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**Faculty of Agrobiology, Food and Natural Resources**

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# WHEAT SEEDS TOLERANCE TO WATER STRESS

Diploma Thesis

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Field of Study: Sustainable Agriculture and Food Security

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## Declaration

I declare that the Diploma Thesis "Wheat seeds tolerance to water stress" is my work and all the sources I cited in it are listed in the Bibliography.

Prague, 20 July 2020 Signature ___________________________

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# Wheat seeds tolerance to water stress

## Abstract

Wheat is traditionally an important crop in the Czech Republic. It has now become evident that in Central Europe, climate change probably increases the frequency of water stress conditions. Hence, the need to enhance the adaptive capacity of crop production. This research reviews existing knowledge on wheat seed development, its ability to germinate and tolerate moisture stress conditions as a function of seed vigour. The hypothesis is "It is possible to select stress tolerant seed lots by the detailed seed testing in stress conditions". The practical part of the research were laboratory experiments conducted at the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences in Prague. Eight wheat cultivars namely Genius, Annie, Matchball, Turandot, Hyfi, Tobak, Bohemia and Julie were chosen for this study to evaluate performance during seed germination and coleoptile emergence. Following the International Seed Testing Association (ISTA) rules, seed lots were prepared in four replications of 100 seeds per seed lot. Seed lots were subjected to two bed moisture levels: higher dose 30 ml (optimal) and lower dose 20 ml (stressed variant) of tap water at two incubation temperatures of 20 °C and 15 °C (set in the climabox). The germination parameters evaluated were germination percentage (TG), germination energy (GE), mean germination time (MGT) and coleoptile emergence (COL). Results were statistically evaluated. The difference between a higher dose of moisture and the lower variant did not have a significant effect on total germination in 5 out of the 8 seed lots assessed: Genius, Annie, Turandot Hyfi and Julie. There were slightly significant differences between germination percentage in Bohemia, Tobak and Matchball. Bohemia showed maximum germination values (100%) in all interactions of moisture and temperatures. However, at 15 °C germination energy was slower, as it were in all cultivars. Under the influence of 15 °C and 20 ml, Annie showed the least germination values (92%). Although Bohemia had the best response, gemination energy was significantly slower at 15 °C than 20 °C. Mean germination time varied between cultivars. Coleoptile emergence was significantly affected by aberrant temperature rather than moisture differences. In conclusion, the hypothesis was accepted on the premise that cultivars performed differently. Aberrant temperature significantly reduced germination energy and coleoptile emergence more than bed moisture differences.

**Keywords:** germination, vigour, wheat seeds, water stress, tolerance

## Content

# 1 Introduction

Seed germination is an intricate physiological process of plant development during which the seed must quickly recover physically from maturation drying, resume a sustained intensity of metabolism, complete essential cellular events to allow for the embryo to emerge and prepare for subsequent seedling growth. Nonogaki et al. (2010) described germination as ‘still a mystery'. From an economic and physiological standpoint, successful germination is ultimately characterised by uniform and optimal seedling numbers that result in higher seed yield (Kaydan and Yagmur, 2008).

One particular area that remains a baffling mystery to seed scientists and breeders is tolerance of orthodox seeds to water stress during germination and seedling developmental stages. It is a phenomenon that requires further exploration because of its complexities. With recent discoveries, studies should model ways to maximise crop productivity in the face of environmental adversities. Water stress tolerance is genetically complex because desired traits are likely to be polygenic and responses between interspecies greatly vary. Tolerance to moisture stress, during germination as in all developmental stages of plant growth, is controlled by many genes (Foolad et al., 2003).

In the process of seed development, there are successive and intricate physiological processes that the seed has to go through to produce the best qualities required for germination. The interaction of the genotype and environment (GxE interaction) provides varying performance during anthesis, fertilization, grain filling and maturation drying before the seed enters into dormancy. These processes aim to produce the best quality of seed as a means for survival. As alluded, seed germination is an important yet vulnerable stage in the life cycle of terrestrial angiosperms and determines seedling establishment and plant growth (Zhang et al., 2010). For a good crop establishment, seeds require a suitable range of temperatures and gases during germination. Some species may require nitrates and/or light. As equally important, seeds require the correct amount of moisture to initiate biochemical processes leading up to germination.

Orthodox seeds such as wheat, rice and maize are regarded as the most important grains for human sustenance. They remain a major part of the global food basket. Bread wheat (*Triticum aestivum*) is the most grown crop on the largest arable area worldwide of approximately 215 million ha and revealing the third-highest grain production of 715.9 million tons after rice and maize (FAO, 2013). Every region in which wheat is grown, the effects of water stress tend to vary in their timing and severity. Water stress is a major problem in agriculture and the ability to withstand such stress is of immense economic importance. Water stress limits global wheat productivity more than any other stress. By definition, stress with a constraint or high unpredictable fluctuations imposed on regular metabolic patterns causes injury, disease, or aberrant physiology (Shao et al., 2008).

Several genetic resources, cultivars or landraces have proven to adapt to specific water stress conditions. The seed manoeuvres some active mechanisms aimed at an adjustment of cell metabolism to a decreased intracellular water content (osmotic adjustment). The aim is to provide cellular protection and cellular repair during rehydration. Seed germination and seed vigour are prerequisites for the successful wheat establishment and under rainfed, conditions of arid and semi-arid regions; low moisture is the main limiting factor for germination (Abdul et al., 2011).

The rate and degree of seedling establishment is an important function of grain yield. Screening of different cultivars for their resistance and tolerance to water and other abiotic stresses is used to find out the best cultivar for planting (Zahir et al., 2007). Seed germination studies have shown variations between maximum germination percentages, shoot length root length, germination rate index, coleoptile length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight and root/shoot ratio under different water stresses.

# 2 Hypothesis and Objectives

**Hypothesis:**

It is possible to select stress tolerant seed lots by detailed seed testing in stress conditions.

**Objectives of the thesis:**

1. To obtain knowledge about wheat seed tolerance to water stress during the germination stage
2. To evaluate the response of wheat cultivars to water stress conditions at specific incubation temperatures during germination and seedling elongation stages

This will provide a theoretical basis for selecting seed lots with tolerant abilities, for quick and uniform seedling establishment in water stress conditions.

The main part of the thesis will be laboratory experiments with 8 different seed lots (8 cultivars) of *Triticum aestivum* L.

# 3 Literature review

## 3.1 The wheat caryopsis:

### 3.1.1 A peculiar grain to the world

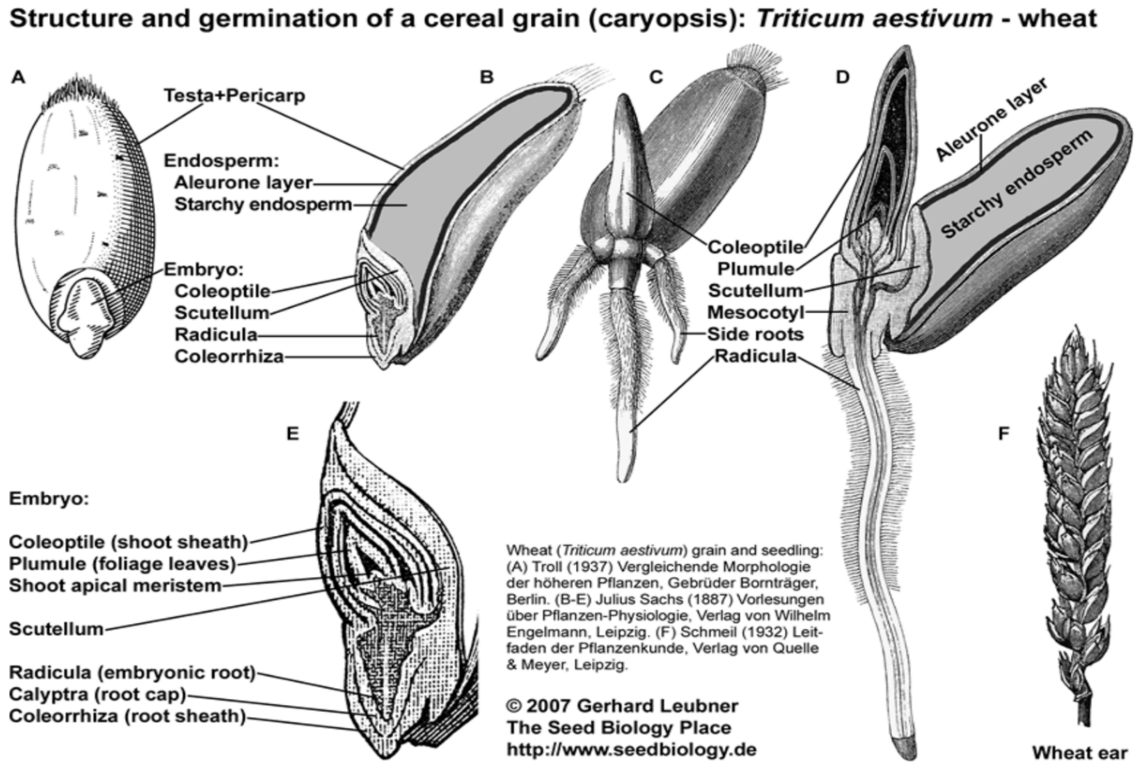
Wheat is a staple food of about one-third of the world's population (Raza et al., 2012). It belongs to the endospermic family of seeds, Poaceae or Gramineae. According to Delcour and Hoseney (2010), wheat contributes approximately 20% of the total caloric intake in humans. The binomial name for Bread wheat is *Triticum aestivum* which refers to the hexaploid (AABBDD) bread wheat. In North America, *Triticum aestivum* wheat is divided into soft and hard wheat. The soft cultivars are used for cookies or biscuits while the hard cultivars are used in breadmaking. The terms hard and soft where coined on how much force is needed to crush them. Soft wheat is easier to crush than hard wheat. In contrast, in some European countries, the term soft wheat is used for both non-breadmaking and breadmaking *Triticum aestivum* wheat. Hard wheat refers to *Triticum durum* which is the raw material for pasta production.

In the whole plant kingdom, it is only wheat caryopsis that has gluten proteins capable of forming the fully viscoelastic dough required to bake leavened bread (Goleman et al., 2019). The gluten proteins which constitute prolamins and glutelins are the storage proteins in wheat. The plant stores these proteins for use by seedling during germination (Delcour and Hoseney, 2010). In cereals, these proteins are only found in the endosperm.

Wheat is propagated in all continents except Antarctica. This implies that it can be grown in many regions with heterogeneous types of weather, elevation, or soil. It is mostly cultivated between the latitudes of 30°N to 60°N and 27°S to 40°S (Nuttonson, 1955; Enghiad et al., 2017), up to 3,000 meters above sea level, and in places with temperatures between 3 °C and 32 °C. It is also known that the land area where wheat is grown receives an average of 375 to 875 mm of annual precipitation. However, Enghiad et al. (2017) noted that wheat can be grown in a wider set of locations where precipitation ranges from 250 to 1750 mm.

