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Towards an impact on food security for Tajikistan:

Improved wheat grain and seed quality through plant breeding as a key

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Cover: Illustration of a typical Tajik bread (*non*) with a reap of matured wheat spikes
(Illustrator: Muhammad Husain; ©Bahromiddin Husenov)

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Abstract

Bread wheat (*Triticum aestivum* L.) is the worlds' most important food crop and it contributes 60% of the daily protein and calories to the population of Tajikistan. This thesis evaluated protein composition and bread-making quality of wheat, presence of and response to wheat seed-borne diseases, relationships between seed-borne diseases and protein quality as well as the major constraints for high yield of good quality in the Tajik wheat production systems.

High variation in protein composition, though with high heterogeneity, was found in the Tajik wheat. High Payne quality score, due to the presence of high molecular weight-glutenin subunit (HMW-GS) 5+10, was found for the majority of varieties/lines. No correlation was found between HMW-GS composition and percentage of un-extractable polymeric proteins in total polymeric protein (%UPP); also the level of the latter neither indicated strong gluten. A local adaptation and similarities with organic farming traditions seemed to prevail.

Overall fourteen and eighteen fungal species, respectively, were identified in seed samples from breeders' versus farmers' fields. *Tilletia laevis*, *T. tritici, Bipolaris sorokiniana* and *Stemphylium* spp. were the major pathogenic fungi, although *Alternaria* spp. was the most common fungi.

Wheat common bunt and loose smut were observed with low incidence in the field, though common bunt inoculum on the seed was >50%; *T. laevis* being the most common cause. Tajik wheat varieties and lines showed high susceptibility towards common bunt. A significant correlation was found between presence of certain fungi and protein quality.

The knowledge status of the farmers was found low. Presence of seed-borne diseases, low protein content and weak gluten hampered the wheat quality, mostly independent of farm type and knowledge of farmers.

To conclude, a concerted action is needed for the wheat production in Tajikistan. This thesis clearly shows a need to increase farmers' knowledge through the use of both the educational and the agricultural extension systems. Educational activities should result in a sustainable use of certified seeds, weed and pest management, crop rotations, etc. without hampering the large biodiversity of Tajikistan. A system for use of certified seeds of novel resistant and high quality varieties also needs to be targeted within the action.

Keywords: bread-making quality, common bunt, field surveillance, plant breeding, seed-borne diseases, sustainability, Tajikistan, *Triticum aestivum* L., wheat protein.

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Dedication

To all my teachers
To my family

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Husenov B, Mahkamov M, Garkava-Gustavsson L, Muminjanov H, Johansson E (2015). Breeding for wheat quality to assure food security of a staple crop: the case study of Tajikistan. *Agriculture & Food Security*, 4(9). DOI 10.1186/s40066-015-0029-1.

II Mahkamov M, Husenov B, Garkava-Gustavsson L, Muminjanov H, Kuktaite R, Prieto-Linde ML, Johansson E. Protein composition in Tajik wheat: opportunities to breed for improved bread-making (submitted manuscript).

III Husenov B, Otambekova M, Mahkamov M, Eshonova Z, Johansson E, Garkava-Gustavsson L, Muminjanov H. Bread-making quality of Tajik wheat; opportunities and draw-backs for improvements in developing countries (manuscript).

IV Husenov B, Asaad S, Muminjanov H, Garkava-Gustavsson L, Yorgancillar A, Johansson E (2017). Evaluation and managing wheat seed-borne diseases: options and suggestions from the case of Tajikistan. *Cereal Research Communications,* 45(1), pp. 124-138. DOI: 10.1556/0806.44.2016.045.

V Husenov B, Asaad S, Muminjanov H, Garkava-Gustavsson L, Johansson E. Seed health and protein quality in farm produced wheat of Tajikistan: Implications for a sustainable increase of food security in developing countries (manuscript).

VI Husenov B, Otambekova M, Muminjanov H, Morgounov A, Asaad S, Garkava-Gustavsson L, Johansson E. Constraints and perspectives for sustainable wheat production in Tajikistan (manuscript).

Paper I is an open access paper and Paper IV is a free access paper.

The contribution of Bahromiddin Husenov to the papers included in this thesis was as follows:

I Planned the experiment together with main supervisor, collected plant (seed) material, performed laboratory analyses and the practical work, and wrote the manuscript together with co-authors.

II Collected plant (seed) material, performed laboratory analyses and the practical work together with first author, participated in drafting the manuscript and in correspondence with the Journal.

III Planned the experiment together with co-authors, collected plant (seed) material, performed practical field works, drafted the manuscript and finalised together with co-authors.

IV Planned the experiment together with supervisors, collected plant (seed) material, performed laboratory analyses and the greenhouse test, drafted the manuscript and finalised together with co-authors.

V Planned the experiment together with supervisors, conducted surveillance, collected plant (seed) material, performed laboratory analyses, drafted the manuscript and finalised together with co-authors.

VI Planned the experiment together with co-authors, conducted field surveillance, drafted the manuscript and finalised together with co-authors.

Abbreviations

%UPP	Percentage of unextractable polymeric proteins in total polymeric protein
AMOVA	Analyses of molecular variance
ANOVA	Analyses of variance
CIMMYT	International Maize and Wheat Improvement Centre
CWT	Centrifuge wash test
FAO	Food and Agriculture Organization of the United Nations
HMW-GS	High-molecular-weight glutenin subunits
ICARDA	International Center for Agricultural Research in the Dry Areas
MYTN	Multi-location yield trial nursery
PCA	Principle Component Analyses
SAS	Statistical Analyses System, statistical software
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE-HPLC	Size Exclusion-High Performance Liquid Chromatography
SLU	Sveriges Lantbruksuniversitet - Swedish University of Agricultural Sciences
SPSS	Statistical package for social sciences
TAU	Tajik Agrarian University
TFI	Tajik Farming Institute
TOTE	Total SDS extractable proteins

1 Introduction

Food security, assuring sufficient food access for the population of the country is a major target for all countries worldwide, being always an impendence for the governments of developing countries (Augustin *et al.*, 2016; Kesavan & Swaminathan, 2008; Ehrlich *et al.*, 1993). Food security still remains a main challenge for Tajikistan, the smallest country in the Central Asian region, with more than 30% of the population being poor and undernourished (FAO, 2015; WFP, 2015). Food security is thus one out of three strategic objectives defined by the government for the country; the other two are ensuring energy independency, and break in the communication deadlock (www.president.tj).

Food security is highly dependent on the availability and use of functional and sustainable agricultural farming systems and a proper handling of the produced commodities (Augustin *et al.*, 2016). Such feasible production systems may be highly lacking for vulnerable groups of people in developing countries, despite the fact that the rural population is mostly highly dependent on their own food production (Harvey *et al.*, 2014).

Wheat is an important food crop which has a great impact on food security at the global level (Shiferaw *et al.*, 2013). The crop is the most important staple commodity in Tajikistan (Robinson *et al.*, 2009), contributing in average with more than 60% of the daily calories and protein intake in the country (Braun, 2003) and thus playing a fundamental role in achieving food security for the Tajik population. Recently, the production volume of wheat in Tajikistan has increased, but still more than 50% of its annual consumption is imported (TajStat, 2017a). Furthermore, the quality of the domestically produced wheat remains poor (Madaminov, 2004).

Wheat production in Tajikistan faces also several other constraints, e.g., seed-borne diseases, which significantly affect the quantity and quality of produced grain, and where the knowledge was scarce up to now (Husenov *et al.*, 2008; Pett *et al.*, 2006). In addition, limited information about quality parameters, especially baking quality in local varieties and breeding materials called for attention to these questions.

The thesis work was also conducted to come to an understanding of the current challenges and point out the key opportunities for improvement of the Tajik wheat production through enhancement of current plant breeding systems, modernization of the seed production system and improvement of farming practices.

The key findings of this work are intended to be utilized by breeders and the seed sector of Tajikistan with an ultimate goal to give its farmers better business opportunities and thus improving the prospects of achieving food security for the population. Furthermore, wheat is the major food crop not only in Central Asia, but also in West Asia and North Africa. By that, the impact of this work will be multiplied since many of the findings will also be applicable in many other developing countries.

2 Background

2.1 Wheat

Bread wheat (*Triticum aestivum* L.), is a hexaploid crop (2n = 6x = 42, AABBDD) (Peng *et al.*, 2011), and the most widely grown wheat species, accounting for about 95% of the total wheat production (Shewry, 2009). The crop is known being a staple for more than 1/4 population of the world and contributes with the highest share of protein for human consumption among the three major cereals: rice, wheat and maize (FAO, 1995). Yearly world wheat production is around 750 mln t/year, during the later years (FAO, 2017). The area of wheat production stretches from 40° S up to 60° N (Curtis, 2002) and accounted in 2014 for more than 220 mln hectares around the world with an average yield 3.3 t/ha (FAOSTAT, 2017). The main wheat producing countries are China, India, USA, Russia and France (FAOSTAT, 2017).

