress conditions and acts as a compatible osmolyte / antioxidant, helping the plants in stress adaptation, recovery and signaling as well as modulating responses to abiotic stress like PHS (Szabados and Saroure, 2009), Kishor et al., (2005) also reported significant accumulation of proline under stress conditions due to increased synthesis. The stress tolerant genotypes were showing less PHS due to increased proline content. Verbruggen and Hermans (2008); Xiao et al., (2012); Kostal et al., (2011) noted that stress conditions induce proline accumulation in a wide range plants. Proline accumulation is believed to be very important as part of the physiological adaptation of plants to stress like PHS. The level of proline that accumulates in plants in response to stress varies greatly and is highly dependent on the plant species.

2.19 Effect pre-harvest sprouting on the economics of wheat cultivation

The pre-harvest sprouting cause severe economic losses to producers as it reduces yield at harvest, because sprouted grains are light and can be blown out of the harvester with chaff, which lead to heavy yield losses (Hogget al., 2005; DePauw et al., 2012). The packing density of sprouted grain is often lower than that of sound
grains, resulting in a lower test weight when delivered to the silo, which entails a monetary penalty to the producers (Trethowan, 1995; Hu et al., 2010). In most wheat-producing countries, grains delivered to silo are tested for sprouting damage and depending on their level, it will be given an appropriate grading. Sprouted grains in Australia are given general purpose (GP) grade or a feed grade, the latter incurring the greater financial penalty.

In Australia, the average yearly loss of income to wheat growers, caused by pre-harvest sprouting, was conservatively estimated as $18 million in 1980 (Derera, 1980; Gerjets et al., 2010). Many researchers like McMaster (1987); Chen et al., (2008) estimated average yearly losses to Australian wheat growers between 25 to 47 million A$ depending on the severity of downgrading (Clarke et al., 2005; DePauw and Clarke 1976; DePauw et al., 2006; Anson et al., 2011; DePauw et al., 2009). The average yearly losses to wheat producers worldwide, due to pre-harvest sprouting and subsequent downgrading of their crops, were estimated to be in excess of US $500 million (Derera, 1990; DePauw et al., 2009).
Review of Literature