OPPORTUNITIES IN TAJIKISTAN TO BREED WHEAT VARIETIES RESISTANT TO SEED-BORNE DISEASES AND IMPROVED BAKING QUALITY

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Summary

Wheat seed-borne diseases and options for improving baking quality of wheat, as well as the role of genotypes for breeding to achieve high yield and quality are the key issues discussed in this introductory paper. The importance of wheat for Tajikistan and how to achieve food security goals in the country is also elucidated. Wheat seed-borne diseases are caused mostly by fungi. Loose Smut (*Ustilago tritici*), Common Bunt (*Tilletia laevis* and *T.caries*), Karnal Bunt (*T.indica*), Dwarf Bunt (*T.controversa*) and Black point (*Alternaria* spp., *Bipolaris sorokiniana* etc.) are all seed-borne diseases that are economically and regulatory important for Tajikistan, and these are therefore discussed in detail. The peculiarities of Tajik bread and requirements to the grain quality are also highlighted.

Preface

The use of high quality seed of a desired variety is known to be important to obtain high yield and quality. Wheat is the main crop for making bread and is also considered as being the main source of protein for mankind, thereby including wheat among the major staple crops. Depending on the end-use product that the wheat is aimed for, quality requests differ. High quality gluten with strong and elastic properties of the wheat flour are the main features for production of Tajik bread. Wheat is attacked by a number of diseases, and when attacked, the result is reduced yield and quality. A number of the diseases are transmitted to new areas and to the new generation mainly by seed. Besides being a reason for diseases dissemination, seed-borne diseases are also affecting the grain quality. There are several factors playing a role for the improvement of quality, one of the main is the choice of variety. Some varieties have high resistance to biotic and abiotic stresses and also better quality potentials. Wheat breeding has until present days led to the development of superior varieties in terms of productivity and quality. Thus some of the challenges facing wheat research and production today are: increasing population of the world, climate change, moving agriculture towards using less pesticides, demand for the quality from end-users. The mentioned goals for research and production cannot be achieved without development of new varieties.

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Introduction

Wheat is one of the most important staple food crops in the world. It is grown on more acreage than any other crop (Curtis, 2002). In 2010, 653.7 mln tons of wheat was produced in the world, while for two of the other main staple food crops, maize and rice (paddy), 840.3 mln and 696.3 mln tons were produced, respectively (FAOSTAT). China and India were the two top producers of wheat in 2010, sharing 30% of the whole world production of wheat grain. The USA and France were the two top exporters of wheat grain in 2010, while Egypt and Italy were the two top countries importing highest quantity of the wheat grain (FAOSTAT).

In Tajikistan, as in the other Central Asian countries, wheat is the main staple food crop (Eshonova *et al.*, 2003; Robinson *et al.*, 2009). Tajikistan is not self-sufficient on wheat and, thus depends on import. In order to achieve self-sufficiency, the current volume of wheat production needs to be doubled mainly by increasing the productivity of the lands (Rahmatov *et al.*, 2010). Simultaneously with the increasing demand of quantity, also the quality of the local wheat needs to be improved (Madaminov, 2004).

The wheat grain is mainly used for making bread and depending on the type of bread, quality parameters may differ (Peña, 2002). Genotype and environment are two major determinants of the quality parameters and, thus improving bread making quality of wheat is among the major goals of many wheat breeding programs (Johansson *et al.*, 2005).

Wheat production is affected by many biotic and abiotic factors, where diseases in severe years can be a reason of significant reduction of the crop yield. Depending on the region and the climatic conditions, the wheat plants may be harmed by a large number of diseases, but only less than 20 of them are globally important (McIntosh, 1998). Among the wheat diseases, wheat seed-borne diseases are important due to reduction of yield and negative influence on the seed and grain quality (Watkins & Prentice, 1997). Breeding varieties resistant to the diseases is the most effective and environmentally friendly way of controlling them (Mathur & Cunfer, 1993; Wilcoxson & Saari, 1996).

1. Wheat

1.1. History and trend of wheat production in the world

1.1.1. Wheat domestication and origin

Wheat belongs to the *Poacea (Graminea)* family of the *Triticea* tribe (Dewey, 1984), like many other cereals. For more than 10,000 years, wheat has been cultivated in southwestern Asia in the current territories of Syria, Iraq, Eastern Turkey and neighboring countries, where the crops was domesticated. Wild species related to wheat are still grown in these countries (Sleper & Poehlman, 2006). Bread wheat (*Triticum aestivum* L.), which is the most widely grown wheat species is hexaploid ($2n = 6x = 42$, AABBDD) and its share is about 95% of total wheat production. Another type of commonly grown wheat is Durum wheat (*Triticum durum* L.) ($2n = 4x = 28$, AABB), which is a tetraploid. Share of durum wheat on overall wheat production is about 5% (Shewry, 2009; Peng *et al.*, 2011). Spelt wheat (*Triticum spelta* L.) another hexaploid type of wheat is grown in limited areas nowadays, similarly as einkorn and emmer wheat (Shewry, 2009). The history of the current hexaploid wheat goes back to 30,000 to 50,000 years before present, where the first *Triticum urartu* (donor of the A genome) was by chance crossed naturally with *Aegilops speltoides* (donor of the B genome) resulting in the occurrence of tetraploid *Triticum diccocoides*, holding both the A and the B genomes (NHM, 2011). Bread and durum wheat, occurred 8,500 years ago and the donor of the D genome to the current bread wheat was most probably *Aeigilops tauschii* (Peng *et al.*, 2011).

1.1.2. Wheat in the world

Wheat is grown and traded more than any other crop. The wheat crop is grown successfully up to 60° N and down at 40° S (Curtis, 2002). According to FAOSTAT, wheat was grown on 217.2 mln hectares around the world in 2010, with average yield of 3.0 t from each ha. The five main wheat producing countries, China, India, USA, Russia and France, are sharing more than 50% of the world production (Table 1).

Table 1. Top 10 wheat producers of the world in 2010

Rank	Country	Production, MT	Per cent of world production, %
1	China	115,181,303	17.7
2	India	80,800,000	12.4
3	United States of America	60,062,400	9.2
4	Russian Federation	41,507,600	6.3
5	France	40,787,000	6.2
6	Germany	24,106,700	3.7
7	Pakistan	23,310,800	3.6
8	Canada	23,166,800	3.5
9	Australia	22,138,000	3.4
10	Turkey	19,660,000	3.0
	Total, world	**653,654,525**	**100.0**

Source: www.faostat.fao.org (acc. 12/12/2012)

1.1.3. Use of wheat

The wheat grain is used for many purposes, although most of it is used as human food. The wheat is the main type of food for nearly 40% of all human being (FAOSTAT). The main component with nutritional value in the wheat grain is protein, and thereby the contribution of wheat with calories and protein within the world's diet is higher compared to any other food crop (Hanson *et al.*, 1982). Wheat flour is used for production of various types of bread, pasta and candy. Lately, use of wheat for ethanol production has also increased in the world and also within this use wheat has a great potential (Kim & Dale, 2004).

Beside the use of wheat grain for food purposes, the grain and other parts of wheat are used for many other purposes. Grain and straw can be used as feed for animals and poultry (Faridi *et al.*, 1989; OSUE, 2012), for making paper, textiles (Hoseney, 1994; Marx *et al.*, 2000), for preparation of fertilizers (Xie *et al.*, 2011), biofuel and bio plastics (Cuq *et al.*, 1998), building purposes, souvenirs etc.

1.1.4. Constraints to wheat production

Diseases, insects, draught and frost are the main constrains to wheat production (Rajaram & van Ginkel, 1996; Miller & Pike, 2002). Higher requirements for the quality of the wheat seed is not only important as related to presence and spreading of seed-borne diseases, but it is also important for prevention of spreading of weed seeds, especially quarantine and noxious species of weeds, as well as pests eggs (Diekmann, 1993; Van Gastel *et al.*, 2002).

1.2. Wheat in Tajikistan

1.2.1. History and trends of wheat cultivation

Farming has been the main source of income for the Tajik people for thousands of years. Ghafurov (1979) has described in his book "Tajiks", that mostly wheat and barley were cultivated in the current territories of Central Asia during the Neolithic age (9-7 thousand years ago). Udachin and Shakhmedov (1984) also suggested to consider Neolithic age as a start of cultivation of wheat in the region. Thus, the wheat cultivation and use in Tajikistan is dated back to old historical time. Wheat was and still is the main and staple food of the people (Ghafurov, 1979). At the beginning, *T.compactum* was widely grown and *T.aestivum* became the main wheat species for the entire region starting from 500 years BC. Beside the aforementioned species for the later periods *T.turgidum* and *T.durum* were also found (Udachin & Shakhmedov, 1984). The tendency of growing cotton instead of wheat was started during the annexing of the Central Asian region first to Russia and later to USSR. Local needs for wheat products were satisfied by importing from other wheat producing republics (Morgounov, 1992). During the Soviet era small grain cereal crops were mainly grown in rainfed areas, including wheat as the main cereal crop (Litvinov *et al.*, 1964; Udachin & Shakhmedov, 1984). A significant increase of growing area and production of wheat started after Tajikistan gained independence (Rahmatov *et al.*, 2010).