Wheat flour is mainly used for baking bread, but also for producing other food items, e.g. pasta and cookies. Wheat is also used for e.g., feed (OSUE, 2012; Faridi *et al.*, 1989), ethanol production (Kim & Dale, 2004), biofuel and bioplastics (Johansson *et al.*, 2013; Cuq *et al.*, 1998) and other purposes (Rasheed, 2015).

2.1.1 Wheat and food security

As a major staple crop for about 30% of the world population, wheat plays a major role in their food system and makes it the most important crop for the Global food security (Shiferaw *et al.*, 2013). Especially, wheat is an important crop for developing countries of Asia and the North African region. There has been a good progress in global wheat production for last 50 years, starting from so called “green revolution” and up to now, average yield has been increased significantly. This allowed improving food access and overcome famine for millions of poor population across the Pakistan, India, Mexico, China and many other countries in Asia, Africa and Latin America (Bonjean, 2016; Shiferaw *et al.*, 2013). Global wheat productivity has been raising with a 1% rate, but in order to meet the growing demand and increase in overall population, there is a need for a continuous increase in wheat productivity with more than 1.6% in a year (Rajaram, 2016).

2.2 Wheat in Tajikistan

Tajikistan is a mountainous country and less than 5% of the total area are under the arable lands (TajStat, 2015). With the population of more than 8.7 mln, 73% lives in rural areas (TajStat, 2017b). Sixty percent of labors are dealing with agriculture, forestry and fishery and these sectors contribute 22% to national GDP. The climate is sharply continental, with hot summers and cold winters. Agricultural production is mainly conducted in valleys, like Vakhsh in South, Hisor in Central part and Ferghana in Northern part. Major field crops are: wheat, cotton, maize, alfalfa. Besides those, a variety of vegetables and pumpkins, as well as fruits are produced in the country.

Tajikistan holds one of the leading positions in the world when it comes to the share that wheat has as a protein source for the human population (FAO, 2017; Braun, 2003).

Wheat has been grown in Central Asia since the Neolithic age (Udachin & Shakhmedov, 1984; Ghafurov, 1979) and a number of archaeological excavations show the long history of wheat cultivation in the present territories of Tajikistan (Spengler & Willcox, 2013). Also, the Tajikistan territories are considered as one of the centres of origin and diversity of bread wheat (Vavilov, 1987).

After the country obtained the independence in 1991, wheat area started to expand and production increased (Fig. 1). Presently, average yield in the country is 2.9 t/ha, which is still quite low and therefore needs improvement (Fig 1). According to official statistical data in Tajikistan, 917 thousand tons of wheat was produced in 2016, while 1.03 mln t of wheat was imported to the country (TajStat, 2017a).

Earlier, the largest share in wheat import was flour, imported mainly from Kazakhstan and Russia. During the last decade the number of milling factories in the country has increased, and consequently, the import of wheat grain has increased, while import of wheat flour has significantly decreased (ENT, 2017; TajStat, 2017a).

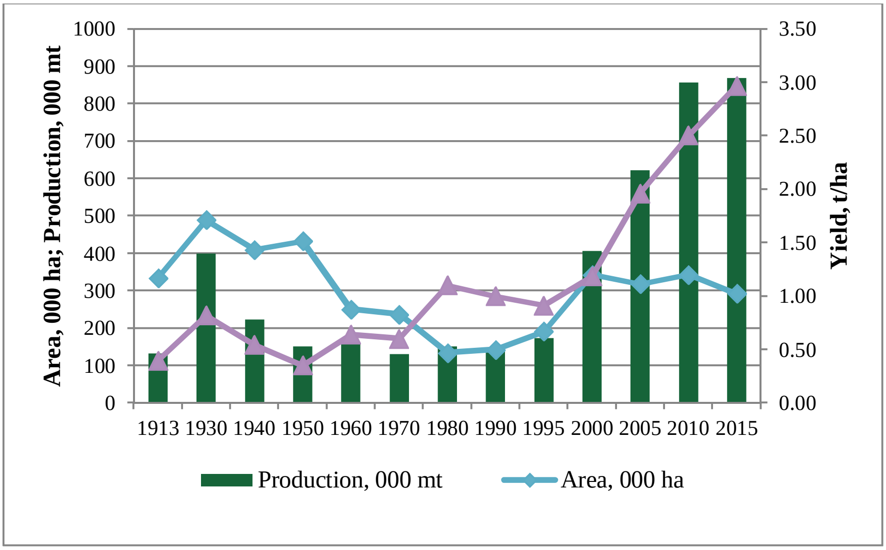

Figure 1. Wheat production trend in Tajikistan, 1913 - 2015

2.2.1 Wheat breeding in Tajikistan

The wheat breeding in Tajikistan started officially with the establishment of a first experimental station in 1932. Local wheat materials were known by their morphological characteristics, like *Surkhak* (red grain), *Safedak* (white grain), geographical locations, e.g. *Safedaki Ishkoshimi*, etc. (Husenov *et al.*, 2015). The station focused on the selection of superior entries from the local landraces and materials received from All-Union Institute of Crop Management (VIR) (Eshonova *et al.*, 2005).

The establishment of the Tajik Research Institute of Farming (later renamed to Tajik Farming Institute (TFI)) allowed to start the breeding of new and high yielding facultative wheat varieties adopted to local conditions (Dorofeev *et al.*, 1987). Due to the fact that cotton was considered as a strategic crop and wheat was not of the highest priority for Tajikistan during the Soviet period, a limited choice of varieties were available at the country's independence in the early nineties. Thus, varieties bread by TFI, e.g. *Navruz* and *Sharora*, showed relatively high yield potential, but they were very susceptible to yellow rust (Morgounov *et al.*, 2005). The high sensitivity to yellow rust, during outbreaks, resulted in that farmers stopped growing *Sharora*. However, the variety *Navruz* is still used as a local check in all official testing trials by the State Commission for variety Testing (SCVT), although the production area under this variety is modest. Despite the fact that the collaboration and exchange of wheat germplasm with the international centres was limited, the Mexican variety *Siette-Cerros 66*, derived from CIMMYT materials was released in the country in 1976, and this variety was popular till late nineties (Morgounov *et al.*, 2005).

A strong and stable collaboration has started with international centres like CIMMYT and ICARDA, after the country became independent. This collaboration has resulted in the development of a number of new wheat varieties (Muminjanov *et al.*, 2015; Morgounov *et al.*, 2005). At present, the Tajik wheat breeding programs are focusing on development and selection of new varieties that combine high yield with resistance to major diseases and pests and adaptation to diverse environments of the country (Muminjanov *et al.*, 2015; Rahmatov *et al.*, 2010). Early maturing varieties is a need for the rainfed and irrigated farming systems, while varieties tolerant to drought, heat and lodging are other important targets (Eshonova *et al.*, 2005). Besides the aforementioned characteristics, new wheat varieties have to bear frost tolerance due to harsh winter conditions, especially in the North and mountains of Tajikistan (Naimov *et al.*, 2005; Eshonova *et al.*, 2003).

Wheat breeding in Tajikistan is currently conducted by a publically funded breeding program at the TFI under the Tajik Academy of Agricultural Sciences and by a privately funded program at the Latif Murodov Production cooperative in cooperation with the Tajik Agrarian University (TAU) (Muminjanov *et al.*, 2015; Eshonova *et al.*, 2005).

These two breeding programs operate in close collaboration with CIMMYT and ICARDA. Along with producing own populations, testing them and selecting superior lines, Tajik breeders receive every year breeding nurseries from CIMMYT for further screening in different agro-climatic conditions. The collaboration with the CIMMYT and ICARDA has been successful and has resulted in the development of a number of new wheat varieties that are currently widely grown by farmers (Muminjanov *et al.*, 2015; Rahmatov *et al.*, 2010; Morgounov *et al.*, 2005).

2.2.2 Challenges in wheat production

A number of constraints in wheat production of Tajikistan have been identified and among them insufficient access to high quality seed of improved varieties were listed as being the major ones (Muminjanov *et al.*, 2015). Furthermore, wheat rusts, seed-borne diseases and insect pests have been pointed out as serious constraints (Pett *et al.*, 2006; Pett & Muminjanov, 2006). Especially the epidemics of yellow rust have been devastating during recent years which have resulted in significant yield losses (Rahmatov *et al.*, 2011; Eshonova *et al.*, 2005). Abiotic stresses, especially frost and drought further add to the challenge in Tajik wheat production (Naimov *et al.*, 2005).

2.3 Wheat protein and quality

Protein content in ripen wheat grain varies from 8 to 17% (Peña *et al.*, 2002; Payne, 1987; Altschul, 1965). Wheat proteins have been classified into different groups based on their solubility in different solvents (Osborne, 1924). Protein content and composition in wheat are the major genetically determined factors influencing flour quality (Shewry *et al.*, 2000; Weegels *et al.*, 1996; Johansson *et al.*, 1994). Gluten proteins, functioning as storage proteins, are located in the endosperm of mature grains and make up to 85% of total wheat flour protein (Shewry, 2009). Gluten proteins consist of two fractions: gliadins (soluble in alcohol) and glutenins (soluble in dilute acids or alkali) (Osborne, 1907). The gliadins are monomeric proteins, divided into four groups: α−, β−, γ−and ω−gliadins (Woychik *et al.*, 1961). The glutenins are polymeric proteins, consisting of high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS). The HMW-GS, comprising about 5-10% of the total protein, determine the elasticity of the gluten and have a higher impact on bread-making quality than LMW-GS, accounting for about 20-30% of the total protein (Gupta *et al.*, 1992; Payne *et al.*, 1979).