Over 90% of the wheat grown worldwide is the *aestivum* species (Goleman et al., 2019). The wheat caryopsis is the reproductive unit of the wheat plant and its structure can be broadly divided into three components: the seed coat 14% and aleurone layer (or bran), the endosperm 83% and the embryo (germ) 3% (White and Edwards, 2008). The endosperm of highly specialized seeds such as wheat, barley and maize can be divided in the starchy endosperm (starch grains, food storage, dead cells, flour) and the aleurone layer (living cell layer surrounding the starchy endosperm) (Leubner-Metzger, 2007). The caryopsis of North

American wheat average about 8 mm in length and weigh about 35 mg. European wheat weighs an average of about 55 mg (Delcour and Hoseney, 2010). The sizes differ among cultivars and the location in the head or spike.



Wheat (*Triticum aestivum*) grain and seedling: (A) Troll (1937) Vergleichende Morphologie der höheren Pflanzen, Gebrüder Bornträger, Berlin. (B-E) Julius Sachs (1887) Vorlesungen über Pflanzen-Physiologie, Verlag von Wilhelm Engelmann, Leipzig. (F) Schmeil (1932) Leitfaden der Pflanzenkunde, Verlag von Quelle & Meyer, Leipzig.

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Figure 1: The structure and germination of a wheat caryopsis. Courtesy of (Leubner-Metzger 2007) Source: The Seed Biology Place.

### 3.1.2 Wheat production in the Czech Republic

Wheat is traditionally an important crop in the Czech Republic. According to Mikulasova (2018) reporting on the Czech Statistical Office records, wheat occupied 33% (approximately 820,000 ha) of the total crop area in the year 2018. This was followed by other major crops: fodder crops 19%, rapeseed 17% and barley 13%. The total yield recorded in 2018 was 4,514,000 metric tons with an average of 5.67 metric tons per ha. In 2000, the total growing area for wheat was 970,000 ha with an average of 4.21 metric tons per ha (Daunay et al. 2004).

In 2018, at least 86 wheat cultivars (classes E, A, B) were evaluated from samples obtained across the Czech Republic. At least 10 most commonly grown wheat cultivars were Genius (E) (8.0%), Julie (E) (7.6%), Tobacco (B) (6.4%), Viriato (A) (5.8%), Dagmar (A) (3.4%), Fakir (A) (3.3%), Matchball (A) (3.3%), Patras (A) (3.1%) and Rivero (A/B) (2, 7%). The yield of the 10 most numerous cultivars ranged on average from 7.2 t/ha (Matchball) to 5.0

t/ha (Fakir). Average of the 10 cultivars was 6.3 metric tons per ha based on data provided by growers (Polišenská et al. 2019).

Wheat improvement has produced cultivars of better seed quality. Wheat flour and rye flour are usually mixed for bread-making hence quality is of utmost importance. There are four categories used to classify the quality of registered wheat cultivars. The first class, E (Elite), is for wheat cultivars with the best breadmaking qualities. Class A (high bread baking quality) is the second category for cultivars with very good baking qualities. Class B is for those qualities with acceptable breadmaking qualities. Last, Class C is not suitable for breadmaking and cultivars are usually produced as fodder for pigs, cattle and poultry. Abiotic stresses pose a great risk to wheat yield and quality in the years to come.

### 3.1.3 Water stress and global risks to wheat production

Agriculture is both a cause and a victim of water stress. There will be 49 million hectares of irrigated wheat growing under high and extremely high-water stress conditions in 2040. The unprecedented increase in global population and decades-long economic shift driven by more resource-intensive consumption patterns means global freshwater use i.e., freshwater withdrawals for agriculture, industry and municipal uses have increased nearly six-fold since 1900 (Ritchie and Roser, 2020). The excessive use and degradation of water resources have over the years been compounded by climate change and intensifying urbanization. Consequently, a growing number of regions will face increasing water crises.

The global production of wheat is currently under threat. It is projected that with the growing human population and increasing per capita income and consumption, global wheat production needs to increase by at least 50% by the year 2030. Despite the reduced land area for production, reduced water for irrigation and extreme weather conditions, this target has to be met. Ironically, the rate of annual growth in wheat production has shown a decline from 3% to less than 1% in recent years (Ray et al., 2012). Gahlaut et al. (2017) asserted that the major constraint for average global wheat productivity is due to water/drought stress and 70% of the cultivated wheat area experiences water stress, globally. Based on the meta-analysis of literature data, (Daryanto et al., 2016) concluded that for under water reduction of approximately 40%, wheat had 20% yield reduction. Seed lots must carry some degree of water stress tolerance. Most plant seeds including cereals, start to lose their resistance to agricultural drought upon germination. Therefore, seedling establishment, including crown root and initial green leaf area development is critical. In drought-prone areas, drying seedbed is a common cause of crop failure (Blum, 1996). This can be averted when the cultivar can tolerate such hindrances.

Producing enough yield for a growing global population in the face of extreme weather conditions presents a significant challenge to society. In the Czech Statistical Office reports of 2018, it is highlighted that the production of basic grains (excluding corn) in the year 2018/2019 was below both five-and ten-year averages. The main reason for a drop in grain yields was cited as adverse weather conditions particularly drought (Mikulasova, 2018). A myriad of factors will reduce the water available for rainfed and irrigated wheat. Climate change has seen different regions having to endure the unprecedented effects of agricultural droughts. Demand by industry, urban populations and demands to maintain environmental flows and water quality will also reduce water available for irrigation. This too will affect global wheat production. The projected yields of crops under a range of agricultural and climatic scenarios are needed to assess for security prospects. It is imperative to increase global production with less available water for irrigation and less reliable rainfall that dryland agriculture in many parts of the world receives (HongBo et al., 2006; dos Santos et al., 2018).

In recent years the World Resources Institute developed Aqueduct Food (Beta), a new tool that maps current and future water risks to crop productivity around the world. New findings from Aqueduct Food show that 32% of all irrigated crop production faces extremely high-water stress, which will increase to 40% by 2040; and the amount of rainfed crop production facing extremely high seasonal variability of water supply will more than quadruple from 2010 to 2040, as climate change takes an increasing toll. One new finding from Aqueduct Food is that more than half of the world's irrigated wheat production is already exposed to extremely high-water stress. By 2040, due to climate change and the growing water demand, 72% of irrigated wheat production may occur in extremely high water-stressed areas (Schleifer and Barton, 2019).

WEF (2019) concluded that extreme weather events, failure of climate-change mitigation and adaptation and natural disasters are likely to be the top five threats most likely to occur in the next 10 years. In one of the projections, World Economic Forum noted that we shall continue to observe a significant decline in the available quality and quantity of fresh water, resulting in harmful effects on human health and/or economic activity. Bizarre as it may sound, water crises might also happen as a result of weather manipulation tools driven by geopolitical tensions. Neighbouring countries may use large-scale cloud-seeding as theft of rain or the reason for a drought.

FAO (2016) reported that the Paris Agreement, an outcome of the 21st Conference of the Parties of the United Nations Framework Convention on Climate Change, provides opportunities for adaptation and mitigation actions in agriculture. These outcomes emphasize enhancing adaptive capacity, strengthening resilience and reducing the vulnerability of climate change, with a view of contributing to sustainable development and ensuring an adequate response. This study presents the possibility of selecting the best possible wheat cultivars that will thrive in such environmental conditions threatened by water scarcity, as a way of adapting and coping with these seemingly inexorable challenges.

## 3.2 Wheat seed development and maturation

### 3.2.1 Abiotic factors influencing wheat seed development and maturation

Although the wheat cultivar (germplasm) plays an important role in seed formation, the rate at which wheat seeds develop largely depends on temperature, the need for a cold period (vernalization) and day length (photoperiod). The seed undergoes anabolic metabolism associated with the formation of the embryo and its surrounding structures and the deposition of the major storage reserves (Bewley et al., 2013). In the end, seed maturation is an important phase of seed development during which embryo growth ceases, storage products accumulate, the protective tegument differentiates and tolerance to desiccation develops, leading to seed dormancy (Gutierrez et al., 2007). Upon maturation, 90–95% of their moisture is lost. During grain filling and maturation, the seeds of wheat develop the ability to withstand significant moisture stress.

Induction of flowering and maturation of winter wheat is hastened following vernalization. Winter wheat should be exposed to a cold period (3 °C to 10 °C) of six to eight weeks before its spikelet formation can begin. Earlier studies proposed that the optimum temperature for vernalization was assumed to be in the range of 0 °C to 7 °C, with temperatures between 7 °C and 18 °C having a decreasing influence on the process (Hanks et al., 1991). However, Sánchez et al. (2014) reported that in a synthesis of 11 studies, it was revealed that the optimum vernalization temperatures lie between 3.8 °C and 6.0 °C. Wheat cultivars vary in their vernalization requirements. Cultivars exhibit different patterns in their response to temperature, vernalization, and photoperiod; to the extent to which these factors interact; and in relative sensitivity to them at different growth stages. Cultivars vary, apparently continuously, in their rates of maturation, thus contributing to the wide adaptation and distribution of wheat in global agriculture. Variation in development rate among varieties, do not arise just because of vernalization requirements. It greatly implies the need for timely planting for specific genotypes to meet vernalization requirements.

Heat stress affects the seed development and maturation of wheat. This also depends on the genotype as some cultivars tend to be tolerant than the others. Quality of seed exposed to heat stress during development can be evinced with the inconsistencies of cultivars with regards to germination and seed vigour. Grass and Burris (1995) researched two wheat cultivars Marzak and Oumrabia, which were subjected to three temperature regimes (20 °C /15 °C, 28 °C /21 °C, 36°C /29 °C) beginning 10 days after anthesis until maturation. High temperatures resulted in low values both seed yield and physical traits of seed quality. Relative heat stress did not affect the germination of Oumrabia. In contrast to germination, seed vigour was adversely affected by heat stress. This decline in seed vigour was reflected in reduced shoot and dry weight, increased shoot/root ratio, reduced root length, low root number per seedling, and high seed conductivity.

Adequate soil moisture is essential during grain filling for transpiration and photosynthesis. Cultivars with relatively higher levels of biomass use a lot of moisture and are at increased risk of running out of water during grain fill. Moisture stress at the grain filling stage may reduce the photosynthetic capacity of the crop by causing the premature defoliation or fall of leaves, making less intended storage reserves available in the caryopsis. The rate at which the carbohydrates are stored in the grain also declines during moisture stress, resulting in a higher percentage of protein. This phenomenon may play a part in precocious germination, an undesirable trait of some wheat cultivars. This is characterized by pre-harvest germination in mature grain whilst on the mother plant. Rainfall triggers the sprouting. Enzymatic activity involving α-amylase will result in the breaking down of starch and protein as part of the germination process (White and Edwards, 2008).