1.2.2. Wheat production and food security issues

In Soviet Tajikistan, as in other republics of USSR, the economy in general and agriculture in particular, was based mainly on planned economy. Only stately based or collectively based farms were active in the sector. Cotton, being a strategic and major crop was given the priority (Udachin & Shakhmedov, 1984). Growing cotton was more profitable and important as compared to wheat both as related to economic and social aspects. Although, wheat did not have a priority role, it was still widely grown and wheat research and breeding was considered important. In Tajikistan wheat, was grown mostly in the rainfed areas and was mostly used as a feed for animals, due to the poor baking quality of the wheat grain. Wheat products were imported mainly from other republics, such as Kazakhstan and Russia. After the collapse of the Soviet Union in 1991, the strong economical, industrial and financial bonds, created during several years between the Soviet republics, were broken. Thereby, Tajikistan faced a lack of wheat and wheat products, leading to an increased need for local production of wheat. People even started to grow wheat in their own kitchen gardens and areas under wheat in irrigated lands started to develop. Overall production started to increase, mostly by increasing the cultivation area, but average yield was and still is low. The country moved towards market economy and new forms of ownership system, such as Private Farms, so called Dehkans (Peasant) Farms started to emerge. The area used for wheat cultivation was 17.5% of the total area used for agricultural crops in Tajikistan in 1991. In 2007 the cultivation share of wheat compared to other crops had increased 2.1 times, resulting in nearly 36% of cultivated lands with wheat (TajStat, 2010).

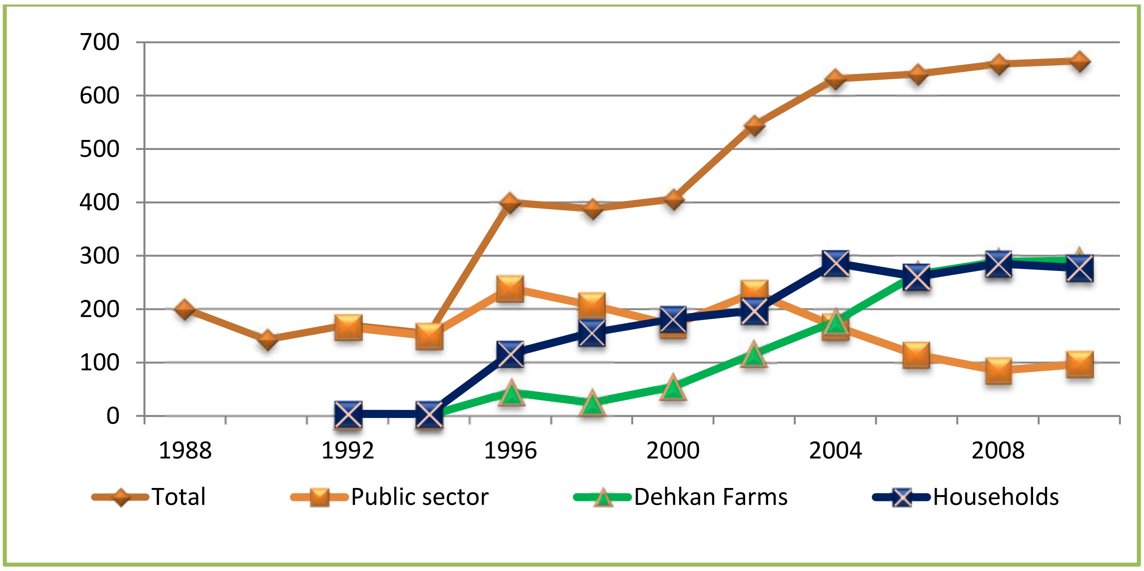

Figure 1. Wheat production in Tajikistan, during the period 1988-2010 (*thousand tons)*

The main increase in wheat grain production in Tajikistan is seen from 1995 and onward (Figure 1). The highest production of wheat ever in Tajikistan was reported for 2009, resulting in 1.1 mln tons, corresponding to 84% of the total amount of cereals (www.stat.tj).

1.2.2.1. Food security and supply in Tajikistan

According to FAO statistics, the wheat consumption per capita in the year 2003 in Tajikistan was 170 kg and the percentage of the wheat products share in the daily calories was 62%. Thus, Tajikistan has the leading position in terms of energy for the population from wheat products. As a comparison, the rate in the other Central Asian countries was: Turkmenistan – 61%, Kyrgyzstan – 58%, Kazakhstan and Uzbekistan – 50%. Similar figures for the European Union and the USA are 21% and 17 %, respectively and totally for the world 20% of the daily energy comes from wheat (FAO-Database, 2003; Çakmak *et al.*, 2004). The figures confirm that wheat is the most important crop in Tajikistan. However to supply the whole population with in-home produced wheat, the annual wheat production has to reach at least 1.5 mln tons. To achieve the goal of being self-sufficient in production of wheat in a mountainous country like Tajikistan, the productivity has to be increased in each piece of land.

To improve the situation for food production and wheat supply, 75 thousand ha of state owned irrigated land were given to the people according to the Presidents order. These lands were mostly planted by wheat allowing for an increase in the wheat-growing area as well as consequently, wheat grain production. To supply the population with grain products is a key issue in the social and economic life in Tajikistan.

1.2.3. Concerns of wheat production and quality

1.2.3.1. Biotic and abiotic stresses

The main wheat pests and diseases in Tajikistan, as well as the main stresses have recently been compiled into one handbook by Pett and Muminjanov (2006), within the GTZ-CIMMYT Project “Regional network for wheat variety promotion and seed production in Central Asia”. Among the most devastating insect pests, Sunn pest (*Eurigaster integriceps*), aphids (*Diuraphis noxia, Schizphis graminum, Rhopalosipum padi)*, leaf beetle (*Oulema melanopa*) and several others were listed. Draught and salinity were mentioned as the main abiotic concerns to wheat production. In case of diseases, mainly fungal caused diseases were reported having significant influence on the wheat production. The following diseases were the main important ones, and these were also observed in many wheat growing parts of Tajikistan:

- Yellow or stripe rust (*Puccinia striiformis,* f.sp. *tritici*);
- Leaf or brown rust (*Puccinia recondita,* f.sp. *tritici*);
- Stem or black rust (*Puccinia graminis,* f.sp.*tritici*);
- Loose smut (*Ustiligo tritici*);
- Common bunt (*Tilletia tritici*, *T. laevis*);
- Tan spot (Yellow leaf spot) (*Drecheslera tritici-repentis* (syn. *Helminthosporium tritici-repentis)* as in not final stage; *Pyrenophora tritici-repentis* as a complete stage);

- Septoria leaf blotch (*Septoria tritici* as in not complete stage, *Mycosphaerella graminis* as a complete stage);
- Septoria leaf and glume blotch (*Septoria nodorum* as in not complete stage; *Stagonospora nodorum* as a complete stage);
- Powdery mildew (*Blumeria graminis* f. sp. *tritici*);
- Helimintosporium leaf blotch (*Bipolaris sorokiniana*, syn. *Helminthosporium sativum*)
- Root rot (*Bipolaris sorokiniana*, *Fusarium* spp.)
- Take-All (*Gaeumannomyces graminis* f.sp. *tritici*, syn. *Ophiobolus graminis*)
- Ergot (*Claviceps purpurea*);
- Black rot (*Alternaria* spp., *Cladosporium* spp., *Stemphylium* spp. *Epicoccum* spp., *Torula* spp. and others)
- Black point (*Fusarium* spp., *Bipolaris sorokiniana*, *Alternaria* spp., *Cladosporium herbarum* and other species).