High molecular weight glutenin subunits (HMW-GS) are related to dough strength (Payne *et al.*, 1979). Specific loci, designated as *Glu-1* or *Glu-A1*, *Glu-B1* and *Glu-D1* on the long arms of the chromosomes 1A, 1B and 1D, control the expression of HMW-GS (Lawrence & Shepherd, 1981a; Lawrence & Shepherd, 1981b; Payne *et al.*, 1981). The composition of HMW-GS subunits in wheat germplasm can be estimated by separation with Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Payne *et al.*, 1979) with a subsequent classification following the system of Payne and Lawrence (1983). Payne and colleagues assigned HMW-GS subunits quality score and higher score indicates stronger gluten (Payne et al., 1987). Usually, wheat varieties possess three to five HMW subunits and the presence of certain HMW-GS e.g. 5+10 have been found to be positively correlated with high gluten strength and thus, good bread-making quality (Marchylo *et al.*, 2001; Johansson *et al.*, 1993). Breeding programs use knowledge on HMW-GS composition to create favourable combinations in new genotypes (Peña, 2002).

Other factors, influencing bread-making quality are the total grain protein content (Gunthardt & McGinnis, 1957; Finney & Barmore, 1948), amount and size distribution of monomeric and polymeric proteins (Johansson *et al.*, 2008; Johansson *et al.*, 2005), genetically determined development time, soil conditions and temperature during the growing season (Malik *et al.*, 2013).

2.3.1 Bread-making quality

Major advantage of wheat compared to other cereals is its ability to form bread due to viscoelasticity and ability of the wheat dough to expand and keep its shape after baking (Marx *et al.*, 2000). Wheat bread is an important component of the human diet as a source of energy due to the high content of carbohydrates as well as proteins (Gellynck *et al.*, 2009). Different combinations of carbohydrates and proteins in the wheat flour allow the production of different types of bread around the world. Since the bread-making quality varies with type of bread as well as cultural traditions and baking technologies, it is not straight-forward to define the universal criteria for bread-making quality (Kuktaite, 2004). The main quality characteristics are flour protein content and composition, water absorption, dough mixing behaviour and loaf volume (Shewry *et al.*, 1995). It is also known that the technological quality of wheat flour is determined by wheat genotype and growing conditions, or interaction of genotype and environment (Johansson *et al.*, 2013; Souza *et al.*, 2004).

Wheat bread-making quality consists of three parts: (i) grain quality, (ii) flour quality and (iii) bread quality (Svec & Hruskova, 2009). The quality of wheat grain in general is described by appearance, test weight, thousand kernel weight, grain hardiness and vitreousness (Svec & Hruskova, 2009). The potential of white flour extraction is measured by test weight and thousand kernel weight, while the feasibility of the grain for different purposes is defined by a genetically determined trait, grain hardiness (Wrigley & Batey, 2003).

The quality of flour is described by protein content and composition, wet gluten content and strength, ash, Zeleny test, falling number, dough rheological and mixing parameters. The protein content in the flour is related to the volume of the resulting bread (Cauvain, 2003). Sprouting damage of the flour is revealed by increased alpha-amylase activity and is measured by the falling number test (Wrigley & Batey, 2003).

The bread quality is affected by several rheological parameters, like mixing time of dough measured with farinograph and mixograph, extensibility of dough and gluten measured with alveograph, dought extensibility measured by extensigraph (Razmi-Rad *et al.*, 2007; Dobraszczyk & Morgenstern, 2003). Bread appearance, taste, flavour and shelf life are all important quality characteristics, but the most important one is bread volume (Finney *et al.*, 1987; MacRitchie, 1984).

In Tajikistan, most of the consumed bread is made in a traditional way, which is commonly called "*non*", and with different varieties and names depending on their ingredients, localities and even master's name (Husenov, 2013). The traditional types of bread in Tajikistan are usually baked in clay made oven, 'tandur' or 'tandyr' (Mack & Surina, 2005). The bread dough is pasted around the hot ovens and should remain there during the baking procedure. This requires a certain level of gluten content and strength, which is indeed low in wheat grain produced in Tajikistan (Sarkisova *et al.*, 2006), due to genotype and environment effects. Therefore, 'improver' flours imported mainly from Kazakhstan and Russia, are used to improve the dough quality (Peña *et al.*, 2003). Thus, directed breeding activities are required to improve technological performance of locally produced wheat by improvement of gluten quality, consequently making Tajikistan less import-dependent.

2.4 Seed-borne diseases

Pathogens which have a significant economic impact on crop production are disseminated by different means, such as air, water, soil, plant residues, etc. (Agrios, 2005). There are a number of pathogens disseminated by seed, and several of them are causes for serious diseases (Diekmann, 1993; Mathur & Cunfer, 1993). When one or more stages of the life cycle of a disease are related to the seed, the diseases are called "seed-borne" diseases (Pearce, 1998). Seed-borne diseases can be problematic in different agricultural systems, however in systems where the use of certified seed is well practiced, seed-borne diseases are often well managed (Bishaw *et al.*, 2013).

2.4.1 Major wheat seed-borne diseases

Almekinders and Louwaars (1999) defined four major seed quality parameters:

- physiological quality (germination, vigour)
- analytical quality (amount of good seed in a given seed lot)
- genetic quality (or varietal quality, such as varietal purity and adaptation), and
- sanitary quality (seed-borne diseases or health status of seed).

A compilation of seed-borne diseases prepared by Richardson (1990) and published by ISTA is widely used as guidance in studies on seed-borne diseases.

The major and most economically important seed-borne diseases of wheat have been described by Mathur and Cunfer (1993), indicating causative organisms and testing methods. Major fungal seed-borne diseases are given in the Table 1.

Knowledge on wheat seed-borne diseases, their prevailed types and major causes are limited in Tajikistan, and therefore as a first step in building solid knowledge, this study focused only on the main ones: in the first hand, common bunt and other prevailing fungal pathogens on seed.

Wheat common bunt

Common bunt, also known as stinking bunt or stinking smut due to the presence of *Trimethilamine* resulting in a fishy smell (Murray *et al.*, 2009), is a widely spread wheat disease (Agrios, 2005). The disease is distributed in almost all wheat growing areas (Hoffmann, 1982) and at present it is well controlled by the use of chemical seed treatments (Gaudet & Menzies, 2012). However, common bunt still remains critical in organic farming, in the developed parts of the world (Steffan *et al.*, 2017; Borgen, 2004), as well as in poor farming systems in the developing countries, where access to fungicides is limited (Pett & Muminjanov, 2006).

Common bunt in wheat is caused by two closely related fungi species: *Tilletia tritici* (Bjerk.) Wint (syn.: *T. caries*) and *Tilletia laevis* Kühn (syn.: *T. foetida*) (Goates, 1996).

Besides the use of chemical fungicides (Murray *et al.*, 2009), more environmentally friendly options to control diseases are also available, e.g. use of resistant varieties (Matanguihan *et al.*, 2011), use of certified seed confirmed free of the pathogen (Husenov *et al.*, 2008), crop rotation and following best crop management practices (Pett *et al.*, 2005). Furthermore, a number of methods and practical advices for controlling common bunt have been developed (Karaca *et al.*, 2017; Gaudet & Menzies, 2012; Goates & Mercier, 2011; Borgen, 2010).

In wheat sixteen genes are known to confer resistance to common bunt, which are designated from *Bt1* to *Bt15* and one *Btp* (Goates, 2012; Goates, 1996). Three of these genes (namely *Bt1*, *Bt4* and *Bt10*), as well as about 15 QTLs conferring resistance to common bunt have known chromosomal locations (Steffan *et al.*, 2017; Matanguihan *et al.*, 2011). Recently Steffan et al. (2017) identified new markers linked to *Bt9*.

Presently, molecular markers are becoming an increasingly useful tool allowing breeders to screen their materials for desired traits. Availability of markers linked to resistance genes and qualitative genetic factors for resistance to common bunt hold promises to enhance the prospects and speed-up breeding wheat varieties with resistance to common bunt (Matanguihan *et al.*, 2011).