Soil fertility is another factor that contributes to successful grain filling. Plants supplied with fertilizers based on major elements including nitrogen, phosphorous and potassium will produce larger seeds than those which are not fertilized. In a study conducted by Abedi et al. (2010), four levels of inorganic fertilizers (0, 80, 160 and 240 kg Nitrogen ha$^{-1}$) were applied to wheat under irrigation. The result showed yield components of wheat; spikes plant$^{-1}$, seeds spike$^{-1}$ and 1000 kernels weight were significantly increased with increasing the level of nitrogen. Agronomic and technological factors can also affect seed development. The fields should be free of weeds to reduce competition for space, water and nutrients. The crop should also be planted at the correct seed rate to maintain the optimum plant population.

Wheat seed development is also influenced and accelerated by exposure to long-days i.e., photoperiod sensitive cultivars are quantitative long-day plants, although short days can sometimes substitute for vernalization. Wheat is usually classified as a long day (LD) plant because most cultivars flower earlier when exposed to longer days. Long day plants are generally induced to bloom when days are long, in summer. Aslam et al. (2017) asserted that growing degree days (GDD) and photoperiod interaction can alter Spring wheat phenology. They further stated that wheat is sensitive to photoperiod. Under normal conditions, the more we have longer days to maturity, the higher the yield. On the contrary, the higher the temperatures wheat is exposed to during the season, the lesser days it takes to mature. Therefore, it affects the overall yield.

Yunze and Shuangsheng (2014) experimented with wheat to increase the energy efficiency of cultivars by regulating the photoperiod. Although the research was conducted in a Controlled Ecological Life Support System (CELSS), a sealed system used in spaceflight to provide astronauts with food and oxygen by plants, a significant influence of photoperiodism was noted at different growth stages of wheat. The light source was red-blue LED (90% red and 10% blue). Results showed shorter photoperiod before flowering (PBF) and longer photoperiod after flowering (PAF) increased both yield and energy-using efficiency of wheat. Shorter PBF promoted the ear differentiation of wheat, increasing spikelet number, floret number and seed number and thus enhancing yield. Longer photoperiod leads to more light energy input and a long time of photosynthesis. Therefore, longer PAF provided more photosynthate and increase seed yield.

### 3.2.2 Stages of wheat seed development and maturation

Seed development begins with anthesis and ends with harvest maturity. In wheat, the outer protective layers of the mature caryopsis are derived from the nucellar epidermis, the inner integuments and the pericarp, which undergo a combination of wall thickening, cell death, and re-absorption during development. The outer integument disintegrates and forms the testa. The inner integument and the nucellar epidermis form the hyaline layer (Evers and Millar, 2002; Fábián et al., 2011).

Initially, the grain fills with water, which is replaced with dry matter (and nitrogen) until a maximum quantity of dry matter per grain is reached (i.e., mass maturity) after which the grain dries to harvest maturity (Gooding, 2017). Seed development may be subdivided into three stages (Jenner et al., 1991; Gooding, 2017). During the first stage of wheat seed development, the following processes occur; double fertilization, syncytium formation, endosperm cellularization and histodifferentiation. The second phase involves cell expansion which comprises endoreduplication and accumulation of storage reserves in the endosperm, and the third phase is maturation drying, which includes desiccation and dormancy.

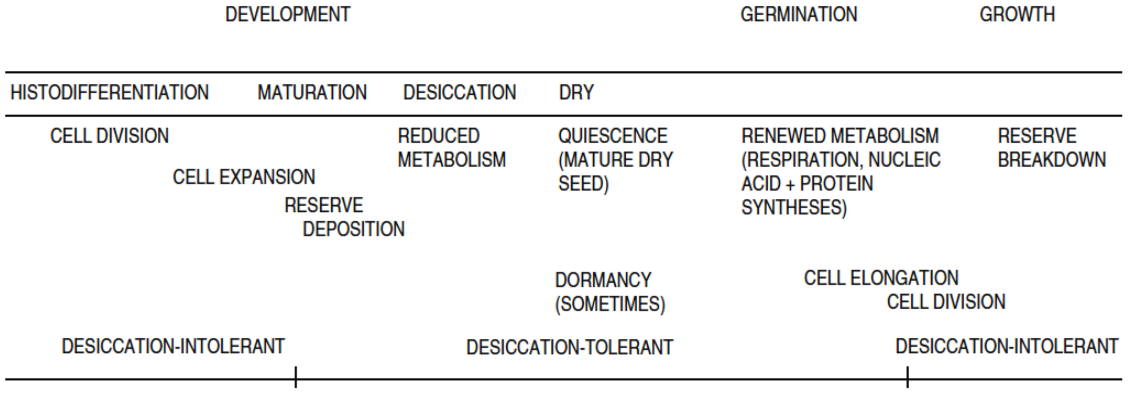

Figure 2: An overview of events associated with seed development, germination, and early seedling growth. From ((Kermode,1986; Bewley et al., 2013). Courtesy of the American Society for Horticultural Science

During the first stage, histodifferentiation involves the differentiation of the embryo and endosperm mostly due to cell division. The grain cells multiply and expand with the rapid accumulation of water into the grain. During this initial phase, the embryo reaches the beginning of the cotyledon stage of development. There is a rapid increase in both fresh and dry weight. The single-celled zygote undergoes extensive mitotic division, and the resultant cells differentiate to form the embryo. At the same time, the triploid endosperm or haploid megagametophyte is formed (Finch-savage, 2003). Embryo differentiation in wheat has a more complex embryo structure. Embryogenesis in wheat includes the proembryo, globular, scutellar, and coleoptile stages. The embryo axis also has a specialized tissue surrounding the shoot and root tissue to aid in emergence during germination. These are the coleoptile and coleorhiza.

The second stage of seed development involves cell expansion and rapid cell enlargement otherwise known as grain filling due to the accumulation of food reserves. This is an active period with large increases in DNA and RNA associated with endoreduplication, and protein synthesis in the seed. The major food reserves include starch, storage proteins, and lipids (Bewley et al., 2013). The wheat caryopsis tends to amass starch in its dry weight. Starch, a carbohydrate, provides essential energy substrates to ensure the survival of the germinating seedling. Food reserves are synthesised in the developing seed from photosynthate being translocated into the seed from the mother plant. Small molecular weight compounds such as sucrose, asparagine, glutamine, and minerals are accumulated into the seed. In wheat and other monocots transfer cells facilitate passage of photosynthate into the endosperms. During the second stage, cell division ceases during seed expansion and deposition of reserves. In most cases, seeds progressively gain desiccation tolerance, although, at the end of development, seeds from different species can differ in their capacity to withstand water loss (Finch-savage, 2003).

During the second phase of seed development, seeds acquire the ability to germinate before maturation drying. The embryo appears to be a powerful sink for nutrients. Smart and O'Brien (1983) noted that the developing wheat embryo is a powerful sink for nutrients supplied to the caryopsis as a whole. Once its development begins, the embryo affects its neighbouring cells strongly, drawing on their reserves, even on their protoplasm, for nutrients. The accumulation of proteins and starch reserves of the embryo appears to occur simultaneously in the scutellum and coleorhiza. The pattern of accumulation of reserves in the embryo is essentially the reverse of that found during the loss of reserves in germinating grain. Following germination, a large process of remobilization of the storage compounds of the starchy endosperm occurs. This process, which supports early seedling growth until the new plant becomes autonomous, requires the synthesis and secretion of hydrolytic enzymes initially by the scutellum epithelium cells (Domínguez and Cejudo, 2014).

Interesting to seed science research, several histological studies have been conducted to see if the development of an embryo and other tissues might affect the germination of the wheat grain. Fábián et al. (2011) noted the effects of drought stress simulated in a controlled environment from the 5th to the 9th day after pollination, on the kernel morphology, starch content and grain yield of the drought-sensitive Cappelle Desprez and drought-tolerant Plainsman V winter wheat (*Triticum aestivum* L.) varieties. As a consequence of water withdrawal, there was a decrease in the size of the embryos and the number of A-type starch granules deposited in the endosperm. Although water withdrawal shortened the duration of development and decreased the size of mature embryos, the embryos remained fully functional at germination. On the contrary, seed vigour was compromised. Due to the reduced amount of carbohydrate reserves in the endosperm, there was a decrease in the number of seminal roots. Seminal roots supply the wheat plants with nutrients between germination and tillering. The drought-tolerant cultivar, however, performed better during recovery of vegetative tissues, seed set and yield.

The third stage of seed development is maturation drying. The process of seed development results in the water content of seeds gradually declining as it is replaced by insoluble storage reserves. Importantly, the water that remains in the seed cytoplasm is assumed to maintain the synthetic activities of the seed. As maturation approaches completion, desiccation (maturation drying) is the normal terminal event for orthodox seeds after which they pass into a metabolically quiescent dry state from several days to many years and retain their viability. Seeds are considered “dry” when they have less than approximately 20% water content (dry weight basis; less than approximately -20 MPa). Seeds become tolerant of desiccation during the development stage. In cereals, such as wheat, barley and maize, the ability of seeds to withstand desiccation occurs at fairly early stages of reserve accumulation (Bewley et. al., 2013). For seeds known to have the capacity to tolerate desiccation, dehydration plays an important role in the transition of seeds from a developmental stage to a germinative one.

It is not known how the seed dehydrates towards maturation, but it is believed to be through evaporation. Dry orthodox seeds can usually remain viable at 3% to 5% moisture. Abscisic acid (ABA) is the main signal for the induction of desiccation tolerance. It is undesirable to have wheat seeds germinate prematurely on the plant without desiccation drying. Such seeds that succumb to this phenomenon undergo precocious germination or vivipary. The seeds go through mutations in the ability to produce or perceive ABA. If the seed is developing well before maturation it won’t germinate on the plant because of high ABA content in the seed. Following maturation drying, the seed can be considered in a quiescent or dormant condition. Quiescent seeds fail to germinate because they are dry. Exposing quiescent seeds to a favourable environment will induce them to germinate. Dormant seeds fail to germinate even under favourable environmental conditions. There are several ecological advantages to seed dormancy and it is a common feature of many seeds. Over years of selection, dormancy has been bred out of most economically important crop species (Dubreucq et al., 2010).