1.2.3.2. Seed quality and variety

The choice of a high quality seed of wheat variety with the best performance under certain climatic conditions is the most important factor for sustainable wheat grain production (Van Gastel *et al.*, 2002). Therefore it is important that the wheat grain farmers are using desirable varieties, which have high yield potential and are resistant to biotic and abiotic stresses. Also, suitable wheat varieties have to be able to provide grain with good quality (Wrigley & Batey, 2003). Since not all wheat varieties are suitable for growing in every region of the world, breeding programs perform variety testing in different climatic and ecological zones, with the purpose to define the most suitable varieties for certain areas. Potentially high yielding semidwarf wheat and rice varieties in combination with improved fertilizer use, irrigation and pest control were the main ground of green revolution during second half of 20th century (Borlaug, 2007). The role of the variety in general and breeding of new varieties in particular, to meet food security problems were specifically underlined in the Second World Seed Conference in Rome in 2009.

Thus, varieties with high yielding capacity and good baking quality are of highest need (Jabborov, 2004). Initiatives of some national and international scientific and research centers and organizations (the Farming Institute of Tajik Academy of Agricultural Sciences, International Wheat and Maize Improvement Centre (CIMMYT), International Centre for

Agricultural Research in the Dry Areas (ICARDA), Sida founded Project giving support to Seed Sector Development, Care Int., German Agro Action and others), as well as seed producing Farms have promoted recent introduction of several new varieties in Tajikistan (Muminjanov, 2008). Contribution of new varieties to the average yield during last decade was significant.

During the later years Tajik farmers have mainly been using farm saved seeds or not certified seeds (Muminjanov *et al.*, 2008). This leads to low seed and grain quality and increased risk of distribution of seed-borne diseases of wheat such as common bunt (*Tilletia tritici*), loose smut (*Ustilago sp.*) and black point (Husenov *et al.*, 2008).

1.2.4. Tajik bread

Traditionally bread is the most important food of the Tajiks and the people show a high respect to the bread. The daily consumed bread in Tajikistan is mainly produced at home. It is generally called "*non*" in the Tajik language and is also famous as "*tandyr*" bread (Peña *et al.*, 2003). Further the Tajik people are using other types of bread in their daily life, prepared with different ingredients and being different in size and shape; eg. *Kulcha* (with milk and butter), *fatir* (with layers and butter in between), *katlama* (with layers and butter in between prepared on fried oil), *chappoti* (flat thin bread). Bread is also prepared with different types of vegetables and with meat and fats, such as lamb fat, onion, pumpkin etc. (Mack & Surina, 2005). Some types of bread is also named from localities, like "*noni Khujandi*" (Khujand bread), "*noni Samarqandi*" (Samarqand bread), or by masters "*noni Sharifjon*" (Sharifjon bread) etc.

1.2.5. Wheat breeding in Tajikistan

1.2.5.1. History of wheat breading

As wheat has been grown in Tajikistan for thousands of years, traditional ways of selection and improvement of the plant material has been preferred. Primitive varieties or landraces were famous such as Surkhaki, Safedaki, Irodi, Bobilo, etc. (Eshonova *et al.*, 2003). Officially wheat breeding started in 1932. Selecting and purifying local landraces as well as selections from the breeding materials of the All-Union Institute of Crop Management (VIR), were the first activities undertaken (Eshonova *et al.*, 2005). The first varieties released from the selection programs were: Surkhak-194, Surkhak-1288, Surkhak-688, Iroda-1006, Khoronko-46 (Eshonova *et al.*, 2005). Tajik Research Institute of Crop Management under the Tajik Academy of Agricultural Sciences was dealing with breeding of facultative wheat with the main aims towards high yield and adaptation to the climatic conditions during the 1960-70th (Dorofeev *et al.*, 1987). In 1999, collaboration with the International Wheat Improving programs was initiated. The GTZ-CIMMYT Project «Regional network of wheat variety promotion and seed production» has introduced and tested new wheat varieties from different countries. Examples of introductions are different breeding lines from CIMMYT, Steklovidnaya 24 from Kazakhstan and a number of new varieties from the Krasnodar Agricultural Scientific Research Institute (Russia). One of the predominantly grown wheat varieties in the early 2000, which contributed to higher production of wheat was the variety Jagger, bred at Kansas University (USA). The variety was also tested within the GTZ-CIMMYT project and later high amount of seed was imported to Tajikistan by the World Bank (Muminjanov, 2008).

1.2.5.2. Wheat breeding programs and objectives

Currently wheat breeding in Tajikistan is mainly conducted by Public Institutions, such as the Farming Institute of Tajik Academy of Agricultural Sciences, the Tajik Agrarian University and the Plant Physiology and Biotechnology Institute of Tajik Academy of Sciences (Eshonova *et al.*, 2005; Naimov *et al.*, 2005; Muminjanov *et al.*, 2008). Besides public breeding, private or cooperative farms have also recently started to work on wheat breeding in collaboration with local and international partners (Rahmatov *et al.*, 2010). The entire interest of the Government of Tajikistan on increasing the production of wheat grain has led to an increasing collaboration with International Agricultural Research Centers (Pett *et al.*, 2005). Significant results on wheat breeding development and improving seed production system in Tajikistan has been achieved within the collaboration with the CIMMYT and ICARDA (Pett *et al.*, 2005). As the result of this collaboration, Tajik wheat breeders receive different breeding nurseries every year and based on the testing of them in different agro climatic conditions selection of the best lines for further release is done (Rahmatov *et al.*, 2008).

Breeding for yield, resistance to diseases and pests, tolerance to hot and dry conditions (Pett *et al.*, 2005; Rahmatov *et al.*, 2008), combined with early ripeness (Eshonova *et al.*, 2005) are described as the main objectives of the Tajik wheat breeding programs. Lately, also quality of the produced grain has become a priority for the Tajik wheat breeders along with the quantity

(Husenov *et al.*, 2010). The recent increased interest for improving the quality resulted in a number of additional tests on new varieties: evaluation of protein content and composition, assessments of wet gluten content and deformation index as well as sedimentation coefficient and some other quality parameters (Niyazmuhammedova *et al.*, 2004; Sarkisova *et al.*, 2006; Husenov *et al.*, 2010). Also there is a group of scientists working with selection of varieties and lines for adaptation to the climatic conditions (Naimov *et al.*, 2005). Historically most of the grown wheat varieties in Tajikistan were spring type, but it was recommended instead to plant wheat as a winter crop, due to the higher productivity of winter sown wheat (Litvinov *et al.*, 1964). Thus, winter hardiness is also considered in the breeding programs.

1.2.5.3. Wheat varieties in Tajikistan

State commission on variety testing (SCVT) is an official authority responsible for testing and listing, as well as protection of new plant varieties in the territory of Tajikistan. SCVT publishes a "National list of plant varieties and hybrids accepted for use in the territory of Tajikistan" which is updated every year. According to the last version of the list from 2011, there are 12 varieties of Bread wheat (Table 2) and three varieties of Durum wheat listed in Tajikistan.

Table 2. Bread wheat varieties officially released in the territory of Tajikistan

Variety	Released year	Breeder, country	Type
Safedaki mahalli	1939	From local landraces, Tajikistan	Facultative
Siete Cerros-66	1976	CIMMYT, Mexico	Facultative
Navruz	1982	Tajik Farming Institute, Tajikistan	Facultative
Eritrospermum-401	1986	Uzbek Scientific Institute "Zerno", Uzbekistan	Facultative
Steklovidnaya-24	2002	Kazakh Farming Institute, Kazakhstan	Winter
Zafar	2004	"Khurokvori" Corporation, Tajikistan	Facultative
Alex	2007	Tajik Farming Institute, Tajikistan	Facultative
Norman	2007	Tajik Farming Institute, Tajikistan	Facultative
Ormon	2008	Tajik Farming Institute, Tajikistan	Facultative
Somoni	2008	Tajik Farming Institute, Tajikistan	Facultative
Ziroat-70	2009	Tajik Farming Institute, Tajikistan	Facultative
Sadokat	2010	Tajik Farming Institute, Tajikistan	Facultative

Source: National list of plant varieties and hybrids accepted for use in the territory of Tajikistan, 2011 edition.

2. Seed-borne diseases

2.1. Importance, assessment and control of seed-borne diseases

2.1.1. Importance

Seed holds the largest share of plant propagation material moving to international trade and therefore seed is considered one common route for the spreading of destructive plant diseases (Neergaard, 1986). When one or more of the phases of the life cycle of a disease is being connected with the seed, the diseases are defined as "Seed-borne diseases" (Pearce, 1998). Seed-borne diseases in plants are caused by a number of different microorganisms: fungi, bacteria, viruses and nematodes. The diseases are enhanced by many factors such as physiological factors (like nutrient deficiency, environment, phytotoxicity, ageing and congenital disorders), as well as mechanical factors (during the processing and damages by insects) (Naumova, 1970; Pearce, 1998). Thus, seeds of cereals may be contaminated with the spores of e.g. bunt and smut diseases mainly during harvesting or processing and thereby become infected. One infected spike with loose smut or common bunt in a field can infect several healthy spikes and thus cause considerable damage (Naumova, 1970). Seed-borne diseases have significant economic effect, especially in the countries where poor farmers cannot afford using chemical treatment (Pearce, 1998).