Table 1. *Wheat major fungal seed-borne diseases*

#	Disease	Cause
1	Alternaria leaf blight	*Alternaria triticina* Prasada & Prabhu
2	Black point	*Alternaria alternata* and *Bipolaris sorokiniana* are the main cause
3	Common bunt	*Tilletia tritici* and *Tilletia laevis*
4	Downy mildew	*Sclerophthora macrospora* (Sacc.) Thirm., Shaw & Naras.
5	Dry seed decay	*Penicillium* spp.
6	Dwarf bunt	*Tilletia controversa* Kühn
7	Ergot	*Claviceps purpurea* (Fr.: Fr.) Tul. (Anamorph *Sphacelia segetum* Lev.)
8	Flag smut	*Urocystis agropyri* (G.Preuss) J. Schrot.
9	Karnal bunt	*Tilletia indica* Mitra
10	Leaf and glume blotch	*Stagonospora nodorum* (Berk.) Castellani & E.G. Germano (Telemorph: *Phaeosphaeria nodorum* (E.Muller) Hedjaroude)
11	Loose smut	*Ustilago tritici* (Pers.) Rostr.
12	Pink snow mold, Leaf blotch and Ear blight	*Microdochium nivale* (Fr.) Samuels & I.C. Hallett (Telemorph: *Monographella nivalis* (Schaffnit) E. Muller)
13	Scab (Fusarium head blight)	*Fusarium graminearum* Schwabe (Telemorph *Gibberella zeae* (Schwein.) Petch)
14	Spot blotch	*Bipolaris sorokiniana* (Sacc.) Shoem. (Telemorph: *Cochliobolus sativus*
15	Tan spot	*Pyrenophora tritici-repentis* (Died.) Drechs. (Anamorph *Drecheslera tritici-repentis* (Died.) Shoem.)
16	Wheat blast	*Pyricularia oryzea* Cavara

Source: Mathur and Cunfer (1993)

3 Aims and Objectives

Major aims of the thesis work were: a) to evaluate options and challenges in strengthening food security by utilising breeding strategies of wheat in Tajikistan; b) to understand opportunities to improve the bread-making quality of local wheat materials; c) to evaluate prevailing fungal seed-borne diseases in Tajik wheat and define strategies for improved seed health; d) to understand major constraints for wheat production, and finally based on the obtained results to define and propose improvements for wheat production to achieve food security for the country.

To reach the major aims, specific objectives of this thesis were to:

- determine composition of HMW-GS in Tajik wheat;
- investigate quality of breeding lines, new wheat materials, as well farmers grown wheat;
- evaluate the type and prevalence of fungal seed-borne diseases in Tajik wheat seed samples;
- define reaction of widely grown wheat varieties and new breeding lines to common bunt;
- investigate major challenges in wheat production in different farming types and define a sustainable way of the crop improvement

Most importantly, the investigations carried out within this thesis work are planned to help understanding constraints of wheat production in a developing country such as Tajikistan. The aim was to contribute with knowledge and tools for wheat breeders to better program their breeding strategies, and for decision makers to focus on areas requiring improvement.

4 Material and methods

4.1 Plant materials

Wheat varieties and lines from Multi location Yield Trial Nursery (MYTN) were studied for their protein composition (Papers I, II, IV). Detailed description of varieties/lines is presented in Paper I, and a revised table with additional information is presented in Table 2. In MYTN, usually last selection cycle is conducted and afterwards the best lines are given names and submitted for official testing. Other plant materials are described in relevant papers.

For analyses conducted on farmers' seed (Paper V), the samples were collected from farmers' fields, simultaneously as the surveys were carried out.

For Paper III, in total 17 varieties were used, where 11 were officially released varieties in the country and six recently bread varieties being under the official trials.

4.2 Protein analyses

The grain storage proteins were fractioned by sodium dodecyl sulphate polyacrylamide gel electrophoresis SDS-PAGE (Papers I, II) at SLU, Alnarp. For prediction of baking quality Payne scores were calculated for each variety/line (Paper I).

Monomeric and polymeric proteins of 22 varieties/lines were analysed with size exclusion-high performance liquid chromatography (SE-HPLC) (Paper II). SE-HPLC was also used for seed samples collected from surveyed farms (Paper V).

Table 2. *Description of the wheat varieties/lines included in the studies, their origin and source*

ID	Variety/line	Origin	Source / nursery	Papers	Variety name
1	Navruz (control)	TJK	TFI	I, II, IV	
2	Alex (control)	TJK/CIMMYT	TFI	I, II, IV	
3	Jagger 9	USA	Kansas State University	I, II	
4	TNMU/MUNTA			I, II, IV	
5	PRINIA/STAR	CIMMYT	6WWEERYT	I, II, IV	Nurbakhsh (TFI)
6	SHARK/F4105W2.1	CIMMYT	5WWEERYT	I, II, IV	Shokiri (TFI)
7	VORONA/KAUZ//1D 13.1/MLT			I, II, IV	
8	TAM200/KAUZ	IWWIP	10FAWWON	I, II, IV	Fayzbakhsh (TFI)
9	1D13.1/MLT//TUI			I, II, IV	
10	ARILW PRONGHORN			I, II, IV	
11	ESKINA-8			I, II, IV	
12	YN/3NPM/VOS83			I, II, IV	
13	PASTOR/3/VORONA /CN079			I, II, IV	
14	SKAUZ BV 92	CIMMYT	25 ESWYT	I, II, IV	
15	VORONA SN079	CIMMYT	25 ESWYT	I, II, IV	Vahdat (CH)
16	SOROCA	CIMMYT	25 ESWYT	I, II, IV	
17	OTUS TOBA 97	CIMMYT	25 ESWYT	I, II, IV	
18	KAUZ2/CHEW//BCN /3MILAN	CIMMYT	25 ESWYT	I, II, IV	IZ-80 (TFI)
19	CHEN/AEGILOPS SQUAROSA/TAUS/ RCN//3/RAV	CIMMYT	25 ESWYT	I, II, IV	
20	CBRD/KAUZ	CIMMYT	25 ESWYT	I, II, IV	AIKT-20 (TFI)
21	HUAVUN INIA	CIMMYT	25 ESWYT	I, II, IV	
22	CMN82A.1294/2*KA UZ//	CIMMYT	12 HRWYT	I, II, IV	
23	Norman	TJK	5FAWWON	IV	
24	Starshina	Russia	Krasnodar	IV	
25	Stava (control)	Sweden	Svalof	IV	

Note: TJK Tajikistan, USA United States of America, IWWIP International Winter Wheat Improvement Programme (Turkey), FAWWON Facultative and Winter Wheat Observation Nursery, ESWYT Elite Spring Wheat Yield Trial, HRWYT High Rainfall Wheat Yield Trial; CH-Chilgazi Farm; TFI-Tajik Farming Institute.

4.2.1 Bread-making quality

Detailed description is given in the Paper III.

For grain and bread-making quality analyses, following tests were conducted: grain moisture and hardiness, total protein content, Falling number, gluten content and its index; flour and bread assessment

Bread-making quality and bread assessment test were carried out at Quality Lab of National Centre of genetic resources and variety testing of Kyrgyzstan, Bishkek.

4.2.2 Seed health testing

Seed health status was evaluated by conventional seed health testing methods. Centrifuge wash test (CWT) was used for identification of common bunt causes in seed samples (Papers IV, V). Seed samples were also planted in order to evaluate prevailing fungal pathogens by Agar plate test (Papers IV, V).

4.3 Reaction of genotypes to common bunt

To evaluate the response of wheat genotypes to common bunt, field experiments were carried out in Turkey, in the Transitional Zone Agricultural Research Institute, Eskisehir (39°46´N; 30°31´E) during the 2013–2014 growing season (Paper IV).

Greenhouse experiments were conducted at greenhouse facilities of the Swedish University of Agricultural Sciences (SLU), Alnarp during the period from October 2012 to March 2013 (Paper IV).

4.4 Field surveillance

Field surveys were conducted in 2011 and 2012 growing season (Paper V) and 2012, 2013 and 2014 (Paper VI). Surveys were conducted in the field by filling the form included as Supplementary material to the Paper V.

4.5 Harvest index and yield features

'Harvest index', which is the ratio of spike's grain weight to its stem weight was calculated for 10 new varieties (Paper III). Other yield characteristics included plant height (PH), single stem weight (SW), spike length (SpL), spike weight (SpW), number of spikelets (SN), number of grains in the spike (SpG), weight of the spike's grain (SpGW) and thousand grain weight (TGW).

4.6 Statistical analyses and calculations

Details of statistics method used in the study are presented in the relevant papers. Basic calculations and data preparation were carried in the studies by Microsoft Excel. The statistical packages Minitab v 16 and 17 (Minitab, 2014; Minitab, 2010), Arlequin (Excoffier & Lischer, 2010), SAS (SAS, 2004) were used for data analyses.

5 Results and Discussion

5.1 Protein and bread-making quality of wheat varieties and breeding lines

As mentioned earlier in this thesis, bread is the absolutely most important food item in Tajikistan, and in average more than 60% of the daily protein intake of the population comes from wheat and its products (Braun, 2003). This fact makes wheat the major staple crop for Tajikistan, and bread-making quality a clear breeding asset together with yield. To assure high yield of good quality of the produced wheat, would contribute largely to the food security of Tajikistan, being one of the countries ranked on the top in the region of the hunger map published by the World Food Program of the United Nations (WFP, 2015). Previous studies have indicated a need for improvements in bread-making quality of the Tajik wheat material (Sarkisova *et al.*, 2006; Niyazmuhammedova *et al.*, 2004). Hence, increased efforts with a focus on improving the bread-making quality should be a target for the breeding programs of Tajikistan.