## 3.3 Wheat Seed Germination

### 3.3.1 Factors affecting wheat seed germination

Along with seed germination, it is imperative to understand seed vigour. According to ISTA Vigour Test Committee (1995), seed vigour is "the Sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence". Seed lots lose their ability to carry out physiological functions that allow them to perform. This process is called physiological ageing or deterioration. It starts before harvest, continues during harvest, processing and storage. Biochemical changes in the seed that happen overtime may ultimately lead to the death of the seed i.e., complete loss of germination. Seeds lose vigour before they lose the ability to germinate. Variations between interspecies to express seed vigour depend on genetic, production and environmental factors which are not yet fully understood.

Germination of the wheat caryopsis commences after a relatively short period of dormancy. Post-harvest dormancy becomes the transition between maturation and germination of seed. Bewley et al. (2013) defined dormancy as the temporary failure of seed to complete germination under favourable conditions. Seed dormancy whilst on the mother plant is somewhat a favourable trait for a wheat variety because it prevents preharvest sprouting (PHS), pre-germination or what was referred previously as precocious germination or vivipary. Freshly harvested seeds can germinate quickly than ageing seeds. Ageing seeds lose the integrity of their cellular systems and show high electrical conductivity when tested, thus compromising overall quality. In cereals, some genotypes produce non-dormant seeds at harvest. The main problem with this early loss of dormancy in crop species is PHS. This phenomenon is a characteristic of cereal species, like rice, barley, wheat and sorghum (Walker, 2011). After harvesting the rate of seed dormancy loss is affected by several factors including poor storage, processing and above all harvesting seeds with great than the required 12.5–13.5 % moisture content. Inhibitory substances found in the seed coat of hard red winter wheat varieties, for example, can strengthen post-harvest dormancy (Edwards, 2007). European and North American red wheat cultivars have a dormancy derived from their seed coat that lasts 3–7 months (GRDC, 2016).

Plant hormones also play a vital role in the release of seed dormancy. Abscisic Acid (ABA) has an inhibitory effect during dormancy. ABA prevents precocious germination of the developing embryo in the ovule. Gibberellic Acid (GA) has a promotive influence during the germination of seeds. Reducing the amount of ABA in seeds allows for the release of seed dormancy. Seeds of various species show ABA increase during the late stages of seed development and decrease during after-ripening. GA is involved in many physiological functions of plant development including germination, vegetative and generative growth. Thus, for successful germination, a seed needs to have either enough supply of endogenous or exogenous GA. Ethylene (ETH), is another plant hormone that often exhibits a promotive effect on germination (Bewley et al., 2013).

Soil moisture influences the speed of germination. The minimum soil moisture content and temperature for germination are 35–45% and 4 °C, respectively, although the speed of germination is faster at higher moisture contents and temperature, with 20 °C–25 °C, being optimal for non-dormant seeds (Evans et al., 1975). The availability of soil moisture strictly determines halting or progression of seed germination. Several studies that simulate moisture stress environments have been conducted. One of the most common methods used is the utilization of Polyethylene glycol (PEG) 6000. Abdul et al. (2011) conducted a study with seeds of five wheat cultivars (GA-2002, Chakwal-97, Uqab-2000, Chakwal-50 and Wafaq-2001) that were subjected to osmotic stress. Moisture stress greatly affects seed germination and vigour.

Germination percentages and Mean Germination Time (MGT) were measured. MGT is expressed as;

$$MGT=\Sigma Dn/\Sigma n, \qquad [1]$$

Where:

MGT: mean germination time
Dn: is the number of seeds which germinated on day D
N: number of days from the beginning of germination test to day D

The number of seeds germinated was counted daily and the germination percentage and mean germination time were estimated. Results from the experiments showed that germination percentage decreased from 100% in control to 59.25% in -8 MPa osmotic stress, while MGT decreased from 17.10 in control to 5.69 under -0.8 MPa osmotic stress. An increase in stress level caused a linear decrease in germination percentage and MGT in all wheat cultivars. Their findings also revealed that moderate stress intensities only delay germination, while high-stress intensities immensely reduce germination percentages. Most importantly, it is imperative for planting to select cultivars that have some degree of water stress tolerance during the germination stage because it gives the plant stability for later growth.

Spanic and Izakovic (2017) noted the differences in seed vigour between cultivars under induced moisture stress conditions. While all genotypes had been affected with low germination percentages, as low as 43% in Žitarka (a wheat variety) under 20% concentration of PEG 6000, some cultivars had an increased root fresh weight.

Wheat can germinate in a wide range of temperatures between 4 °C and 37 °C, but the ideal temperature range for a good stand establishment is between 12 °C and 25 °C. Seed germination increases as temperature rises, for most species (Nyachiro et al., 2002). In a study conducted by Sánchez et al. (2014) it was noted that lethal limits for wheat to be -17.2 °C±1.2 °C. The rate of imbibition, the diffusion of oxygen and the rate of biochemical reactions of the seed are all affected by temperature (ISTA, 2015). In wheat, the speed of germination is driven by accumulated temperature, or degree days (GRDC, 2016). This is also referred to as seed vernalization. Degree-days are the sum of the average daily maximum and minimum temperatures over consecutive days. In wheat, visible germination occurs after seed attains 35 degree-days. It takes 27 degree-days for the root to be visible and 35 degree-days for the coleoptile to be visible (Passouira, 2005) and this marks the completion of germination.

Temperature is a modifying factor in germination since it can influence the rate of water absorption and other substrates supply are necessary for growth and development (Essemine et al., 2007; Buriro et al., 2011). In a study done in Pakistan, temperature significantly influenced germination and related traits of various wheat varieties. The highest germination (97%) was recorded in Abadgar-93. Abadgar-93 was subjected to 30 °C, TJ-83 and Imdad-2005 were subjected to 10 °C and 20 °C respectively. Seeds that were subjected to 20 °C or 30 °C responded well for germination, seed vigour index, shoot and root length, fresh and dry weight (Buriro et al., 2011).

However, high and extremely high temperatures also affect wheat productivity. Global wheat production is estimated to fall by 6% for each °C of further temperature increase and become more variable over space and time (Asseng et al., 2015). Hossain et al. (2012) noted that it takes more days for wheat grown in a post-anthesis heat stressed environment i.e., very late sowing, to germinate than in an optimum sowing environment. In the optimum sowing environment, the maximum temperature during germination was above 28 °C with a minimum of 15 °C. In the very late sowing conditions where temperatures were a high of 23 °C and a low of 10 °C germination was later due to low temperatures.

Salinity is a global challenge and it affects crop production, particularly in arid and semi-arid regions. Irrigation may enhance crop productivity but also lead to increased soil salinity. Irrigation water may contain from 0.1 to 4 kg/m$^3$ of salt which, considering 1.0 to 1.5 m of irrigation water applied annually, contributes from 1 to 60 metric tons of salt per hectare (Shannon, 1997). Salinity can affect germination and growth either by creating an osmotic pressure that prevents water uptake or by toxic effects of sodium and chloride ions (Soltani et al., 2008; Al-saady and Al-Razak, 2015). In a study by Akbarimoghaddam et al. (2011) the effects of increasing Sodium Chloride (NaCl) levels on germination percentage of six bread wheat cultivars were evaluated after 48 and 96 hours (final germination). The results showed that by increasing NaCl concentration, germination is delayed and decreased in all cultivars. Seeds of three different wheat cultivars were sown in Petri dishes and three salinity levels (6.8, 13.2 and 19.0 dS m$^{-1}$) were imposed by developing NaCl solution concentration of 0.5, 1.0 and 1.5%, respectively. Results revealed that increasing concentration of NaCl solution resulted in a gradual reduction in seed germination and suppression of early seedling growth in all wheat cultivars (Hussain et al., 2013).

Germination of three wheat cultivars was assessed using three replicates of 50 seeds in a factorial laid out in Completely Randomized Design (CRD) testing combinations of three levels of salinity (0, -0.6 and -1.2 MPa NaCl. Results showed that by increasing concentrations of NaCl there was a substantial reduction in germination percentage, radicle length, hypocotyl length, seedling fresh and dry weights, radicle and hypocotyl dry weight, but increased hypocotyl dry weight at the potential -0.6 MPa (Akbari et al., 2007).

Light is another germination factor that acts in both dormancy induction and release. Light effects on germination can involve both quality (wavelength) and photoperiod (duration) (Hartmann et al., 2002). Wheat is not a light-requiring seed hence the influence on germination is minimum to none. In other plant species, the spectral quality of light also influences photomorphogenic responses such as germination and phototropism. Other requirements for successful germination are nitrates, oxygen and other gases. Gases may be dissolved in soil moisture. Soil porosity allows for the diffusion of gases needed for germination. Waterlogged soils and heavy soils may restrict molecular diffusion of gases and the oxygen content in the gaseous may drop considerably below that of air. Germination and early seedling growth generally require oxygen at atmospheric levels (Bewley et al., 2013).

## 3.4 Seed water relations during triphasic stages of wheat germination

Wheat is a C3 crop and it is efficient at photosynthesis in cool, wet climates. However, it is a widely adaptable crop that can be grown from temperate, irrigated to dry and high rainfall areas and from warm, humid to dry, cold environments. The process of germination largely relies on the water potential of cells in the seed and embryo (Hartmann et al., 2002). Water potential and temperature are the most important factors affecting seed germination (Lindstrom et al., 1976). Water potential ($\Psi$) is an expression of the free energy status of water, and in Plant Physiology, it is generally expressed in terms of pressure units. Cell water potential ($\Psi$cell) of the seed is expressed:

$$\Psi cell = \Psi m + \Psi \pi + \Psi p \qquad [2]$$

Where: $\Psi$cell = Water potential

$\Psi$m = Matric potential

$\Psi\pi$ = Osmotic potential

$\Psi$p = Pressure potential

Hartmann et al. (2002); Woodstock (2014) stated that it is matric potential (Ψm) of cell walls and macromolecules such as starch and proteins that are primarily responsible for germination. The Ψm accounts for the effects of the tight association of water to tiny capillaries (e.g., among the polymers comprising the cell walls) and the surfaces of macromolecules such as starch and proteins. This binding lowers the energy content of water, so Ψm values are so negative. Ψm is an important component of Ψ in air-dried wheat seeds and is responsible for the Ψ gradient that initially drives water uptake (Bewley et al., 2013). Osmotic potential is a measure of the osmotically active solutes in a cell. Osmotic potential of a cell increasingly becomes negative with more osmotically active solutes (i.e., both non-ionic such as sugars and ionic such as potassium) present in a cell. The greater the solutes in the cell, the lower (negative) is the Ψπ and hence the Ψcell. Consequently, this can result in more water moving into the cell because of a Ψ gradient that has been created. This will determine the radicle protrusion phase of seed germination (Woodstock, 2014). Pressure potential is the opposing force and the ability of the cell wall to exist and resist outward pressured exerted by water inside. Suffice to say, since water is incompressible, the movement of water into the cell increases the internal pressure that raises the energy status of water. Values of Ψp are expressed as positive and they increase Ψcell. The result is radicle protrusion due to the loosening of the cell walls covering radicle tissues.