2.1.2. Seed-borne diseases assessment

Studies on seed-borne diseases and pathogens are covered by a range of topics within plant pathology and most of it is included in the topic of Seed Pathology (Agarwal & Sinclair, 1997). However, some scientists describe the seed pathology aspect using a broader concept, e.g. Munkvold (2009) formulates Seed Pathology as "the study and management of diseases affecting seed production and utilization, as well as disease management practices applied to seeds". Thus, beside seed-borne diseases, also other diseases affecting seed production are considered by the definition of Munkvold. Furthermore, development and application of seed treatments, standardization of seed health testing methods and phytosanitary regulations for research and production are also included in the definition. The aim of the seed health testing is to evaluate if pathogen are present in the seed, causes of seed-borne diseases as well as estimation of the rate of the infection in the seed lot (Reeves, 1995). There are a number of technics and methods used to identify causal agents (McGee, 1995) and by development of molecular technics, identification and description of the causal agents are improving (Richardson, 1996; Pearce, 1998; Munkvold, 2009). The International Seed Testing Association (ISTA) as an internationally recognized organization providing standardized methods for routine seed quality testing, provides a number of methods of seed health testing (ISTA-SHT). Seed health testing methods applied are depending on the nature of the pathogen and on the plant species evaluated. In general studies on seed-health were started by the establishment of the first seed labs. Paul Neergaard is known as a “father” of seed pathology for his valuable works during the 70th of the last century on standardization of seed health testing methods. The methods developed or compiled by Neergaard are still used in the seed health testing and seed quality control all around the world (Nameth, 1998). There are a number of other organizations and institutions working on the seed health studies and testing aspects at international level, e.g.: International Seed Health Initiative (ISHI), African Seed Health Centre (AfSHC), National Seed Health System of USA (NSHS), Danish Seed Health Centre (DSHC). On national level of many countries seed health testing are mostly carried out either through Plant Quarantine or through Seed Quality Control Services (Asaad, 2010).

During the last decades rapid and sensitive diagnostic methods have been developed quickly and are also applied for identification of seed-borne diseases (Mathur & Cunfer, 1993; Richardson, 1996; Pearce, 1998). Thus, seed-borne diseases detection methods can be subdivided into conventional and advanced methods. Conventional methods include: direct visual examination, sediment suspension examination, staining method, growing-out test, blotter test, agar plate test, selective or semi selective media test, embryo extraction test (Neergaard, 1977; Reeves, 1995; Pearce, 1998; Taylor *et al.*, 2006). Advanced or molecular methods for seed health testing are: Conventional PCR method, Real time PCR, RT-PCR detection using specific Fluorescent probes, Bio PCR, DNA chips (Taylor *et al.*, 2006; Munkvold, 2009).

2.1.3. Control of seed-borne diseases

The best way to achieve a healthy crop, free of seed-borne diseases is to use healthy seed material of resistant varieties. Thus, the most effective measures of controlling the diseases and improving the crop are to breed resistant varieties and to carry out seed health status assessment (Agrios, 2005; Taylor *et al.*, 2006). Chemical treatment of the seed and soil, hot water treatment and application of pesticides have shown good results as protection against seed-borne diseases (Agrios, 2005). Seed treatment is the method that has been suggested as the most effective treatment (Buhariwalla *et al.*, 2012). However, increasing interest for organic farming and for minimized use of chemical treatments in the industrial countries might lead to more eco-friendly and cost-effective solutions of the problem. Similarly, poor farming conditions and lack of funds to use chemicals in many developing countries requests for reduced use of inputs (McNeil *et al.*, 2004; Ciuca & Saulescu, 2008). Breeding for resistance, development of the most effective seed certification schemes, regular control of the crop and routine tests have proven their effectiveness for this purpose (Van Gastel *et al.*, 2002; Goyal *et al.*, 2010). Nowadays, seed-borne diseases of wheat are rather common and important in countries such as Tajikistan, as the results of poor farming and the use of untreated and uncertified seeds (Husenov *et al.*, 2008).

In order to achieve the best quality of seed production, a number of pre harvest controls has to be taken into consideration: using disease resistant variety, disease free or certified seed, isolation of seed from sources of potential infection, good crop rotation, treating seed, rouging of infected plants, applying pesticides (if needed), control of insects vectors, avoiding or reducing some growing practices which can facilitate spread of the diseases, such as overload irrigation. Some additional measures to improve health status of the seed can be applied after harvesting: to separate infected seed (if known) and other inert materials, chemical treatment, hot-water treatment, and surface disinfection (Copeland & McDonald, 2001). In many countries seed-borne diseases are also prevented by seed certification authorities as well as phytosanitary inspections.

2.2. Wheat seed-borne diseases

Among all the diseases of wheat, seed-borne diseases were the first diseases studied scientifically by Tillet in 1755 (Agrios, 2005). There are a number of wheat diseases spread in the world and the most devastating pathogenic forms for wheat are various types of fungi (Zillinsky, 1983). Fungi are as well the main pathogenic organisms causing seed-borne diseases of cereals (Richardson, 1996). Among the fungi caused seed-borne diseases of wheat, smuts and bunts are found in most wheat growing areas (Wilcoxson & Saari, 1996; Matanguihan *et al.*, 2011; Knox & Menzies, 2012). There are five smut and bunt diseases in wheat: loose smut, flag smut, common bunt, Karnal bunt and dwarf bunt (Wilcoxson & Saari, 1996). Other fungi caused seed-borne diseases of wheat are black point or smudge kernel, scab, tan spot and ergot (Mathur & Cunfer, 1993). In the ISTA handbook “An annotated list of seed-borne diseases” edited by Richardson (1978) 32 pathogenic fungi, 6 bacterias, 1 virus and 1 nematode are listed as potential seed-borne pathogens for wheat. Mathur and Cunfer (1993) compiled the wheat seed-borne diseases and seed health testing methods for them in a handbook “Seed-borne diseases and seed health testing of wheat”. In this handbook, 16 diseases caused by fungi, 8 by bacterias, 2 by virus and 1 by nematode are described.

A report from the Seed health testing laboratory of the International Centre for Agricultural Research in the Dry Areas (ICARDA) has identified the most commonly found seed-borne fungi and nematode of wheat and wild relatives of wheat for the region of Central and West Asia and North Africa (Asaad & Abang, 2009), which also includes Tajikistan. The study covered 13 fungal pests causing 14 different diseases (Table 3). In total, 22.65% of the Tajik seed samples were found to be infected and they were affected by *Tilletia caries*/*T. laevis*, *Helminthosporium* spp., *T.indica* and *Fusarium* spp., where *T.indica* was reported for the first time. However, not any nematode was reported to be present in the samples of wheat from Tajikistan.

Table 3. Seed-borne fungi encountered in CWANA region (Asaad & Abang, 2009)

Pathogen	Disease (common name)	Present in Tajik samples
Pyrenophora tritici-repentis (Died.) Drechs.	Tan spot	Yes
Bipolaris sorokiniana (Sacc.) Shoemaker	Spot blotch	No
Fusarium spp.	Head blight, scab, foot and root rot, snow mold	Yes
Septoria tritici Roberge in Desmaz.	Septoria leaf blotch	No
Septoria nodorum (Berk.) Berk. In Berk. & Broome	Septoria glume blotch	No
Tilletia caries (DC.) Tul. & C.Tul. and T.*foetide* (Wallr.) Liro	Common bunt	Yes
Tilletia indica Mitra	Karnal bunt	Yes
Tilletia controversa Kuhn in Rabenh	Dwarf bunt	No
Ustilago tritici (Pers.) Rostr.	Loose smut	Yes
Urocystis agropyri (G.Preuss) Schrot.	Flag smut	No
Bipolaris sorokiniana (Sacc.) Shoemaker, *Fusarium* spp., *Alternaria* spp., *Cladosporium* spp.,	Black point	Yes

According to the studies conducted in Tajikistan, the most wide spread seed-borne diseases of Tajik wheat were: loose smut, common bunt, black point and tan spot (Pett *et al.*, 2006; Pett & Muminjanov, 2006; Husenov *et al.*, 2008), and especially bunt and smuts were often seen in farmers' fields (Pett *et al.*, 2005). Until now, as relating to wheat bunts causes, only *Tilletia laevis* (syn. *T. foetide*) and *T.tritici* (syn. *T.caries*) has been reported from the local studies in Tajikistan (Pett & Muminjanov, 2006). However Asaad and Abang (2009) reported for the first time presence of *Tilletia indica* or Karnal bunt in the wheat samples from Tajikistan (Table 3), which is not confirmed until now by other studies. Karnal bunt is considered as a quarantine pest in Tajikistan, and dwarf bunt is a regulatory important pest. Due to the increasing import and export of wheat seed and grain, studies on the presence of undesirable pathogenic species, including *Tilletia* species are needed in the forms of field surveys and lab tests.