Bread-making quality of wheat is determined by the appearance and performance of the bread itself. However, within breeding programs there is a need to use high precision, cheap and fast methods (Watson *et al.*, 2017). Thus, a range of methods have been developed to be used both in breeding programs and by millers and bakers to evaluate bread-making quality, including e.g. Zeleny sedimentation volume, glutograph, mixograph, alveograph, farinograph etc (Razmi-Rad *et al.*, 2007; Johansson *et al.*, 2006; Branlard *et al.*, 2001).

Proteins are known as the component in the wheat grain with the largest impact on bread-making quality (Johansson, 1995). Thus, reliable fast and cheap methods have been developed to evaluate protein related factors affecting the bread-making quality. Protein related factors known for a high correlation with bread-making quality are the specific protein composition (Johansson *et al.*, 1993), i.e. mainly the composition of high-molecular-weight glutenin subunits (HMW-GS), and the amount and size distribution of polymeric proteins (Johansson *et al.*, 2001b). The former of these factors is measured applying electrophoresis and the later by size exclusion-high performance liquid chromatography.

This thesis has evaluated bread-making quality in Tajik wheat both from plant breeders' wheat material (Papers I, II, III) and from farmers' wheat (Paper V), applying electrophoresis (Paper I), HPLC (Papers II, V), gluten index, farinograph and baking tests (Paper III).

5.1.1 Specific protein composition (HMW-GS) of Tajik wheat

Electrophoresis (SDS-PAGE) determining the HMW-GS composition, revealed a general relatively high variation in the Tajik wheat material (Papers I, II). Thus, the protein composition indicated the presence of three, five and four different alleles at the *Glu-A1, Glu-B1*, and *Glu-D1* loci encoding the protein composition in the wheat varieties and lines (Papers I, II). However, the HMW-GS compositions 2*, 7+9, and 5+10 encoded on *Glu-A1*, *-B1* and *-D1*, respectively were highly dominating being present in 66.5%, 62.5 % and 74.3% of the analysed samples. All these three HMW-GS combination are to different degrees reported to be correlated with high gluten strength (Johansson *et al.*, 1993). The high content of the mentioned HMW-GS is also the reason for the generally high Payne quality score (Payne *et al.*, 1987) found in the Tajik wheat material. In total, 19 out of the 22 evaluated varieties and breeding lines showed a Payne quality score above 8 (possible values=3-10, and higher value is linked to higher gluten strength; Paper I).

High Payne quality scores are normally found in countries with a tradition of baking bread of French bread types requiring strong mixing while countries applying more gentle mixing use wheat with lower Payne score, e.g. in Sweden normal Payne quality score value of winter wheat is around 5 (Johansson *et al.*, 1995). Despite of the generally high Payne quality score found in wheat varieties and breading lines evaluated in this thesis, Tajik wheat has been reported as holding not enough good baking quality for the local requirements (Sarkisova *et al.*, 2006; Niyazmuhammedova *et al.*, 2004). By the use of the opportunities to combine different HMW-GS known to contribute with high gluten strength, it is possible to increase the Payne quality score even further in the Tajik wheat material. As to the combination of HMW-GS present in the Tajik wheat material, subunits contributing to the highest possible score are already present encoded from *Glu-A1* (2*) and *Glu-D1* (5+10). However, the HMW-GS 13+16 encoded on *Glu-B1* are known to contribute with higher gluten strength than 7+9 (Payne *et al.*, 1987), presently being the dominating composition from *Glu-B1* (Paper I). Thus, one opportunity for the breeders to improve the gluten strength of the Tajik wheat material is to change the HMW-GS composition of the *Glu-B1* locus.

High variation in protein composition was not only observed between the varieties and lines, but also within most of the varieties and breeding lines (Paper I). Differences observed in presence/absence of various HMW-GS in different varieties and breeding lines varied also over the two evaluated locations (Paper I). Thus, the only variety or breeding line found being homogenous for HMW-GS encoded from all three *Glu-1* loci was *ESKINA-8* (Paper I). However, *ESKINA-8* was the line revealed to have the lowest Payne quality score of all the varieties and breeding lines evaluated, and is therefore from a plant breeding for bread-making quality view not a relevant line for further use.

Presence of genetic variability is a pre-requisite for the breeding work, to be able to introduce novel characters and to select for the best options (Michelmore *et al.*, 2013). The high variability of HMW-GS in the Tajik wheat material could therefore be seen as beneficial in order to obtain novel varieties with improved bread-making quality. However, the variability was found higher within varieties (87%) than between varieties (13%) within the Tajik wheat (Paper I). Within variety variability is always a bit of an issue within plant breeding, due to the fact that the breeder cannot predict genetic composition of the offspring as any relative composition of the available gene combinations is possible. Despite this fact within variety variations as well as variety mixtures grown on the field can also be potentially beneficial, e.g at disease outbreaks when different parts of the grown population might hold various resistance genes protecting at least part of the crop (Cox *et al.*, 2004). Selection of plants in each of the evaluated varieties and breeding lines with HMW-GS contributing with gluten strength could, however, result in lines homogeneous for HMW-GS and with improved bread-making quality, still holding other beneficial characters as e.g. resistance genes or yield potential genes, suitable for local wheat production.

Protein composition analyses followed by clustering analysis applying Manhattan Distance resulted in varieties and lines with the highest Payne quality score gathered into one cluster. This cluster of varieties included the variety *Alex* and the breeding lines: *TNMU/MUNTA*, *SHARK/F4105W2.1* (*Shokiri*), *VORONA/KAUZ//1D13.1/MLT*, *YN/3NPM/VOS83*, *VORONA SN079*

(*Vahdat*) and *CMN82A.1294/2*KAUZ//* (Table 3). Thus, the Payne quality score analyses together with the cluster analysis allowed selection of lines with the highest amount of plants containing HMW-GS known to contribute to high gluten strength. Despite this, the full picture of the reason for the reported not enough good bread-making quality of the Tajik wheat was not depicted by the revealed HMW-GS composition.

5.1.2 Amount and size distribution of polymeric protein in Tajik wheat

Similarly as for specific protein composition determined with SDS-PAGE, a high variation in amount and size distribution of polymeric protein determined by SE-HPLC was revealed for Tajik wheat varieties (Papers II, V). This high variation was obviously independent if breeding material (Paper II) or farmers samples were evaluated (Paper V). However, no significant correlation was seen between specific protein composition and amount and size distribution of polymeric proteins in the Tajik breeding material. Thus, the specific protein composition of the Tajik wheat material was not a determinant of the development of protein polymers in the Tajik breeding lines (Paper II). Generally, specific protein composition, especially of HMW-GS is supposed to be the reason for variation in amount and size distribution of polymers in wheat, mostly determined as %UPP (Johansson *et al.*, 2013). However, the literature reports various degrees of correlations among these two protein factors (Zhang *et al.*, 2008), and most studies have used only one of the two methods to determine protein quality.

Previous studies have shown both genotypes and environments as important contributors to the determination of amount and size distribution of polymeric proteins (Johansson *et al.*, 2003; Johansson, 2002; Johansson *et al.*, 2002; Johansson *et al.*, 2001a). The work in this thesis clearly showed that present varieties and breeding lines played a limited role for the revealed variation in amount and size distribution (Paper II). Instead, cultivation localities (Paper II) and locations of farms (Paper V) and cultivation year (Paper V) played a significant role for the variation in amount and size distribution of polymeric protein. Previous investigations have clearly shown the impact of environment on the amount and size distribution of polymeric protein, where nitrogen availability, crop development time and temperature have been reported as the most important determinants (Johansson *et al.*, 2013; Malik *et al.*, 2011). Furthermore, soil properties have been reported to be of relevance for amount and size distribution of polymeric protein in barley (Malik, 2012). However, the varietal variation, mainly determined by specific protein composition and variety determined development time, use to be of similar significance as the environment (Rasheed *et al.*, 2016; Malik *et al.*, 2013; Johansson *et al.*, 2001b), if the genetic base is not too narrow.

Environmental variation related to differences in farm size and differences in altitude of cultivation did not significantly affect the amount and size distribution of polymeric proteins in the Tajik wheat material (Paper V). Differences in mechanisation due to farm size and differences in altitude are factors that may influence crop performance and development (Xiao *et al.*, 2008) and thereby quality, but these factors seemed of less significance for the Tajik wheat. Instead a local adaptation of certain varieties to specific environment seemed to prevail (Paper II). Local adaptation is a character often reported for organic production (Moreira-Ascarrunz *et al.*, 2016). Wheat production in Tajikistan is relatively traditional and low intense (Paper VI), thereby partly resembling organic production, which might be the explanation for the local adaptation of the Tajik wheat.