Water is critically an important factor for successful wheat germination. The uptake of water occurs in three phases. The first phase (Phase I) is the rapid uptake otherwise known as imbibition. This is followed by the plateau phase (Phase II). In the last phase (Phase III) a further increase in water uptake takes place as the embryo axis elongates and breaks through the covering layers to complete germination (Schopfer and Plachy, 1985). It is, therefore, the plant hormone, Abscisic Acid (ABA) that inhibits water uptake. The wheat seed has an embryo that is surrounded by two covering layers: the endosperm and the testa. Eventually, visible germination is marked by the protrusion of the radicle as a result of cell expansion (Finch-Savage and Leubner-Metzger, 2006).

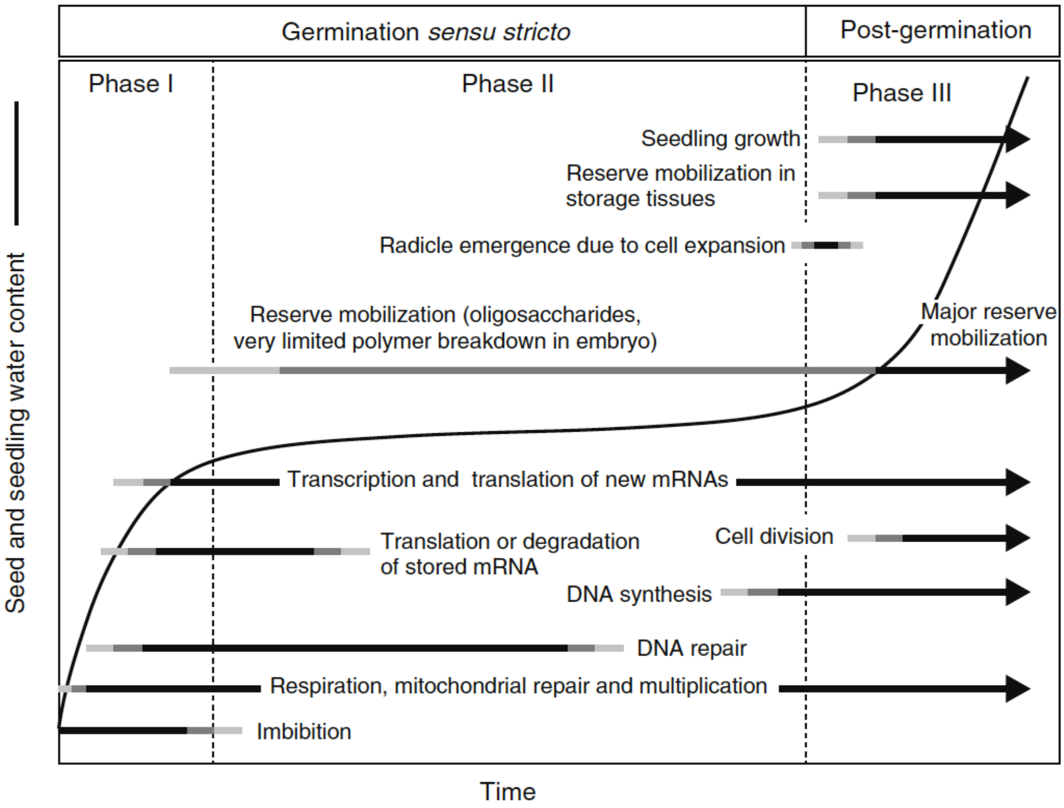

Figure 3: Time course of water uptake and some important changes associated with germination and early seedling growth. Nonogaki et al. (2010) Courtesy of Elsevier

### 3.4.1 Phase I

Caryopsis germination commences with the uptake of water by imbibition by the air-dried seeds (Finch-Savage and Leubner-Metzger, 2006). This intricate process increases seed fresh weight as a result of increased water uptake and the subsequent biochemical processes. As noted above, initial water uptake occurs largely due to Ψm. The basic requirements for germination are water, oxygen and appropriate temperature. Other factors may include light and or nitrate and they to influence germination in some species. Germination commences once dry seeds come in contact with water under favourable conditions (Weitbrecht et al., 2011).

Dry orthodox seeds have very low water potentials between -350 and -50 MPa corresponding to a moisture content of only 5–10% (Walker, 2011; Obroucheva et al., 2017). Pure water has the highest potential, and by convention, it is assigned a zero value (0 MPa). The rate at which seeds imbibe water depends in the first place on the gradient of water potential between water and seed. Low soil water potential limits or prevents germination of rainfed winter wheat. Singh et al. (2013) concluded that seed germination of the five commonly sown wheat cultivars in dryland Northwest USA had above 90% at high water potentials (0 to -1.5 MPa). At the lowest water potentials (-1.0 to -1.5 MPa) all cultivars had less than 73% germination. Therefore, germination commences when the seed imbibes water from the surrounding soil.

The movement of water into the seed is due to both diffusion and capillary action with water moving from a region of higher to lower potential (Woodstock, 2014). On seed morphology, the extent to which water imbibition occurs is dependent on three factors: composition of the seed, seed coat permeability and water availability. Water uptake occurs regardless of whether the seed is dormant or non-dormant, viable or nonviable. Cells in viable seeds can build up a pressure potential but the resulting Ψp counteracts further water uptake. Ψp in nonviable seeds remains zero because cell membranes are injured and as a result, solutes are released into the apoplast, allowing continued water uptake (Bewley et al., 2013). This implies selecting good quality seeds (without abnormalities) for planting.

Imbibition triggers the activation of basic metabolic processes. Water content increase of up to about 18–20% will increase seed respiration due to the activation of glycolysis and the Krebs cycle. In a parallel manner, amino acid metabolism also occurs due to enzyme activation (Obroucheva et al., 2017). In wheat, higher protein seeds took up water more rapidly (Lopez, 1972). The principal component of seeds that is responsible for the imbibition of water is protein. Proteins are zwitterions that exhibit both negative and positive charges that attract the highly charged polar water molecules (Copeland and McDonald, 2001). The uptake of water during the first phase is often accompanied by leakage of cellular solutes. The leakage will, therefore, accelerate germination by lowering inhibitor concentrations within the seed (Matilla et al., 2005). During imbibition, in maize, a DNA is found in single-strand breaks, most of which is attributed to imbibitional damage. Subsequently, DNA ligase works to repair the DNA strands, otherwise, this would hinder germination (Weitbrecht et al., 2011).

### 3.4.2 Phase II

At the end of the first phase, the rate of water uptake starts to subside and Ψm becomes less negative as the cellular components and cell walls become hydrated. The water potential gradient also decreases. The soil water potential and the cell water potential are at equilibrium at this stage. Thus, water uptake approaches a lag or plateau phase where there is a steady but slow increase of water content. Changes in seed size and shape start to stagnate because of cell expansion, then the embryo produces hormones that stimulate enzyme activity (Weitbrecht et al., 2011). Specific enzymes are responsible for cell wall loosening in the embryo or tissues surrounding the embryo. Enzymes are also responsible for storage reserve metabolism. The result is the production of osmotically active solutes such as sucrose that can lead to a change in the water potential of cells during the expansion of the embryonic axis (Hartmann et al., 2002). The breakdown of phytin in wheat can release ions that are important osmolytes in the cell.

There is an interlink between an increase in temperature, enzyme activity and water potential realised during the lag phase of germination. Within hours of imbibition, the mitochondria are rehydrated and its membranes become enzymatically active. The implication of this is that respiration and ATP synthesis substantially increase. After seed hydration, polysomes and new proteins are formed with the completion of imbibition. New proteins are required for seed germination. Phase II continues until the rupture of the seed coat, the first visible sign of germination (White and Edwards, 2008). If the seed imbibes water and go on to Phase 2, but suddenly the soil almost dries out without the seed transitioning to Phase 3, that seed remains viable. This can happen when dry sowing is followed by a small amount of rain that keeps the soil moist for a few days before drying out. When the next fall of rain comes, the seed resumes germinating, taking up water and moving quickly through Phase 2, so that germination is rapid (Poole, 2016).

Palmiano and Juliano (1973); Hameed et al. (2015) stated that respiratory enzymes activation, breakdown of food molecules and the mobilization of seed nutritional reserves occur in germinating seeds. They discovered that during the germination of the seed of cereals, the aleurone layer is the site of production of hydrolases. Subramani et al. (2011); Hameed et al. (2015) stated that during seed germination, starch digestion is primarily controlled by α-amylase, protein digestion by different proteases and by a group of esterases which catalyses the hydrolysis of different types of esters. Other processes that take place during the lag phase are the restoration of cellular integrity, reformation of the cytoskeleton and repair of damage to DNA accumulated during storage (Bewley, 2013).

### 3.4.3 Phase III

From a physiological standpoint, the first visible sign of germination is the protrusion of the radicle. Barroco et al. (2012) stated that initial radicle protrusion is probably accomplished through cell enlargement rather than cell division. Cell division initiates in the root apical meristem before root protrusion (Masubelele et al., 2005). Therefore, once cell elongation starts to occur in the radicle, cell division can be detected in the tip of the radicle. The biomechanical process of radicle protrusion takes place as a result of opposing forces between the growth potential in the embryo and the physical resistance of the seed coat. For radicle protrusion to finally occur, the water potential of the cells in the radicle become more negative due to metabolism of storage reserves, the cell walls in hypocotyl and radicle become more flexible to allow cell expansion. The tissues around the embryonic axis also have to weaken to allow cell expansion in the radicle (Hartmann et al., 2002). Water uptake at this stage is no longer by imbibition but by the decrease in $\Psi\pi$ that arises as a result of osmotically active substances. A water potential gradient is created for water uptake movement relative to soil water potential or water absorbed on a filter paper during seed quality testing.

## 3.5 Coleoptile emergence

As the first primary roots appear the coleoptile bursts through the seed coat and begin pushing towards the surface. Emergence is when the coleoptile or the first leaf becomes visible above the soil surface (White and Edwards, 2008). In areas prone to moisture stress, selecting wheat cultivars with long coleoptile is an important component of improving emergence, weed suppression and grain yield. Singh and Khanna-Chopra (2010) stated that coleoptile length plays a major role in seedlings emergence, early plant vigour and successful establishment of wheat. The wheat seeds can be planted at a depth between 8 and 12 cm. However, optimum moisture conditions that enable smooth and uniform coleoptile germination are between 2 to 4 cm. Low depth makes non-uniform emergence of seed plants. Therefore, coleoptile length together with seed size have tremendous effects on seed vigour and less on germination (Alaei et al., 2010)

## 3.6 Osmopriming

Osmopriming is a seed priming technique that involves soaking seeds in solutions of different organic osmotica or solutions of low water potential. The main purpose is to partially hydrate the seed to a point where germination processes commence but not completed (Shabala and Munns, 2012). The use of Polyethylene glycol (PEG), an organic osmoticum, is commonly used in studies aimed at screening or comparing germination and vigour between wheat seed cultivars under simulated moisture stress conditions. In laboratory studies, seed germination as a function of water potential is often tested with soil adjusted to desired water potentials or by using PEG. PEG effectively lowers the water potential of an aqueous solution (Singh et al., 2013).