2.2.1. Loose smut

2.2.1.1. Importance

Loose smut (Figure 2) is a cereal disease present around the world (Agrios, 2005). It was known by man already during the historic time. Thus, Roman people recognized the disease and designated it *Ustilago*, meaning *burn* in Latin (Wilcoxson & Saari, 1996). The disease can cause both yield and quality reduction of wheat. Smuts along with the rusts were the main concern of farmers until the 20th century in most of the wheat growing areas (Agrios, 2005). Loose smut is mainly spread by infected seed, it can also be spread by air, but not for long distance (Nielsen & Thomas, 1996). The loose smut disease is internally seed-borne, i.e. the spores remain in the seed embryo, and thereafter in a presence of favourable conditions it affects the new plant from the same infected seed. In Tajikistan, loose smut has been recorded in all main wheat growing areas (Pett *et al.*, 2006). The disease is usually seen at higher elevations, and wet and cool weather is favourable for the disease (Saari *et al.*, 1996).

2.2.1.2. Pathogen

Loose smut is caused by different species of the fungus in each type of the cereals, e.g. wheat, barley and oat. In wheat the disease is caused by *Ustilago tritici* (Persoon) Rostr. (syn. *U. nuda* var. *tritici* Scaff.) (Saari *et al.*, 1996). The fungus is a hetero-basidiomycetous and is the only *Ustilago* species affecting the wheat plant (Nielsen & Thomas, 1996). A number of different races of the pathogen are found in nature (Nielsen, 1987). Different races of *U.tritici* can cause significant losses both in durum and bread wheat, respectively (Randhawa *et al.*, 2010).

Figure 2. Wheat loose smut. **A.** Infected wheat field; **B**. Mass of spores replacing the spike; **C**. Infected embryo, where mycelllium is seen on the surface of scutellum. [Photos by the author]

2.2.1.3. Cycle and symptoms

After germination of the new seedling from the seed affected by loose smut, the mycelium grows and settles close to the apical meristem the culm nodes, and the floral primordia. Before the emergence of the ear, cells of the hyphae turn to new teliospores (Murray *et al.*, 2009). Instead of healthy and intact spike, deformed spike filled with spores covered by a thin membrane appears first in the plant. After only some days, the membrane becomes dry and breaks allowing the spores to be released (Agrios, 2005). The spores are transferred by air and wind and are thereby reaching the floret of neighboring newly merging healthy spikes of wheat and thus new infection occurs (Murray *et al.*, 2009).

The first symptoms of loose smut can be seen after the ear emergence, when infected ears usually emerge earlier than healthy ears and the infected ears have a dark brown color and are also visible through their increased height compared to the healthy plants. The ears of infected plants are usually all covered by a mass of spores, but also partial infection of the ears can be seen.

2.2.1.4. Seed testing methods of loose smut

ISTA has adopted a method based on extracting and testing of the embryo to evaluate seeds for presence of loose smut in barley. This method nowadays is also used routinely in seed health laboratories for the detection of loose smut infection of wheat seed (Figure 2c). Sperlingsson (2011) and other colleagues presented an improved method of Embryo extraction for the detection of *Ustilago* mycellium and now the method is adopted to ISTA Rules as an alternative to the existing method.

2.2.1.5. Control measures

The use of certified seeds, free from the pathogen, is the most effective way of disease control. Other effective ways to control the disease are to use resistant varieties or to treat seeds with fungicides (Agarwal *et al.*, 1993; Murray *et al.*, 2009).

The disease is controlled by systemic fungicides (Wegulo, 2009). Although, the use of chemicals are the principal methods and has a significant output for controlling the disease occurrence, these methods have to be minimized, due to the environmental and health concerns. If the disease occurrence is higher than the thresholds, it is recommended to change the whole seed material (Pett *et al.*, 2005). The use of "certified smut-free" seed has been suggested as the best way of controlling loose smut (Agrios, 2005). Thus, growing resistant varieties and using certified seed are the most effective and environmentally friendly solution.

2.2.1.6. Breeding strategies

Breeding of wheat varieties resistant to loose smut can help to manage the disease, however new varieties have to be tested against the races dominantly spread in the region of where these varieties are to be grown (Nielsen & Thomas, 1996). Resistance genes against loose smut have been reported. Nielsen and Thomas (1996) summarized conclusions from many previous studies on resistant genes to loose smut:

- Genes can be partially or completely resistant and its resistance can be either dominant or recessive;
- Resistance genes can stop the growth of the pathogen at one or more sites, e.g. in the ovary, embryo or seedling;
- Although the embryo is genetically susceptible, the penetration of the fungi can sometimes be blocked by resistant maternal tissue of the ovary.

By crossing parents known as resistant with parents known as susceptible to loose smut, four major and one minor QTL were identified (Knox *et al.*, 2010).

2.2.2. Common bunt

2.2.2.1. Importance

Common bunt (also called stinking bunt, stinking smut) is the most spread type of bunt (Hoffmann, 1982; Asaad & Abang, 2009) and this disease is thereby of significance in most wheat growing areas in the world, primarily on winter wheat (Murray *et al.*, 2009) (Buhariwalla *et al.*, 2012; Gaudet & Menzies, 2012). Presence of Trimethilamine in bunted grain contributes to a bad fishy like smell and has resulted in the names of bunt or “stinking smut” (Laroche *et al.*, 2000; Murray *et al.*, 2009). Bunt was described by Hoffmann (1982) as a “devastating” pathogen of the main cereal crops. Economically the disease is important, since it results in loss of yield and quality (Figure 3). Even some traces of the disease can be a reason to consider the grain as being “smutty” and resulting in a price of low quality grain (Lipps, 2013). Common bunt has since domestication of wheat been a reason of loss in wheat yield and quality in various parts of the world (Gaudet & Menzies, 2012). The first scientific work on bunt was carried out by Tillet in France in the 18th century (Tillet, 1755). Results of studies carried out during the last centuries on common bunt losses have shown that the disease have been reduced significantly. More resistant varieties being bred, increased use of chemical treatments, as well as improved management practices have allowed a total control of the disease (Gaudet & Menzies, 2012). However, with the increasing interest for organic agriculture and due to reduced fungicides use, the disease is currently re-emerging and thereby becomes important in organic farming in Europe and North America (Borgen, 2004; Matanguihan & Jones, 2010). For the region of Central-West Asia and North Africa the disease was considered the second most significant disease after rusts during the 1970th (Hoffmann, 1982), and is still important (Asaad & Abang, 2009; Gaudet & Menzies, 2012; Hajihasani *et al.*, 2012). Common bunt has been recorded in all wheat growing areas of Tajikistan, and its occurrence was found to be influenced by the environment, as well as by genotypic factors (Pett *et al.*, 2006).

2.2.2.2. Pathogen

Two fungi are causing common bunt (Saari *et al.*, 1996):

1. *Tilletia tritici* (Bjerk.) Scientific synonyms: *T.caries* (DC.) Tul., *T.tritici* (Bjerk.) R.Wolff. Common names: Common bunt, stinking bunt, covered smut, hill bunt, complete bunt, low bunt, high bunt;
2. *Tilletia laevis* Kühn *(*syn. *foetide).* Scientific synonyms: *T.levis* Kühn, *T. foetida* (Wall.) Liro, *T.foetens* (Berk & Curt.) Schröt. Common names: Common bunt, stinking bunt, covered smut, hill bunt, complete bunt, low bunt, high bunt

The disease symptoms caused by each of these two fungi are identical, although the fungi have different spore morphology (Wilcoxson & Saari, 1996; Murray *et al.*, 2009):

2.2.2.3. Cycle and symptoms

The pathogens are both seed-borne and soil borne, nonetheless seed-borne teliospores are considered as a primary source for the disease spread in the world, and they can also remain in the soil viable for some years (Gaudet & Menzies, 2012). The teliospores usually germinate at relatively moist soils and at temperature of 5 to 15°C (Murray *et al.*, 2009). The germinated teliospores form 8-16 primary basidiospores, and from the primary basidiospores secondary basidiospores are produced. These secondary basidiospores usually germinate on the host plant or close to it, and thereafter enter to the plant tissues, mostly through the coleoptiles. Within the plant, the fungal mycelium grows and reaches the apical meristem. The hyphae replaces the young ovary tissues and then develops into teliospores, filling the grain (Murray *et al.*, 2009). The first symptoms of the disease in the plant can be seen after the stem has started to elongate. A plant infected with common bunt is slightly decreased in height and has spreading tillers. Bluish green color, spread glumes and owns are common symptoms found in the diseased spikes (Agrios, 2005). The infected grain is shorter and thicker and filled with bunt spores (Wilcoxson & Saari, 1996). Sometimes bunted grains can be easily seen. Infected spikes keep the green coloration longer than healthy spike, thus delaying maturation. When the infected grain is matured and become dry, the teliospores are easily released during the harvesting, thus infecting all grains of the same harvest or can even spread to neighbouring fields by the wind and by mechanical contacts. If the disease occurrence is high, undamaged bunted sori can easily be found by visual check of grain or seed lot (Murray *et al.*, 2009). Presence of infected seed can affect other healthy seeds both during harvesting and processing, thereby reducing both seed and market quality of the grain (Agrios, 2005).