Generally, low grain protein concentration and low gluten strength was seen in the Tajik wheat material (Papers II, V). However, values of TOTE and %UPP were somewhat higher in the breeding material (Paper II) than in the farmers' material (Paper V). The higher values in the breeding material could be a positive result of the breeders' effort to improve bread-making quality of the Tajik wheat, but could also be a result of better farming practices in breeders' fields than in farmers' fields. As limited variety variation was found, the latter might probably be the main explanation for the variation in the results between the evaluated materials. Thus, for increased values of amount and size distribution of polymeric protein, there is a need for breeders to introduce novel genetic background into the breeding material that can contribute with these traits.

5.1.3 Grain, flour, dough and bread quality of Tajik wheat

The results from the grain, flour, dough and bread-making quality analyses carried out within this thesis work, showed limited variation in these characters among Tajik wheat varieties (Paper III), corresponding with the limited variation found in amount and size distribution of polymeric protein (Paper II). Despite the limited variation, PCA analyses contributed to revealing specific characters of interest for further breeding in some of the evaluated varieties. Thus, the varieties *Norman* and *Ziroat 70* were found to have high protein/gluten content and high values for some of the dough quality parameters respectively (Paper III).

The grain, flour, dough and bread-making quality analyses of the Tajik wheat varieties (Paper III) did not indicate more recent wheat material to perform better for these characters than that being a bit older. Thus, the differences reported in amount and size distribution of polymeric protein among farmers' varieties and breeding lines (Papers V versus II), indicating an improvement in bread-making quality in breeding lines, were not verified as a continuous improvement in quality in novel varieties (Paper III).

Despite the fact that previous studies have indicated a need for improvement of the baking quality in Tajik wheat (Sarkisova *et al.*, 2006; Niyazmuhammedova *et al.*, 2004), as was also indicated by the HPLC data (Papers II, V), baking of the varieties ended up in bread with high values for bread surface and shape, and crust and crumb color (Paper III). However, the Tajik wheat varieties generally received lower values for porosity and elasticity indicating opportunities for improvement (Paper III).

5.1.4 Targets and opportunities to improve bread-making quality in Tajik wheat

This study revealed the need to strengthen the gluten quality, and to improve porosity and elasticity of bread doughs of Tajik wheat varieties. Improvements of these wheat varieties may be possible if protein composition in the present wheat material is made more homogeneous, through selection of plants with protein composition known to correlate with high gluten strength. Through the use of electrophoretic methods, breeders have the ability to select suitable protein composition on plant basis (Johansson, 1995). Also, the varieties and lines pointed out in this study with highest TOTE, %UPP (Paper II) and best bread performances (Paper III) can be selected and used in further breeding programs. However, limited variation for the trait was generally seen in the Tajik wheat material, and therefore, the need of introduction of novel variety variation might also be a must.

The fact that a local adaptation was seen in the material, often also found important in organically cultivated crops (Moreira-Ascarrunz *et al.*, 2016), indicated similarities between the farming systems applied in Tajikistan with those being organic. The work in this thesis also revealed a low degree of mechanisation and farms mostly being small in Tajikistan (Paper VI), which further point in the direction of such similarities. The need of local adaptation should thereby to be taken into consideration in breeding for improved bread-making quality of Tajik wheat. Furthermore, baking requirements of the Tajik wheat needs to be further evaluated. Baking industry around the world is known to have different desires as to strength of gluten for various applications (Johansson, 1995).

5.2 Status of seed-borne diseases

Poor seed quality has been identified as one of the major problems in wheat production of Tajikistan (Husenov *et al.*, 2008; Pett *et al.*, 2005). The health status of seed is often related to presence and quantity of so-called seed-borne diseases, transmitted as potentially pathogenic microorganisms through the seed to the next generation of plants (Munkvold, 2009; Pearce, 1998; Richardson, 1990). Tajik farmers are known to commonly use non-certified farm saved seeds (Muminjanov *et al.*, 2008), and such farming practice has in previous investigations been shown to have relatively higher level of seed-borne diseases (Bishaw *et al.*, 2013).

Fungi are the main cause of the majority of the seed-borne diseases in wheat (Richardson, 1996; Richardson, 1990). Prevalent seed-borne diseases in wheat globally are common bunt and loose smut although also black point, fusarium head blight and glume blotch are common (Wilcoxson & Saari, 1996; Mathur & Cunfer, 1993). Common bunt is historically well known, it is impacting yield and flour quality and is caused by two related fungi (*Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn) (Wilcoxson & Saari, 1996). Loose smut is caused by the fungus *Utsilago tritici* (Pres.) Rostr. (Wilcoxson & Saari, 1996). Black point is a collection name of a number of diseases causing discoloration of the wheat seed, although the most common fungi causing this discoloration are *Alternaria* spp., *Dreschlera* spp. and *Bipolaris sorokiniana* (McIntosh, 1998; Mathur & Cunfer, 1993; Culshaw *et al.*, 1988).

This thesis has identified presence and composition of seed-borne fungi both in wheat breeding material (Paper IV) and in wheat seed used by farmers (Paper V). Furthermore, field incidences of common bunt (Papers IV, V, and VI) and loose smut (Papers V, VI), as well as black pointed seeds (Paper IV, V) were evaluated. Presence of common bunt (Papers IV, V) was also evaluated by the CWT test in lab conditions, and black pointed seeds were correlated to presence of fungi in the seeds. Responses of the Tajik wheat towards common bunt were reported in Paper IV.

5.2.1 Presence and composition of seed-borne pathogenic fungi in Tajik wheat

Totally, fourteen fungal species were identified in seed samples collected from breeding nurseries during the 2010 and 2011 harvesting seasons (Paper IV). Furthermore, a total of eighteen fungal species were found in seed samples of farmers' wheat sampled over two years (2011 and 2012) in 20 farms (Paper V). The most commonly identified fungal species in breeding materials was found to be *Tilletia laevis*, *T. tritici*, *Bipolaris sorokiniana*, *Stemphylium* spp., and *Drechslera* spp. (Paper IV). In farmers' seeds, the most prevalent fungal species found beside *T. laevis*, were *Alternaria* spp., *Nigrospore* and *Aspergilus niger* (Paper V), the two latter ones not reported at all for the breeders' samples (Paper IV).

In general, the pathogenic presence on the seeds of the Tajik wheat must be seen as rather high. This might be the result of limited use of chemical treatments and of the use of farmer saved seeds (Paper VI). Some of the identified seed-borne fungi are known to be saprophytic, causing damages to the seed or plant in some stages of the life cycle of the pathogen (Mathur & Cunfer, 1993), or being toxic or allergenic to humans (Lõiveke *et al.*, 2004; Richardson, 1990). Thus, the high content on the Tajik wheat seeds cannot be seen as beneficial within crop husbandry. However, the focus of this thesis work was on potentially pathogenic fungi. Thus, this thesis was able to identify *T. laevis*, *T. tritici*, *B. sorokiniana*, *Stemphylium* spp., *Fusarium* spp. and *Cladosporium* spp. as the major pathogenic fungi in both breeders' and farmers' wheat, and additionally the pathogenic fungi *Drechslera* spp. was found in breeders' wheat and *Curvularia* spp. was found in farmers' wheat (Papers IV, V). Reasons for the somewhat different composition of fungi in breeders' and farmers' wheat were not further revealed in this thesis, but possible reasons might be differences in year and/or regions of growth of the wheat material (Paper IV, V).

In both breeders' and farmers' wheat it was clearly revealed that *T. laevis* was the most prevalent of the two fungi causing common bunt and thereby also the major cause for this disease in Tajik wheat (Papers IV, V). Furthermore, it was clear from this thesis work that although a large amount and rather high content of pathogenic fungi was present on the seeds, plants on the field was not diseased to a similar rate, neither in breeders' material (Paper IV), nor in farmers' material (Paper V). Similar findings of fungi present in the majority of fields but with low level of diseased plants have also been reported in other studies from low input wheat farming (Kayaly *et al.*, 2011).

5.2.2 Field incidences of common bunt and loose smut, and presence of black pointed seeds

The field incidences of common bunt and loose smut was generally found to be low in both breeding trials (Paper IV) and farmers' fields (Papers V, VI), with highest presence in the field survey of farmers' fields in 2012-2014 (Paper VI) with 11% and 5%, respectively. However, in cases with incidences, disease rate was occasionally found to be high, thereby contributing to large yield losses (>50%; Paper V).

Field incidences of common bunt varied over locations (Paper IV) and years (Paper V) of evaluation and one reason for these variations might be differences in climatic conditions, with more humid conditions contributing to increased occurrence.

Black pointed seeds were present in most of the Tajik wheat material (Papers IV, V), with frequencies of up to 28% in farmers' wheat (Paper V). Previous studies have indicated the major causes of the black point disease as either a susceptibility of varieties grown (Conner & Thomas, 1985) or late rain at the end of growing season, which enhances the disease (Conner, 1989).