## 3.7 Seed quality testing

Late germination has been the focus of seed research for many decades. Seed testing is done to assess seed lot attributes and determine overall quality and value for seedling production. Seed testing standards, preferably those that align with International Rules of Seed Testing (ISTA), provide a set of procedures for institutions to conduct tests uniformly and ensure comparable results for seed owners. In the Czech Republic, similar standards are ensured by the Central Institute for Supervising and Testing in Agriculture (CISTA) which officials control seed testing and related activities (CISTA, 2020). This study, however, was conducted following the guidelines of ISTA rules, which are internationally recognized.

Obtaining high-quality seeds is fundamentally the key objective in commercial seed production. Seed vigour and seed germination are both important parameters of determining seed quality of a seed lot but it is the latter that is more essential for seed trade. Seed vigour tests for maize and wheat are based on the simulation of environmental stress conditions encountered during storage or during field emergence (CIMMYT, 1996). Since the ISTA rules of 1931 definitions and instructions for each step in the germination testing have been documented (growing media, material and apparatus, test procedure, retesting, calculation and expression of results, reporting results and germination methods) (Milivojević et al*.,* 2018). Over the years there have been some amendments made for several genotypes. The ISTA rules prescribe the substrates and growing media (organic matter, sand, filter paper), temperatures and test durations, recommended procedures for breaking dormancy, and additional directions and advice. Seeds can be placed on top of filter paper (TP), or between filter paper (BP) (ISTA, 2015).

A typical institution following the ISTA rules is the Alberta Forest Genetic Resource and Conservation Management Standards (FGRMS) of Canada. According to the Alberta Seed Testing Standards, each test must consist of four hundred seeds which are drawn from the working sample and then randomly divided into four replicates of 100 seeds (Government of Alberta, 2016). Germination testing for International seed trade (for international certification) is based on 400 seeds. Testing less than 400 seeds, but not less than 100, was allowed in 2011 for high valued seeds (Milivojević et al., 2018). The FGRMS stipulates that a registered seed lot owner do germination tests on their seed or to do different tests. A Provincial Seed Officer will officially record all germination test results from the seed lot owner. The testing facilities are strictly defined by FGRMS and permitted for use by the seed lot owner. Covered germination containers or boxes should allow for adequate and uniform spacing of seeds and replicates. The germination/incubation cabinets (climabox) must allow an even distribution of temperatures and all samples receive a temperature within the prescribed limits for the test/treatment ±2 °C. Cabinets should also control light. A suitable substrate is also recommended by the FGRMS. A clean paper that has open and porous nature with the capacity to hold sufficient water for the duration of the test period is recommended, for example, a Grade 3 filter paper. Other substrates could be water agar or sand that meets ISTA regulations for grain size, pH and conductivity (Government of Alberta, 2016).

According to CIMMYT Seed Testing of Maize and Wheat: A Laboratory Guide, the use of seed germination/vigour tests may include quality control programmes, an indication of storage life, evaluation of the effect of seed treatments and other critical operations, consumer demand and identification of good quality seed lots. In the end, seed germination protocols involve evaluating normal seedlings, abnormal seedlings, ungerminated seeds and maximum tolerated ranges between replicates (CIMMYT, 1996).

# 4 Materials and Methods

## 4.1 Location

Experiments were conducted in the seed laboratory of the Department of Agroecology and Crop Production at the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences in Prague.

## 4.2 Materials and sampling

This study was carried out by testing eight seed lots of wheat. The seed lots came from cultivars registered for use in the Czech Republic. The following cultivars of categories "A, B and E" of wheat quality were evaluated: Genius E, Annie E, Matchball A, Turandot A, Hyfi B, Tobak B, Bohemia A and Julie E. Seed lots were obtained from a harvest stored for not more than 12 months. Before sampling, all seeds were mixed in their respective bags because lighter seeds tend to settle on top than heavier ones. Sampling was done to obtain samples that are suitable in size and representative of the lot being tested. To minimize changes in seed moisture, the sampling exercise was done rapidly.

## 4.3 Establishment of germination tests

**Standard germination test ($G_{st}$)** was established in the laboratory, following International Rules for Seed Testing by the International Seed Testing Association (ISTA). Seed lots were placed in germination containers that were put in the climaboxes. In each germination container, 3 plane filter papers (Hahnemühle) were placed at the bottom and a single pleated filter paper on top. Seed lots were prepared in four replications per 100 seeds per cultivar. Seed lots were subjected to two incubation temperatures of 20 °C and 15 °C (set in the climabox) and two moisture levels; higher dose 30 ml (standard/optimal) and lower dose 20 ml (stressed variant) of tap water. Fluorescent lighting in the climabox provided illumination throughout the experiments.

## 4.4 Evaluation of germination and coleoptile emergence

Seeds with primary root and cotyledons were evaluated as normal seedlings from day 2 to day 8 after 'planting'. Evaluation of germinants was done primarily by calculating germination percentages of four replications of 100 seeds for each seed lot. A germinated seed was identified as having three roots and a visible coleoptile at least 3 mm long. Total germination was calculated as the sum of daily values (cumulative germination). Germinated seeds were removed using tweezers and counted every 24 hours until the end of the experiment. Ungerminated seeds were left in the germination container, counted and returned to the climabox. Abnormal seeds were counted and removed using tweezers. After each day's evaluation, the germination containers were returned to the climabox for counting cumulative germination after every 24 hours.

At day 8, all germinated and abnormal seeds were counted and removed. Any seeds that had germinated, i.e. radicle visible, but not achieved the 4x seed length rule were to be classified as 'low vigour'. Ungerminated seeds must be cut tested and classified into either 'empty' or 'filled ungerminated'. An 'abnormal' seedling is any seedling that falls into the abnormal category according to Section 5.2.8 of ISTA (2015) rules. Coleoptile emergence was measured on day 4 and day 5.

The following germination parameters were evaluated:

**Total Germination (TG)** is an indicator of the basic characteristics of viable seeds that are capable of further development from germination, subsequent embryo growth to the formation of an adult plant. It is the percentage of germinated seeds out of the total seed rate.

**The germination energy (GE)** is a quality indicator given by the ratio of the number of germinated seeds in the average sample at the beginning and at the end of the set time. Germination energy is the number expressing the percentage (%) of fast-germinating seeds. Each cultivar has its time during which it can germinate.

**Mean germination time (MGT)** expresses the rate and equilibrium of seed germination for 24 hours. In other words, it is a measure of the rate and time-spread of germination. It varies depending on the botanical species and the quality of the seed (Ranal et al., 2009). The expression for Mean Germination Time is:

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \qquad [3]$$

Where

$t_i$: time from the start of the experiment to the *i*th observation (day for the example)

*ni*: number of seeds germinated in the *i*th time (not the accumulated number, but the number correspondent to the *i*th observation), and

*k*: last time of germination.

## 4.5 Statistical Evaluation

The obtained data were evaluated using SAS statistical programs, version 9.4 (SAS Institute, Cary, USA) and the Analysis of variance (ANOVA) was determined. Differences between mean values were evaluated by the Tukey's HSD (Honest Significant Difference) method at a significance level of $P \leq 0.05$. The influence of tested factors i.e., moisture and temperatures were estimated as F-values from a classical additive ANOVA model. The higher the F-values, the higher the significant influence of the factor.

# 5 Results

Eight wheat cultivars namely Genius, Annie, Matchball, Turandot, Hyfi, Tobak, Bohemia and Julie were chosen for this study to evaluate performance during seed germination and coleoptile emergence between standard and water stress conditions in a laboratory setup. Evaluation of germination parameters shown in the tables and graphs have the following abbreviations: germination energy (GE), germination energy on the second day (GE2), germination energy on the third day (GE3), total germination (TG), mean germination time (MGT), coleoptile emergence (COL4) on the fourth and coleoptile emergence (COL5) on the fifth day, minimum significant differences according to Tukey's (HSD). In the tables, values marked with the same letters do not differ statistically, at the significance level $\alpha \leq 0.05$.

## 5.1 Analysis of germination and coleoptile emergence in Genius

Table 1 shows the seed quality parameters and recorded average values in bed moisture conditions (30 ml and 20 ml) and incubation temperatures (20 °C and 15 °C) throughout germination. There were statistically significant differences between GE2, GE3, COL4 and COL5 between temperatures. There were slight significant differences between GE3 and COL4 between moisture conditions. There were no significant differences between total germination at the end of the experiment signifying that the difference in moisture quantities had no overall effect on total germination. There was no significant difference between MGT in both moisture levels. However, MGT was significantly shorter at 20 °C than 15 °C.

Table 1: Influence of factors on seed quality parameters in Genius (values are means for each factor)

| **Factor** | **GE2 (%)** | **GE3 (%)** | **TG (%)** | **MGT (days)** | **COL4 (%)** | **COL5 (%)** |
|---|---|---|---|---|---|---|
| 30 ml | 20.5a | 74.5a | 96.5a | 3.09a | 41.5a | 73.8a |
| 20 ml | 15.3a | 65.0b | 97.8a | 3.22a | 36.3b | 72.5a |
| **HSD** | **5.47** | **4.66** | **1.57** | **0.15** | **4.37** | **7.44** |
| 20 $^{0}$C | 35.8a | 95.5a | 97.3a | 2.67b | 67.0a | 91.5a |
| 15 $^{0}$C | 0.0b | 44.0b | 97.0a | 3.64a | 10.8b | 54.7b |
| **HSD** | **5.47** | **4.66** | **1.57** | **0.15** | **4.37** | **7.44** |

Means with the same letter are not significantly different. HSD = Tukey's honestly significant difference test (Tukey's HSD) $P \leq 0.05$.

The differences in temperature on performance and seed quality parameters were more evident than differences in moisture levels. When measured, on day 2 (48 hrs), GE2 was 0% at 15 °C and 35.8% at 20 °C. There were statistically significant differences between GE2 and GE3 in temperatures during seed germination. GE3 was significantly higher at 20 °C with 95.5% compared to 44% at 15 °C. On the final day of counting, there were no significant differences between total germination in both moisture and at temperature conditions. MGT between the two temperatures remained significantly different. There was a significant difference between coleoptile emergence at both temperatures. On day 5, coleoptile with at least 3 mm long were 91.5% at 20 °C compared to 54.7% at 15 °C.