2.2.2.4. *Testing methods*

There are a number of methods used for detecting common bunt on seed (Mathur & Cunfer, 1993). Visual test of seed sample and centrifuge wash tests are the routine tests used in Seed Health Testing Laboratories (Pearce, 1998). The Seed Health Laboratory of ICARDA is using the mentioned methods for detecting teliospores of *T. laevis* and *T. tritici* (Asaad & Abang, 2009). The seed wash filter test is used in the Seed Health Laboratory of CIMMYT (Mezzalama, 2010).

Figure 3: Wheat common bunt. **A**. Heavily damaged field; **B**. Infected spike; **C**. Infected unbroken seed (left) and healthy seed (right); **D**. Infected broken seed (left) and healthy seed (right); **E**. Teliospores of *T. laevis* under the microscope. [Photos by the author]

2.2.2.5. *Control measures*

Common bunt can effectively be controlled by the use of chemical fungicides, like carboxin, benzimidazols, and difenoconazole (Murray *et al.*, 2009), as well as Vitavax, Fundazole, Raksil etc. (Pett *et al.*, 2005). Varieties resistant to the disease are an important and effective tool to control the disease. Different races of the pathogens do exist, and this should be taken into account while selecting the “right” variety. Farming practices, where certified seeds of wheat free of the pathogen are used as well as proper crop rotation and shallow sowing can be helpful for controlling the common bunt. Crop rotation and avoiding monocropping of wheat, as well as control of the weeds are other tools to avoid development of common bunt (Pett *et al.*, 2005). Planting seeds in warmer soil, i.e. earlier planting of winter wheat and late planting of spring wheat can help to reduce inoculation by common bunt (Gaudet & Menzies, 2012).

A number of methods to control the disease in organic agriculture have been developed (Borgen, 2010a). Examples of methods applicable in organic agriculture is to use the fungi *Muscodor albus,* which have been shown effective in the USA (Goates & Mercier, 2011). Further, treatment of the wheat seed with a combination of ultrasound and steam was shown effective without affecting the vigour of the wheat seed (Borgen, 2010a).

Threshold of common bunt exists in a number of countries and in e.g. Denmark the threshold is 10 spores/g seed, or less than 1 diseased spike on each 1000 m^2 of seed crop (Borgen & Kristensen, 2010).

2.2.2.6. Breeding strategies

As a result of the opportunities to control common bunt with the use of fungicides, efforts towards breeding new varieties resistant to common bunt has been given low priority in many wheat growing countries. Example of regions with low priority for breeding common bunt resistant varieties are USA, Europe, North Africa and West Asia (Matanguihan *et al.*, 2011). Bread wheat has generally been found more resistant to *T. laevis* compared to durum wheat, which is susceptible to both species (Mamluk & Zahour, 1993). Inoculation of winter wheat lines by a number of races of *T. tritici* and *T. laevis* showed presence of new genes or gene combinations in some of the tested material from USA (Matanguihan & Jones, 2010). Evaluation of species from several genus of *Triticum* other than *T.aestivum,* showed high differences in susceptibility to common bunt (Borgen, 2010b).

Bunt resistance genes: To date, there are 15 genes for common bunt, so called *Bt* genes, reported to contribute with resistance to bunts (Goates, 1996). From these resistance genes, six have been mapped on the wheat chromosomes and for only one of them – *Bt10*, a linked marker has been found (Laroche *et al.*, 2000; Matanguihan *et al.*, 2011). The *Bt1* gene has been found encoded on chromosome 2B of wheat (Sears *et al.*, 1960), while *Bt4, Bt6* and *Bt5* have been mapped to 1B (Scmidt *et al.*, 1969; McIntosh *et al.*, 1998); *Bt7* to 2D (McIntosh *et al.*, 1998) and *Bt10* to chromosome 6D (Menzies *et al.*, 2006).

The *Bt10* is known as an effective resistance gene to common bunt in wheat originating from many places in the world, although it did not show effectiveness against bunt populations in eg. Latvia (Priekule, 2010).

Pathogen races: Several races of the disease are known. Races caused by *T.laevis* are labeled "L", while races caused by *T.tritici* are labeled "T". In 1996 30 T and 16 L races was listed by Goates (1996). More recently, additional previously unknown races of the disease have been identified in the USA (Matanguihan & Jones, 2010).

2.2.3. Karnal bunt and dwarf bunt

2.2.3.1. Importance

Some of the bunt diseases, caused by *Tilletia* species are considered as quarantine pests at regional and national levels i.e. Karnal bunt (*Tilletia indica* Mitra) (European and Mediterranean Plant Protection Organization-EPPO) and dwarf bunt (*Tilletia controversa* Kuhn) (Buhariwalla *et al.*, 2012). Karnal bunt is a reason of economic losses, and the disease has been distributed from India to the region around (Mathur & Cunfer, 1993) and further to some other parts of the world. Karnal bunt is widely spread in India, Afghanistan and Pakistan (Wilcoxson & Saari, 1996), the neighboring countries to Tajikistan. The disease was spread to Mexico in 1980 and was also found in some parts of USA in late 1990th (Agrios, 2005). So far the disease has not been recorded officially in Tajikistan, but it is listed as a quarantine pest for Tajikistan (Rautapaa & Kroeker, 2010). Compared to dwarf bunt, Karnal bunt is a higher risk for Tajik wheat production, due to the spread in neighboring countries. The economic relation and trade between Tajikistan and the Southern neighbors are increasing every year, thus increasing the potential risk for introduction of the disease. In order to prevent disease entrance to the territory of Tajikistan, precise control under quarantine commodities need to be conducted at the entry border points. Furthermore, regular surveys in agricultural fields close to the border have to be carried out (Rautapaa & Kroeker, 2009).

Karnal bunt is a relatively novel bunt disease and it is caused by *Tilletia indica*, first reported in 1931 from the Indian region called Karnal (Mitra, 1931). The disease affects mainly bread wheat, durum wheat, triticale and other related species. The disease is easily disseminated by seed and many countries have a zero tolerance (Kumar *et al.*, 2008).

Dwarf bunt, caused by *Tilletia controversa* Kuhn, is a serious disease mainly for winter wheat (Buhariwalla *et al.*, 2012). An affected plant is dwarfed and is therefore not easy to identify in the field (Figure 4a) (Goates, 1996). The disease infects mainly tillers of winter wheat during winter time. Dwarf bunt can be completely controlled by systemic seed treatment by Difenoconazole (Keener *et al.*, 1995).

2.2.3.2. Pathogens

Pathogens causing the diseases are:

- Karnal bunt: *Tilletia indica* Mitra, other common names: partial bunt, new bunt, kernel bunt, KB. Scientific synonyms: *Neovossia indica* (Mitra) Mundkur (Figue 4c).
- Dwarf bunt: *Tilletia controversa* Kühn, other common names: short smut, stunt smut, stubble smut, TCK smut. Scientific synonyms: *T.contraversa* Kühn, *T.brevifaciens* Fisch (Figure 4d).

Figure 4. Karnal bunt and dwarf bunt. **A**. Dwarfed tillers, showing affect by dwarf bunt (left); **B**. Sori of Karnal bunt; **C**. Teliospores of *T.indica*; **D**. Teliospores of *T.controvers* [Photos: CIMMYT courtesy]

2.2.3.3. Cycle and symptoms

Karnal bunt teliospores can keep viability up to five years in the soil and more on the wheat seed (Agrios, 2005). Karnal bunt occurrence in the area, where disease is prevalent highly depends on the climatic conditions (Sharma *et al.*, 2012). The symptoms of Karnal bunt are different from other bunts, as it mostly affects plant leaves and infected ears are not totally destroyed, but only few of the grains in the spike are infected (Mathur & Cunfer, 1993).
The symptoms of dwarf bunt are similar to the symptoms of common bunt, although infected plants are dwarfed to half of the normal plant height or even more (Mathur & Cunfer, 1993).