5.2.3 Common bunt on seeds and reasons of black point

The use of the CWT test showed presence of common bunt inoculum on the majority of the seeds from both breeders' (Paper IV) and farmers' (Paper V) fields. *T. laevis* was found to be the predominant of the two common bunt causing fungi present on the wheat seeds during both evaluated years (2011, 2012), both locations of breeders' fields and on farmers' fields, although also *T. tritici* was present (Papers IV, V). Differences in frequency of presence of the fungi were found for the different locations (Paper IV) and for different farms (Paper V). However, no obvious reasons for the found variations were revealed.

The most commonly found fungi on black pointed seeds was *Alternaria* spp. (Papers IV, V), which has also been reported in previous studies (Toklu *et al.*, 2008; Cromey & Mulholland, 1988; Rees *et al.*, 1984). Furthermore, this thesis showed *Aspergilus niger, Bipolaris sorokiniana, Fusarium* spp., and *Drechslera* spp., also being present on black pointed seeds, the latter one also being reported as a cause by other authors (Pett *et al.*, 2005). The major drawback reported from black pointed seeds is a degradation of grain quality (Sisterna & Sarandón, 2010; Wallace & Sinha, 1975), and a decrease of the germination capacity of the seed (Toklu *et al.*, 2008; Rees *et al.*, 1984), although reports are not consistent (Cromey & Mulholland, 1988).

Table 3. *Overall results of studied varieties/lines*

	Paper I			Paper II	Paper IV (reaction to common bunt)		
ID*	Payne score (ave.)	HMW-GS from *Glu-D1*	cluster	%UPP	field reaction	reaction in greenhouse test	infection percentage (average)
1	8.86	5+10/2+12	II	0.45	S***	S	79.2abcd**
2	9.59	5+10/2+12	III	0.48	S	S	100.0a
3	8.98	5+10/2+12	I	0.47	n/a	n/a	n/a
4	9.50	5+10/2+12/4+12	III	0.48	S	S	96.3ab
5	8.93	5+10/2+12	II	0.48	MR	S	86.1abc
6	9.77	5+10/2+12	III	0.49	MR	S	19.8f
7	9.59	5+10/2+12/4+12	III	0.48	S	S	70.6cd
8	7.98	2+12	IV	0.53	S	S	62.7de
9	8.91	5+10/2+12	I	0.48	S	S	92.2abc
10	9.00	5+10	I	0.52	S	S	94.4ab
11	6.00	4+12	IV	0.48	S	S	86.0abc
12	9.95	5+10	III	0.50	S	S	43.0e
13	8.86	5+10/2+12/4+12	I	0.43	S	S	96.9ab
14	8.93	5+10/2+12	II	0.51	S	S	87.5abc
15	9.39	5+10/2+12/2+10	III	0.46	S	S	87.8abc
16	8.18	2+12/5+10	IV	0.48	S	S	94.8ab
17	7.11	2+12/5+10	IV	0.46	S	S	92.0abc
18	8.86	5+10/2+12	I	0.51	S	S	100.0a
19	8.07	2+10/5+10/2+12	IV	0.47	S	S	82.4abcd
20	9.23	5+10	II	0.51	S	S	94.4ab
21	9.02	5+10/2+12	II	0.50	S	S	96.7ab
22	9.86	5+10	III	0.59	S	S	94.5ab
23					S	S	95.5ab
24					S	S	74.5bcd
25						R	0f

Note: * IDs correspond to varieties/lines given in Table 2.

** Tukey comparison: means that do not share a letter are significantly different ($P < 0.05$).

*** Reaction of varieties/lines to wheat common bunt: S-Susceptible, MR-moderately resistant; R-resistant;

5.2.4 Evaluation of genotypes' response to common bunt

Reaction of Tajik wheat varieties and advanced breeding lines towards infection of common bunt showed no distinct resistance to be present in (Papers IV, VI). Only one breeding line (*SHARK/F4105W2.1*) showed moderate resistance in the field test (5 to 10% infection) and lowest infection percentage (19.8%) in the greenhouse test (Table 3.). As discussed in previous chapters, *T. laevis* was found to be a predominant common bunt cause in Tajikistan. According to (Goates, 1996) only 10 races of *T. laevis* are known and with the exception of *Bt1*, *Bt2* and *Bt7*, most of the other *Bt* genes are effective against the *T. laevis* races. Thus, a further action within the Tajik wheat breeding should therefore be to identify the most widely spread races of common bunt in Tajikistan. Thereafter, wheat material from outside Tajikistan with known *Bt* genes against most spread races should be introduced into the Tajik wheat breeding material.

5.3 Relationships between bread-making quality and seed-borne fungi in Tajik wheat

This thesis work indicated presence of an effect of seed-borne fungi on the wheat seeds, on grain protein content and composition (Paper V). Similar negative relationship between presence of seed-borne fungi and a decrease in quality have also been reported in previous studies (Petrov & Filipović, 2011). Thus, as protein composition in the Tajik wheat material, generally has a composition normally correlated to high gluten strength (Paper I), and despite this bread-making quality is not sufficient (Paper III), one might speculate whether the high prevalence of seed-borne fungi might be part of the explanation of the not satisfactorily quality (Paper V).

5.4 Major limiting factors of wheat production in Tajikistan

Within this thesis, field surveys were conducted in 2011 and 2012 (Paper V) and 2012 to 2014 (Paper VI), allowing to identify the major limiting factors in wheat production. Important key factors identified to target for increased yield and quality within wheat production in Tajikistan was i) to improve farmers' knowledge (Papers V, VI), ii) to use certified seed for production (Papers V, VI), and iii) to use suitable management systems, in particular for weed management (Paper VI). The low knowledge level of the farmers, not being aware of what variety they were using (Papers V, VI), using farmers saved seeds (Papers V, VI), low level of mechanization and lack of use of modern technologies (Paper V), and low levels of inputs (Paper VI) influenced to a high extent the performance of the crop on field (Paper VI). Also, several of the factors interacted with each other, low technical knowledge level of farmers were related with use of farmer saved seeds with lack of resistance and co-harvested with the weeds from the previous year, as well as with low crop management (Paper VI). A change from the use of farm saved seeds to certified seeds is impacted by availability and trust on certified seed (Bishaw, 2004), but also by the farmers' willingness to use certified seed, status of knowledge, and financial abilities (Ali *et al.*, 2015). Seed certification has been implemented in many countries around the world to control spreading of diseases and major yield losses (Diekmann, 1993). In Tajikistan, implementation of seed certification is not properly carried out yet (Husenov *et al.*, 2008).

This thesis evaluated impact of farm management (Paper V), regions (zones; Paper V), altitudes (Papers V, VI), farms of different sizes (Paper V) on farming practices. None of these variations explained to a similar extent as the key factors described above the yield constraints within wheat production in Tajikistan. Therefore, capacity development activities, combined with variety development and certification, and development of farm management practices should be the objectives to develop a social, economic and environmentally sustainable wheat production system in Tajikistan. This thesis showed human resources developments (HRD) activities being a need for all types of farmers, due to the fact that knowledge and success in wheat production seemed to be lacking in all types of farms (Paper V). Thus, the capacity development activities needed are both formal trainings in schools, as well as demonstration and disseminations activities to spread present and novel knowledge to farmers as related to sustainable crop production, major diseases and weeds and opportunities to improve the bread-making quality of wheat.

6 Conclusions and recommendations

Based on the results obtained within this thesis work, the following major conclusions can be drawn:

- Large variation in specific protein composition was found in the Tajik wheat material, allowing the breeders to work further with improvement of bread-making quality of wheat varieties. However, heterogeneity was observed to a high extent within the varieties and lines, which clearly points towards the need for improvement of maintenance breeding, as well as early generation seed production.
- The Tajik wheat generally showed a high Payne quality score and a high content of HMW-GS indicating high gluten strength. Despite this, amount and size distribution of polymeric protein as well as baking data did not show exceptional quality or strength within the Tajik wheat, neither much of variation in quality.
- Local adaptation was found for the Tajik wheat in terms of quality measurements, similar as is often found for organic wheat. Thus, breeding for quality in Tajik wheat should adapt to procedures applied for organic wheat quality breeding.
- Seed-health status of Tajik wheat was found depressing and thus, use of certified seeds is highly recommended together with the decrease of the use of farmer saved seed.
- The present Tajik wheat material was found to lack resistance towards common bunt, thus, introduction of resistance genes from wheat material with such resistances is highly recommended.
- To increase yield and quality of Tajik wheat production, the major keys are; to educate and train the farmers', to use certified seeds of good quality and to improve crop management not least as related to weed handling.