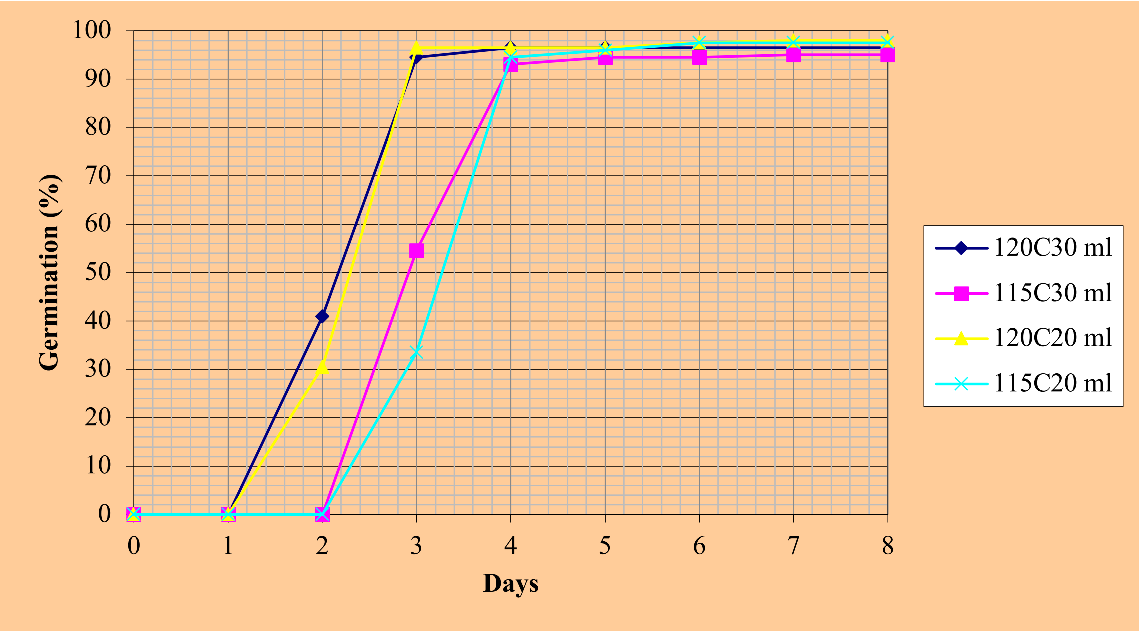

Figure 4: Real values of daily germination in Genius

Germination curves present daily germination of the cultivar, Genius. The difference between GE2 between conditions 20 °C/30 ml and 20 °C/20 ml was 11%. However, the difference between GE2 between conditions 15 °C/30 ml and 15 °C/20 ml was 0. Germination was faster under the influence of 20 °C. The difference between GE3 between conditions 15 °C/30 ml and 15 °C/20 ml was 21%. The highest GE3 was 96% recorded under conditions 20 °C/20 ml. The least GE3 was 33% recorded under conditions 15 °C/20 ml. The highest total germination was 97% recorded under the conditions 20 °C/30 ml and 15 °C/20 ml. The least total germination was 95 % recorded under 15 °C/30 ml as shown in Fig. 4.

## 5.2 Analysis of germination and coleoptile emergence in Annie

Table 2 shows the seed quality parameters and recorded average values in bed moisture conditions (30 ml and 20 ml) and incubation temperatures (20 °C and 15 °C) throughout germination. There were statistically significant differences between GE2, GE3, COL4, and COL5 between temperatures. There were slight significant differences between GE3 between moisture conditions as shown in Table 2. However, there were no significant differences between GE2, COL4, and COL5 between moisture conditions. At the end of the experiment, there were no significant differences between total germination between both temperature and moisture conditions. MGT significantly decreased under the influence of 20 °C than 15 °C. There was no significant difference between MGT in moisture conditions.

Table 2: Influence of factors on seed quality parameters in Annie (values are means for each factor)

| **Factor** | **GE2 (%)** | **GE3 (%)** | **TG (%)** | **MGT (days)** | **COL4 (%)** | **COL5 (%)** |
|---|---|---|---|---|---|---|
| 30 ml | 18.3a | 68.8a | 96.8a | 3.13a | 45.3a | 72.8a |
| 20 ml | 20.5a | 61.5b | 94.0a | 3.17a | 40.3a | 71.5a |
| **HSD** | **5.89** | **4.52** | **4.30** | **0.07** | **10.78** | **5.78** |
| $20^0$C | 38.8a | 93.0a | 97.0a | 2.65b | 74.5a | 95.3a |
| $15^0$C | 0.0b | 37.3b | 93.8a | 3.65a | 11.0b | 49.0b |
| **HSD** | **5.89** | **4.52** | **4.30** | **0.07** | **10.78** | **5.77** |

Means with the same letter are not significantly different. HSD = Tukey's honestly significant difference test (Tukey's HSD) $P \leq 0.05$.

The influence of temperature on seed quality parameters was observed during germination. Low temperatures will slow germination energy. GE2 was 0% at 15 °C and 38.8% at 20 °C. There was a significant difference between the influence of temperatures on seed germination. GE3 was significantly faster under the influence of 20 °C, with an average of 93% versus 37.3% at 15 °C. Hence, there was a significant difference between GE between temperatures. There were no statistically significant differences between total germination on the final day of counting germination. MGT between the temperatures remained significantly different, being shorter under the influence of 20 °C than 15 °C. There was a significant difference between coleoptile emergence at incubation temperatures. On day 5, coleoptile at least 3 mm long was 95.3% under the influence of 20 °C compared to 49.0% at 15 °C.

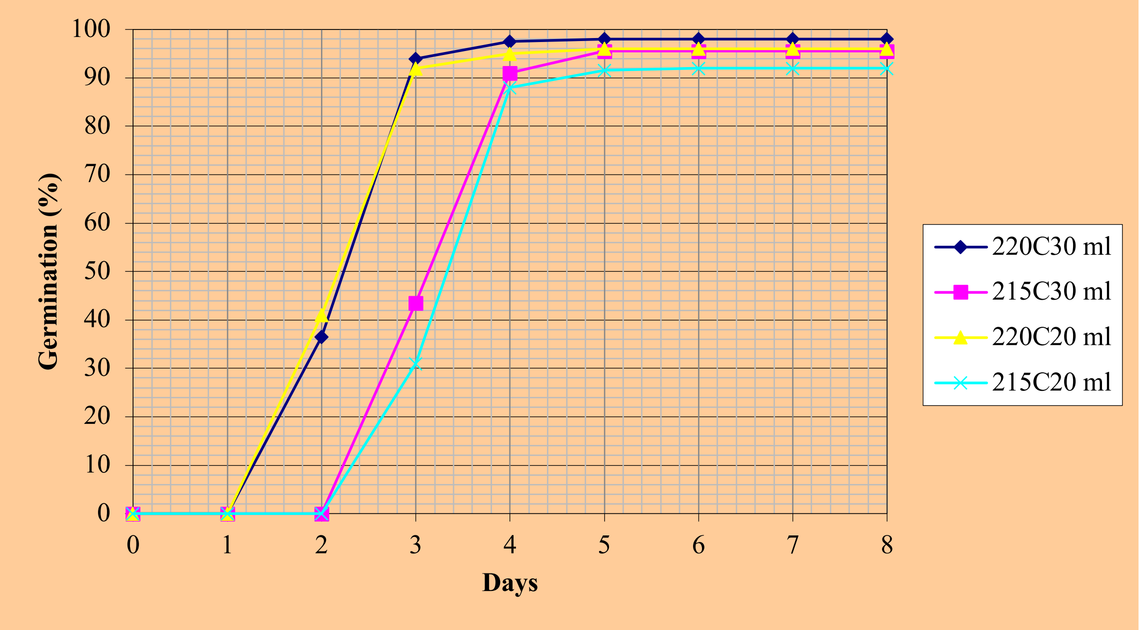

Figure 5: Real values of daily germination in Annie

Germination curves present daily germination of the cultivar, Annie. The difference between GE2 observed between conditions 20 °C/30 ml and 20 °C/20 ml was only 5%. However, the difference between GE2 between conditions 15 °C/30 ml and 15 °C/20 ml was 0. The difference between GE3 between moisture conditions at 15 °C was quite higher. The difference was 12%. The highest GE3 was 94% recorded in conditions 20 °C/30 ml. The least GE3 was 31% recorded in conditions 15 °C/20 ml. The highest total germination was 98% recorded in conditions 20 °C/30 ml. The least total germination was 92% recorded under conditions 15 °C/20 ml as shown in Fig. 5.

### 5.3 Analysis of germination and coleoptile emergence in Matchball

Table 3 shows the seed quality indicators and recorded average values in bed moisture conditions (30 ml and 20 ml) and incubation temperatures (20 °C and 15 °C) throughout germination. There were statistically significant differences between GE2, GE3 and COL4, COL5 between incubation temperatures. There were slight significant differences between GE3 and TG between moisture conditions for this cultivar. This indicates how a slight change in moisture conditions may affect germination. However, there were no significant differences between GE2 and COL5 between moisture conditions. There was no significant difference between total germination in both factors, but MGT was significantly shorter at 20 °C than 15 °C.

Table 3: Influence of factors on seed quality parameters in Matchball (values are means for each factor)

| **Factor** | **GE2 (%)** | **GE3 (%)** | **TG (%)** | **MGT (days)** | **COL4 (%)** | **COL5 (%)** |
|---|---|---|---|---|---|---|
| 30 ml | 16.3a | 75.3a | 98.3b | 3.1b | 46.3a | 77.3a |
| 20 ml | 14.5a | 65.3b | 99.5a | 3.2a | 32.8b | 76.0a |
| **HSD** | **3.65** | **8.33** | **1.13** | **0.11** | **6.43** | **5.16** |
| $20^0$C | 30.8a | 96.5a | 98.5a | 2.72b | 65.3a | 95.8a |
| $15^0$C | 0.0b | 44.0b | 99.3a | 3.58a | 13.75b | 57.5b |
| **HSD** | **3.65** | **8.33** | **1.13** | **0.11** | **6.43** | **5.16** |

Means with the same letter are not significantly different. HSD = Tukey's honestly significant difference test (Tukey's HSD) $P \leq 0.05$.

The influence of temperature on seed quality parameters was observed between the two temperatures. GE2 was 0% at 15 °C and 30.8% at 20 °C. There was a statistically significant difference between the influence of temperatures on seed germination. After 72 hrs, GE3 was significantly faster at 20 °C with an average of 96.5% versus 44% at 15 °C. Therefore, a significant difference between the GE between temperatures was observed. There was a slightly significant difference between total germination in bed moisture conditions. There was no significant difference between total germination between temperature conditions. However, MGT was significantly shorter at 20 °C than 15 °C. There was a significant difference between coleoptile emergence under both temperatures. On day 5, the average total emergence was 95.8% at 20 °C compared to 57.5% at 15 °C.