2.2.3.4. Seed testing methods

Seed wash filter test is used for detecting the spores of *T. indica* and *T. controversa* in the Seed Health Laboratory in CIMMYT (Mezzalama, 2010). Direct inspection of seed samples for the presence of bunted balls and centrifuge wash test are used in Seed Health Laboratory of ICARDA (Asaad & Abang, 2009). With the purpose to identify *T.indica* in the quarantine control, molecular markers have been developed in China (Zhang *et al.*, 2010).

2.2.3.5. Control measures

Compared to other bunts, the control of Karnal Bunt is difficult and, therefore quarantine measures in countries were the disease is absent has to be a priority. Also, the cultivation of disease resistant wheat varieties is promising (Murray *et al.*, 2009).
Controlling dwarf bunt by chemicals is not as effective as the control of common bunt, due to the late occurrence of infection, usually during the winter time (Mathur & Cunfer, 1993) and also because of the soil borne nature of the pathogen (Purdy *et al.*, 1963).
Wheat breeding programs are searching for possible resistance sources for the diseases. Four main sources of resistance to Karnal bunt have been identified at CIMMYT, with the origin from India, China, and Brazil and from wheat produced synthetically at CIMMYT (Singh & Rajaram, 2002).

2.2.3.6. Quarantine regulations

According to the EPPO data sheet, Karnal bunt is present in 5 countries in the Asian continent and 3 in the American continent. It is considered as an A1 quarantine organism, i.e. the pest is absent in the territory of EPPO members (EPPO-A1, 2012). The disease presents a high risk for wheat growers, if it is spread to a new area and generally it is difficult to eradicate while present (EPPO, 2012).

Compared to Karnal bunt, the dwarf bunt is not in the EPPO list and is not regulated at International level. However, some countries e.g. China has zero tolerance for the disease, (Mathur & Cunfer, 1993).

2.2.4. Black point

2.2.4.1. Importance

Black point is present in many countries and is also known by different names and caused by a number of fungi (Mathur & Cunfer, 1993). Some scientists have reported that the disease is mostly present in durum wheat (McIntosh, 1998; Sharma, 2012b), while others have found it also to be common in bread wheat (Hannan *et al.*, 2005). In Tajikistan, the disease is a relatively new subject of study. However, in the studies carried out the disease has been observed in wheat grains in all the wheat growing areas and usually in winter wheat varieties, although genotype and year influenced the occurrence. Based on a survey in Tajikistan, Pett *et al.* (2006) reported symptoms of the disease with up to 11% infection in wheat grains.

2.2.4.2. Causes of the disease

The pathogens *Alternaria alternata* (Rees *et al.*, 1984) and *Bipolaris sorokiniana* (Muller *et al.*, 2005) are the main causes of this disease (Figure 5). Other fungi have also been found associated with black point, such as *Fusarium* spp. (Dexter & Matsuo, 1982) and *Stemphylium* (Cristea *et al.*, 2008). The fungal species mostly identified in Tajikistan were: *Alternaria* spp., *Drechslera* spp., *Bipolaris* spp. (Pett *et al.*, 2005; Pett *et al.*, 2006; Pett & Muminjanov, 2006). Beside affecting the grain vendibility, the disease was found to affect the germination rate of infected seeds (Figure 5a) (Pett *et al.*, 2006). Rainfall and high humidity during the ripening of wheat plants are suitable for the disease (Sharma, 2012a).

2.2.4.3. Symptoms

Different discolorations of wheat grain are found, from red to black (Fernandez & Conner, 2011). Discoloration of grain at the embryo site is usually defined as black point, while in other parts discoloration is defined as smudge (Dexter & Matsuo, 1982). It is not difficult to distinguish black pointed seed from healthy ones, or even from bunted seeds visually (Mathur & Cunfer, 1993).

2.2.4.4. Testing methods

Conventional blotter testing methods described by ISTA is usually used for detection of black point causes. Simply, infected seed is placed in an agar media and fungi are allowed to grow, so that they later can be identified (Mathur & Cunfer, 1993). Freezing blotter is a modification from the conventional blotter test, where the active wheat seed embryo is killed by freezing. The freezing of the wheat embryo increase the growth of pathogen, leading to easier identification. The freezing blotter test is a routine test for detecting presence of fungi pathogens on wheat seed in Seed Health Laboratories of CIMMYT (Mezzalama, 2010) and ICARDA (Diekmann & Asaad, 1989; Asaad & Abang, 2009).

Figure 5. Black point. **A**. Black point effect to wheat germination; **B**. Growth of the *Helminthosporium* on a surface of wheat seed; **C**. Conidia of *Alternaria alternate*; **D**. Conidia of *Helminthosporium* sp. [Photo: A by B. Pett ; B, C and D by the author)

2.2.4.5. Control measures

The use of resistant varieties is an effective method for preventing high incidence of the disease (Pett *et al.*, 2005). Chemical treatments of seed have also been helpful for controlling black point, and additional types of seed-borne diseases (Agarwal & Verma, 1975). Storing seed under optimal conditions, with lower relative humidity and late planting of susceptible varieties can help to avoid severity of the black point (Mathur & Cunfer, 1993).

2.2.4.6. Breeding strategies

It is possible to breed varieties resistant to the disease (Mathur & Cunfer, 1993). Historically artificial inoculation were used to screen for diseases resistance in wheat (Parashar & Paracer, 1965) and nowadays new technics of breeding are also available for detecting black point resistance in wheat (Sharma, 2012a). The SSR markers, *gwm319, wmc048 and gwm341* have been applied to detect 3 QTLs for black point resistance to be used for selection in bread wheat populations (Christopher *et al.*, 2007).

3. Wheat baking quality

3.1. *Wheat bread-making quality features*

3.1.1. Wheat bread

The main advantage of wheat in relation to other cereals is its unique ability to form the bread (Marx *et al.*, 2000). Being a staple food for thousands of years, bread is also an important part of the diet due to the high content of starch and complex carbohydrates (Gellynck *et al.*, 2009). The wheat bread is mainly being baked from the following components: flour, water and yeast, and addition of ingredients, such as: salt, milk, fat, sugar etc. Depending on the type of bread and location where its produced, wheat bread is divided into three general types: leavened, flat and steamed (Peña, 2002). Sometimes flour made from other cereals, such as rye, barley and oat are added to wheat flour for prolongation of bread's shelf-life, as well as to enrich the breads nutritional value and taste (Eliasson & Larsson, 1993; Gellynck *et al.*, 2009).

3.1.2. Bread-making quality attributes

Wheat quality parameters are depending on consumers demands and processing techniques and therefore the bread-making quality can be described variously (Chung *et al.*, 2003; Osman *et al.*, 2011). Thus, wheat bread-making quality depends on culture, type of bread and way of baking (Kuktaite, 2004). According to Marx *et al.* (2000), the following three important properties describe the wheat's ability for baking bread: viscoelasticity of the wheat dough, abilities of the dough to expand and options to keep the same shape after baking.

Wheat bread-making quality depending on product and specific process requirements and it can be explained and subdivided into three parts: wheat grain quality, wheat flour quality and bread quality (Svec & Hruskova, 2009). Below a number of main quality characteristics are listed, although some of the main can be described as: flour protein, water absorption, dough mixing behavior, and loaf volume (Shewry *et al.*, 1995).

3.1.2.1. *Wheat grain quality*

Test weight, thousand kernel weight, grain hardiness, vitrescense, and appearance are the main parameters describing the quality of the wheat grain (Svec & Hruskova, 2009).
By test weight plumpness of wheat grain is measured and an average grain weight is measured by thousand kernel weight. These measurements in general describe the potential of white flour extraction (Wrigley & Batey, 2003). Grain hardiness is a genetical trait and this trait is measured in grain trade and milling industry for defining feasibility of the grain for different purposes (Wrigley & Batey, 2003).