7 Future prospects and strategies towards wheat production improvement in Tajikistan

Based on the results from this thesis work, the below future prospects and strategies are of relevance for improvements of wheat production in Tajikistan:

- Bread-making quality requirements for bread made in Tajikistan should be determined and characterized;
- A detailed study, evaluating impact of specific composition of glutenin subunits, and amount and size distribution of protein, on the typical Tajik bread should be carried out. From these results, it should be determined if the specific protein composition or/and the amount and size distribution of proteins is of relevance to be used as a tool in the breeding programs while breeding for improved bread-making quality.
- Impact of heat stress and seed-borne fungi on the protein composition should be further elucidated in terms of interactions contributing to effects on bread-making quality.
- Local adaptation, similarities to organic systems of cultivation and eventual wishes to change the production systems should be dealt with and strategies of how to cope with this in the future needs to be developed.
- Prevailing races of *Tilletia* spp., causing wheat common bunt in Tajikistan should be identified;
- Common bunt resistance test experiments should be developed and broadened to include major varieties, as well as local landraces;
- A national strategy with concreate actions and recommendations should be developed to cope with wheat production challenges.

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Möjligheter att påverka livsmedelssäkerheten i Tajikistan: En förbättring av vete- och utsädeskvalitet är centralt för framgång

Brödvete (*Triticum aestivum* L.) är världens viktigaste livsmedelsgröda och i Tajikistan bidrar vete med 60 procent av befolkningens dagsbehov av protein och kalorier. I denna avhandling utvärderades proteinsammansättning och brödbakningskvalitet samt förekomst av fröburna sjukdomar i Tajikiskt vete. Vidare så utvärderades sambandet mellan fröburna sjukdomar och proteinkvalitet samt de huvudsakliga begränsningarna för hög avkastning av bra kvaliet hos vete i de Tajikiska produktionssystemen.

Det Tajikiska vetet uppvisade hög variation i proteinsammansättningen men samtidigt en hög heterogenitet. Majoriteten av de analyserade sorterna/linjerna uppvisade höga Paynepoäng, eftersom de högmolekylära gluteninsubenheterna (HMW-GS) 5+10 var vanligt förekommande. Ingen korrelation kunde påvisas mellan HMW-GS-sammansättningen och förhållandet mellan svårextraherbara polymera proteiner och total mängd polymera proteiner (%UPP). Vidare, indikerade %UPP ett starkt gluten i det Tajikiska vetet. Tajikiskt vete och dess brukande uppvisade likheter med ekologisk odling av vete, bla genom lokalanpassning av genotyperna.

Totalt identifierades fjorton och arton olika svamparter i fröprover från växtförädlarnas respektive böndernas fält. De mest betydelsefulla svamparna i materialen var *Tilletia laevis*, *T. tritici*, *Bipolaris sorokiniana* och *Stemphylium* spp., medan *Alternaria* spp. var de vanligaste förkommande svamparna.

Förekomst av stinksot och flygsot var generellt låg i fält trots att inokulun av stinksot fanns på mer än 50% av de undersökta vetekärnorna; *T. laevis* var mest vanligt förekommande. De Tajikiska vetesorterna och linjerna saknade i hög grad resistens mot stinksot. Förekomst av vissa svampar korrelerade signifikant med proteinkvaliteten hos vetet.

Böndernas kunskapsnivå var generellt låg. Närvaro av fröburna sjukdomar, låg proteinhalt och svagt gluten påverkade vetekvaliteten negativt, oberoende av typ av jordbruk och böndernas kunskapsnivå.

Sammanfattningsvis behövs samordnade åtgärder för att förbättra veteproduktionen i Tadzjikistan. Avhandlingen visar tydligt på behovet av att öka kunskapsnivån hos jordbrukarna genom förbättring av utbildnings- och rådgivningssystemen. Ökad utbildning borde leda till en hållbar användning av certifierat utsäde samt ogräs- och skadedjursbekämpningsmedel, och en förbättrad växtföljd, etc. samtidigt som en negativ inverkan på Tadzjikistans biologiska mångfald bör undvikas. Systemet för att möjliggöra användning av certifierat utsäde av nya resistenta och högkvalitativa vetesorter kräver också riktade åtgärder.

Дар роҳи таъмини амнияти озуқавории Тоҷикистон: беҳдошти сифати дон ва тухмии гандум бо истифодаи селексияи растанӣ

Гандуми мулоим (*Triticum aestivum* L.) зироати муҳими ғизоӣ барои аҳолии дунё мебошад ва зиёда аз 60% талаботи рӯзмарраи мардуми Тоҷикистон ба сафеда ва калория аз маҳсулоти нонӣ таъмин мегардад. Дар кори илмии мазкур таркиби сафеда ва нишондиҳандаҳои сифати нони гандум, касалиҳои асосии бо тухмӣ паҳншаванда ва таъсири онҳо ба ҳосил ва сифати дон, устувории навъу намунаҳои гандум ба ин касалиҳо, иртиботи касалиҳо ва сифати сафеда, ҳамчунин душвориҳои асосии парвариши гандум дар Тоҷикистон таҳти омӯзиш қарор гирифтанд.

Ташхиси дони навъу намунаҳои гандуми аз Тоҷикистон ҷамъоварӣ гардида имкон дод, ки дигаргунии васеъ дар таркиби сафедаҳо исбот гардад. Дар ин раванд инчунин омехтагии навъҳои гуногун мушоҳида шуд. Таҳқиқот тасдиқ намуд, ки бештари навъу намунаҳои гандуми Тоҷикистон дорои нишондиҳандаи баланди сифатии Payne (Payne quality score) мебошанд, ки мавҷудияти сафедаҳои вазни молекулавиашон бузург (HMW-GS), махсусан 5+10 (*Glu-D1*), ба ин мусоидат кардаанд. Исбот гардид, ки байни нишондиҳандаи таркиби сафедаҳои вазни молекулавиашон бузург (HMW-GS) ва миқдори сафедаҳои полимерии ҳалнашаванда нисбати миқдори умумии сафедаҳои полимерӣ (%UPP) вобастагӣ вуҷуд надорад ва ин нишондиҳандаи сифати хуби ширешаки хамир шуда наметавонад. Мутобиқгардии навъҳои гандум ба шароити маҳал ва бо усулҳои монанд ба кишоварзии органикӣ парвариш намудани гандум бештар ба чашм мерасанд.

Дар намунаҳои тухмии аз қитъаҳои селексионӣ ва истеҳсолӣ ҷамъоваришуда ҳаждаҳ намудҳои гуногуни замбӯруғҳои барангезандаи касалӣ муайян гардиданд. *Tilletia laevis, T. tritici, Bipolaris sorokiniana* ва *Stemphylium* spp. намудҳои асосии замбӯруғҳое мебошанд, ки сабабгори касалиҳои гандум мегарданд. Тасдиқ гардид, ки намояндаи замбӯруғи *Alternaria* spp. дар тухмии гандум аз ҳама бештар паҳн гардидааст.

Гарчанд, ки таи солҳои омӯзиш сиёҳаки сахти гандум ва сиёҳаки гарднок дар гандумзор нисбатан камтар мушоҳида шуда буданд, аммо мавҷудияти спораҳои сиёҳаки сахт дар аксарияти намунаҳои тухмӣ зиёда аз 50%-ро ташкил медод. Муайян гардид, ки барангезандаи асосии сиёҳаки сахт дар Тоҷикистон замбӯруғи *T. laevis* будааст. Дар натиҷаи санҷиш тасдиқ гардид, ки навъу намунаҳои гандуми Тоҷикистон ба спораҳои сиёҳаки сахт устувор нестанд. Аз ин сабаб ҳангоми васеъ паҳншавии ин касалӣ ҳосили зиёд талаф меёбад. Муқаррар гардид, ки байни сифати сафедаи гандум ва баъзе аз касалиҳо иртиботи назаррас мавҷуд аст.

Омӯзиш инчунин муайян намуд, ки савияи дониш ва имконияти аксарияти деҳқонон дар парвариши гандум ба такмилёбӣ ниёз доранд. Аммо, новобаста аз донишу малакаи деҳқонон ва шакли хоҷагидорӣ, пайдоиш ва паҳншавии касалиҳои бо тухмӣ паҳншаванда, сифати пасти сафеда ва ширешаки сусти хамир, инчунин ҳавои гарму хушк дар давраи пухтарасии дон ба сифати нони гандум таъсири манфӣ мерасонанд.

Аз ин лиҳоз, бо мақсади рушди соҳаи гандумпарварӣ ва таъмини амнияти озуқаворӣ бояд чораҳои мушаххас ва зарурӣ андешида шаванд. Дар асоси натиҷаҳои таҳқиқот тавсия дода мешавад, ки савияи дониши деҳқонон бо роҳҳои омӯзиш ва машваратҳои кишоварзӣ баланд бардошта шаванд. Деҳқонон бояд батадриҷ барои истифодаи тухмии сертификатсияшуда, мубориза устувор бо касаливу зараррасонҳо ва бо алафҳои бегона, риояи киштгардон ва дигар қоидаҳои парвариши гандум, ки дар умум ба саломатии инсон ва табиат безарар мебошанд, комилан ошно гардида, барои бадастории ҳосили дилхоҳ муваффақ шаванд. Дар ин раванд ҷорӣ намудани системаи бонизоми истифодаи тухмии сертификатсияшудаи навъҳои ба касаливу заррарасонҳо устувор ва дорои сифати хуби истеъмолӣ, яке аз тавсияҳои муҳими корӣ илмии мазкур мебошад.

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