3.1.2.2. *Wheat flour quality*

The main quality parameters measured in the wheat flour are: protein content and composition, wet gluten content and strength, ash, Zeleny test, falling number, dough rheological and mixing parameters.
Flours with higher protein content are holding ability to form bread with larger volume (Cauvain, 2003). Falling number test is used for measuring alpha-amylase activity of the flour (Perten, 1964) or sprouting damage (Wrigley & Batey, 2003). Result of the test is shown as seconds (AACC-56-81.03), where above 300 seconds refers to good quality of wheat flour and less than 250 seconds is a sign of high enzyme activity, which is mainly due to sprout damage (KSU, 2008).
Rheological parameters of dough play a crucial role during the baking process and affect the bread quality (Mondal & Datta, 2008). Rheological properties are measured by many testing methods, e.g. mixing time of dough measured with mixers (farinograph and mixograph), its extensibility with extensigraph, extensibility of dough and gluten with alveograph etc. (Dobraszczyk & Morgenstern, 2003; Razmi-Rad *et al.*, 2007).

3.1.2.3. *Bread quality*

Bread volume is the main feature of wheat bread (MacRitchie, 1984), while taste, flavor, appearance, shelf-life are also important quality parameters of wheat bread (Finney *et al.*, 1987).

3.1.3. *Wheat protein and baking quality*

3.1.3.1. *Wheat protein*

Protein content of the wheat grain varies among cultivars, which is due to genetic factors as well as due to differences in cultivation conditions. Thus, protein concentration in the grain normally ranges from 8 to 17 % (Altschul, 1965; Payne, 1987; Peña, 2002). Although, the wheat grain protein concentration is lower than in pulses, wheat is the main source of protein for mankind (Goldschein, 2013). Wheat protein compositions have been studied extensively around the world for last decades (Johansson *et al.*, 1994; Weegels *et al.*, 1996; Shewry *et al.*, 2000). According to solubilities of proteins in different solvents, Osborne (1924) subdivided proteins into different groups (Table 4). However in systematic studying of cereals proteins Beccari was the pioneer, who first extracted gluten from wheat flour by washing it with salt solution around the mid of 18^{th} century (Mills *et al.*, 2003).

Table 4. Classification of proteins based on their solubility (Osborne, 1924)

<table>
<tr><td>Soluble in water</td><td>Albumins</td><td rowspan="2">Non-gluten proteins</td></tr>
<tr><td>Soluble in 0.5-1.0 m salt</td><td>Globulins</td></tr>
<tr><td>Soluble in 60-70% aqueous ethanol</td><td>Prolamins</td><td rowspan="2">Gluten proteins</td></tr>
<tr><td>Dilute acid or alkali extractable proteins</td><td>Glutenins</td></tr>
</table>

3.1.3.2. *Gluten proteins*

The main proteins influencing baking properties are the gluten proteins, amounting to about 85% of the total flour proteins (Shewry, 2003). Gluten consists of glutenins, and gliadins (Payne, 1987). Glutenins are subdivided into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (Payne *et al.*, 1979). The HMW-GS are controlled by specific genes located on the long arms of the chromosomes 1A, 1B and 1D and the loci are designated *Glu-1* or *Glu-A1*, *Glu-B1* and *Glu-D1* (Payne *et al.*, 1981). Wheat varieties theoretically may have six HMW-GS, but usually three to five major HMW-GS are present in most varieties: two, one or none subunits are expressed by the A genome, one or two are expressed by the B genome and one or two are expressed by the D genome (Lawrence & Shepherd, 1981; Payne *et al.*, 1984; Margiotta *et al.*, 1996; Ali *et al.*, 2010). According to assigned for HMW glutenin subunits *(Glu-1)* quality score by Payne and colleagues, wheat varieties with higher score have stronger gluten than varieties with less score (Payne *et al.*, 1987). However, it has also been revealed that HMW subunits are differently affecting the gluten properties, some more positively than others from the same locus and some has less contribution to gluten strength (Kolster *et al.*, 1991; Johansson *et al.*, 1995; Peña, 2002). Glutenin subunits can be easily determined by separation with Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Payne *et al.*, 1979) and they can thereafter be classified according to the system developed by Payne and Lawrence (1983). This technic is nowadays used widely for breeding purposes and has been proven effective for obtaining desirable combinations of HMW-GS in a number of wheat lines/cultivars (Peña, 2002).

Figure 6. Tajik Bread. **A**. Baker shapes bread from the daugh; **B.** Bread is ready for baking; **C.** Bread in the oven (Tandyr); **B**. Ready Tajik bread. [Photos by Marufkul Mahkamov]

3.1.4. Bread making quality of Tajik wheat

Most of the varieties of traditional Tajik breads are baked in clay made ovens '*tanur*' or "*tandyr*" (Figure 6). Some alternative bread types are present like "*katlama*", a layered bread fried in a pan, although the majority of bread varieties are "*tandyr*" bread (Mack & Surina, 2005). The shape of tandyrs is depending on the region and preference varies, including different sizes and placement on the ground: some are vertical and some are horizontal. Firewood, coal, cotton plant residues, electricity and gas are the main energy sources for heating tandyrs (Peña *et al.*, 2003). Dough is pasted around the hot oven to be baked (Figure 6c). This baking procedure requires bread dough with enough gluten strength, so that dough does not fall down to fire, during baking. Locally produced wheat grain in Tajikistan usually has relatively low gluten content and strength (Sarkisova *et al.*, 2006) and therefore "improver" flours with higher gluten content is imported mostly from Kazakhstan and Russia and this flours are used in mixtures (Peña *et al.*, 2003). The general problems of the wheat grain produced locally in Tajikistan has been defined as low test weight, low protein content, low and weak gluten (Peña *et al.*, 2003). Although, causes of such quality loss can be explained with different factors, main reason of downgrading of Tajik wheat can be explained due to genotypes and environment effects. Some studies have shown a negative effect of heat or warm environment to the bread-making qualities (Wrigley & Batey, 2003). Longer grain maturity period was reported to have positive correlation with gluten strength and grain protein concentration (Malik *et al.*, 2011). High temperature during the grain filling and short grain ripening period can be observed in many wheat growing areas of Tajikistan.

3.2. Breeding of wheat for improved baking quality

Making bread from wheat grain with desirable quality is complex, and involves many players, including breeders, farmers, millers and bakers (Osman *et al.*, 2011). Wheat breeding therefore has to consider effect and interactions of genotype, environment and management to the bread making quality (Osman *et al.*, 2011). Currently many wheat breeding programs around the world set their goals towards fulfilling end-users demands: both producers and consumers. Breeding for improved quality together with high yield is therefore two of the major objectives (Bushuk, 1998).

Traditional breeding

In the process of making bread from wheat flour, gluten elasticity plays a crucial role (Payne, 1987). Relationship between HMW-GS composition and bread-making quality has been proven by a number of scientists (Payne *et al.*, 1984; Marx *et al.*, 2000). Further a scoring system have been developed, which opened new opportunities for wheat breeders to improve baking quality (Payne *et al.*, 1987). The HMW-GS alleles are suggested to be the major genes connected to bread-making quality and therefore they can be used by plant breeders in breeding towards improved bread-making quality (Johansson, 1995).

The availability of a reliable and fast test at early stage is important for plant breeders. At present classical dough tests and baking bread, as well as newer methods based on molecular technics and electrophoresis are used to evaluate bread-making quality. Electrophoretic method is very useful during early breeding programs, whereby using a half of embryoless seed can be used to determine protein compositions and if needed another half with embryo can be planted for further multiplication (Bietz & Wall, 1975; Johansson, 1995). By the rapid development of DNA markers and novel methods to describe quality traits, choice for early detection of desirable traits is increasing (Korzun, 2003).

3.3. Influence of wheat seed-borne diseases on the baking quality

Seed-borne diseases are directly affecting the wheat grain, as some of the diseases influence both the grain vendibility as well as baking quality (Watkins & Prentice, 1997; Fuentes-Dávila *et al.*, 2002). A high level of infection by common bunt affects both the odour and the taste of the product (Fuentes-Dávila *et al.*, 2002; Wegulo, 2009). Karnal bunt affects the quality and flour properties, as well as gluten quality and dough strength (Gopal & Sekhon, 1988). Wheat products made of the grain with more than 3% infection by Karnal bunt is not recommended for human consumption (Sekhon *et al.*, 1980), although it is not toxic (Agrios, 2005). Food products made from kernels affected by black point may have smell and degrade the quality (Sharma, 2012a). Due to its effect on the grain appearance, black point is a reason for reducing the price of the grain (Solanki *et al.*, 2006), E.g. maximum two per cent of darkened grains is allowed in USA wheat grading for grade 1 and four per cent for grade 2 (Watkins & Prentice, 1997). In comparison to other seed-borne diseases of wheat, loose smut does not have any effect at all or only limited effect on the grain quality (Watkins & Prentice, 1997; Wegulo, 2009).

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