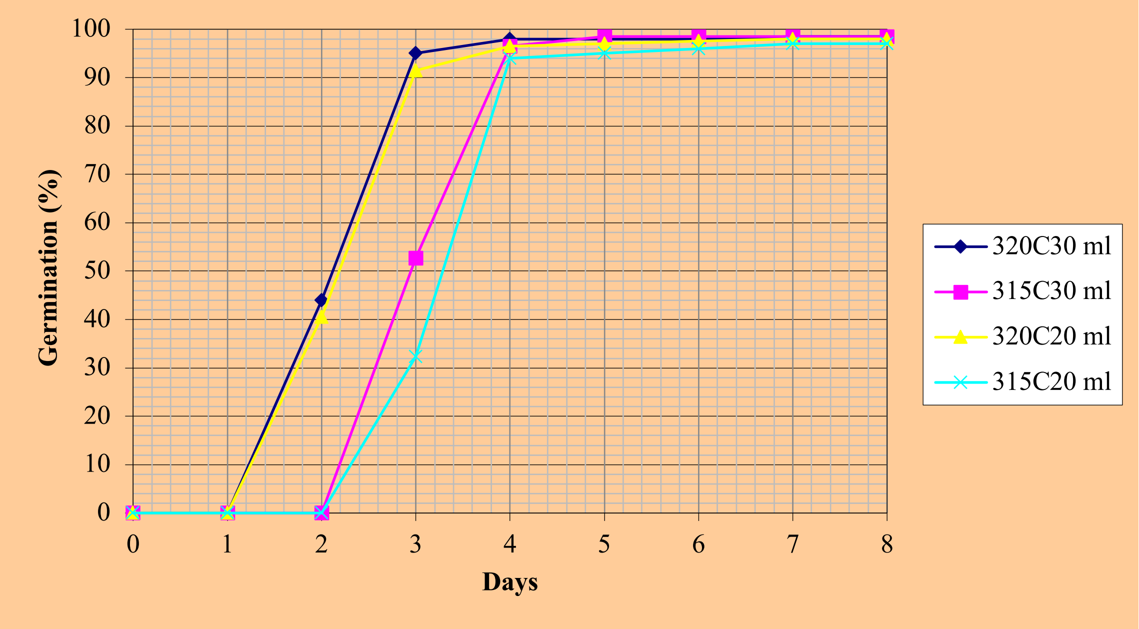

Figure 6: Real values of daily germination in Matchball

Germination curves present daily germination of the cultivar, Matchball. The difference between GE2 observed between conditions 20 °C/30 ml and 20 °C/20 ml was only 4%. However, the difference between GE2 between conditions 15 °C/30 ml and 15 °C/20 ml was 0. The difference between GE3 between conditions 15 °C/30 ml and 15 °C/20 ml was quite higher. The difference was 20%. The highest GE3 was 95% recorded in conditions 20 °C/30 ml. The least GE3 was 32% recorded in conditions 15 °C/20 ml. The highest total germination was 98% recorded in conditions 15 °C/30 ml. The least total germination was 97% recorded in conditions 15 °C/20 ml as shown in Fig. 6.

### 5.4 Analysis of germination and coleoptile emergence in Turandot

Table 4 shows the seed quality parameters and recorded average values in bed moisture conditions (30 ml and 20 ml) and incubation temperatures (20 °C and 15 °C) throughout germination. There were statistically significant differences between GE2, GE3, and COL4, COL5 between temperatures. There were slight significant differences between GE3 between moisture conditions. However, there were no significant differences between GE2 and COL5 between moisture conditions. There was no significant difference between total germinations under the influence of both factors, but MGT was significantly shorter at 20 °C than 15 °C. There was a slightly significant difference between MGT under moisture conditions proving that moisture stress may lengthen MGT.

Table 4: Influence of factors on seed quality parameters in Turandot (values are means for each factor)

| **Factor** | **GE2 (%)** | **GE3 (%)** | **TG (%)** | **MGT (days)** | **COL4 (%)** | **COL5 (%)** |
|---|---|---|---|---|---|---|
| 30 ml | 22.0a | 73.9a | 98.5a | 3.0b | 46.7a | 80.2a |
| 20 ml | 20.3a | 61.9b | 97.5a | 3.2a | 34.8b | 76.0a |
| **HSD** | **6.44** | **6.61** | **2.22** | **0.14** | **7.55** | **8.55** |
| $20^0$C | 42.3a | 93.3a | 98.3a | 2.6b | 71.5a | 94.8a |
| $15^0$C | 0.0b | 42.5b | 97.8a | 3.6a | 9.9b | 61.4b |
| **HSD** | **6.44** | **6.61** | **2.22** | **0.14** | **7.55** | **8.55** |

Means with the same letter are not significantly different. HSD = Tukey's honestly significant difference test (Tukey's HSD) P ≤ 0.05.

The differences between moisture levels and temperatures had no significant influence on germination percentage as shown in Table 2. GE2 was 0% and 42.3% at 15 °C and 20 °C respectively. There was a statistically significant difference between the influence of temperature on germination energy on the second day. There were significant differences between MGT at both incubation temperatures and in moisture levels. MGT was shorter at 20 °C than 15 °C and in 30 ml than 20 ml. There was a significant difference between coleoptile emergence at both temperatures. On day 5, the total emergence of coleoptile was 94.8% at 20 °C compared to 61.4% at 15 °C.

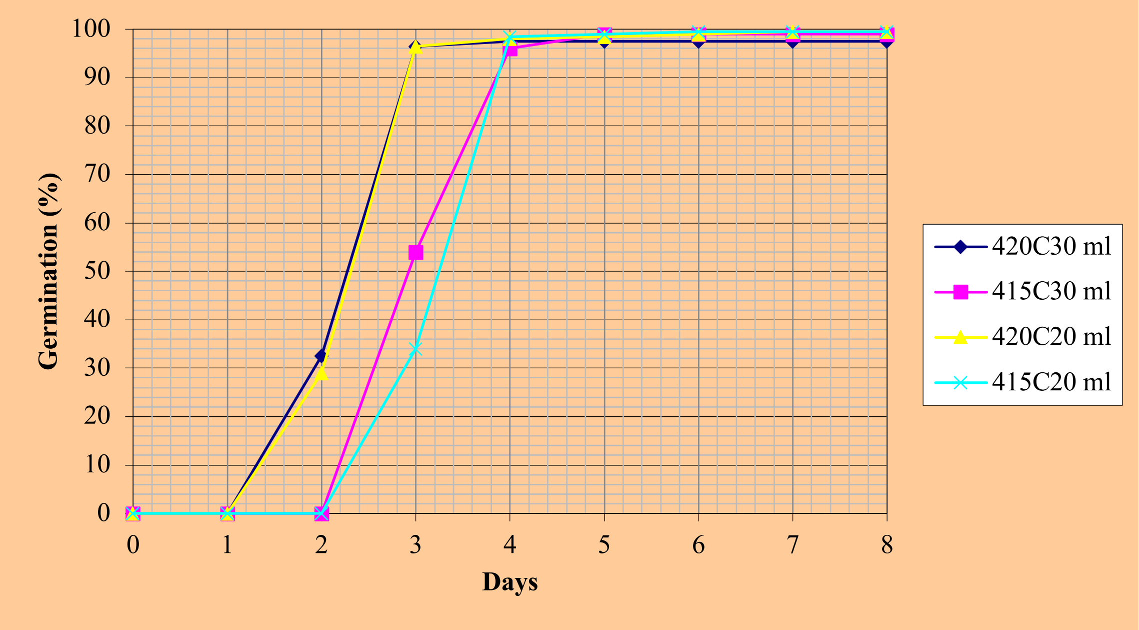

Figure 7: Real values of daily germination in Turandot

Germination curves present daily germination of the cultivar, Turandot. The difference between GE2 between conditions 20 °C/30 ml and 20 °C/20 ml was only 3%. However, the difference between GE2 between the moisture conditions at 15 °C was 0. The difference between GE3 between conditions 15 °C/30 ml and 15 °C/20 ml was quite higher. It was 20%. The highest GE3 was 96% recorded under conditions 20 °C/20 ml and 20 °C/30 ml. The least GE3 was 34% recorded in conditions 15 °C/20 ml. The highest total germination was 99 % recorded in conditions 15 °C/30 ml, 20 °C/20 ml and 15 °C/20 ml. The least total germination was 97% recorded in conditions 20°C/30 ml as shown in Fig. 7.

### 5.5 Analysis of germination and coleoptile emergence in Hyfi

Table 5 shows the seed quality indicators and recorded average values in bed moisture conditions (30 ml and 20 ml) and incubation temperatures (20 °C and 15 °C) over the germination period. There were statistically significant differences between GE2, GE3, COL4 and COL5 between moisture conditions. There was no significant difference between total germination between moisture conditions. There were significant differences between GE2, GE3, COL4 and COL5 between temperatures. MGT was significantly shorter at 20 °C than 15 °C and in 30 ml than in 20 ml.

Table 5: Influence of factors on seed quality parameters in Hyfi (values are means for each factor)

| **Factor** | **GE2 (%)** | **GE3 (%)** | **TG (%)** | **MGT (days)** | **COL4 (%)** | **COL5 (%)** |
|---|---|---|---|---|---|---|
| 30 ml | 17.3a | 73.2a | 97.0a | 3.1b | 44.0a | 77.8a |
| 20 ml | 9.0b | 65.5b | 98.0a | 3.3a | 26.8b | 68.8b |
| **HSD** | **7.34** | **6.47** | **1.53** | **0.16** | **11.67** | **7.74** |
| $20^0$C | 26.3a | 93.2a | 98.5a | 2.8b | 58.5a | 93.0a |
| $15^0$C | 0.0b | 45.4b | 96.5b | 3.6a | 12.3b | 53.5b |
| **HSD** | **7.34** | **6.47** | **1.53** | **0.16** | **11.67** | **7.74** |

Means with the same letter are not significantly different. HSD = Tukey's honestly significant difference test (Tukey's HSD) P ≤ 0.05.

The temperature differences had a significant influence on performance and seed quality parameters of the cultivar, Hyfi. GE2 was 0% at 15 °C and 26.3% at 20 °C. There was a statistically significant difference between the influence of temperatures on seed germination. GE3 was significantly higher at 20 °C with an average of 93.2% versus 45.4% at 15 °C. Therefore, there was a significant difference between GE between temperatures. There was no significant difference between total germination between moisture conditions, but there was a slightly significant difference between temperatures. There was a significant difference between MGT in both factors even though MGT was quicker at 20 °C. There was a significant difference between coleoptile emergence between temperatures. On day 5, coleoptile emergence was 93% at 20 °C and 53.5% at 15 °C.

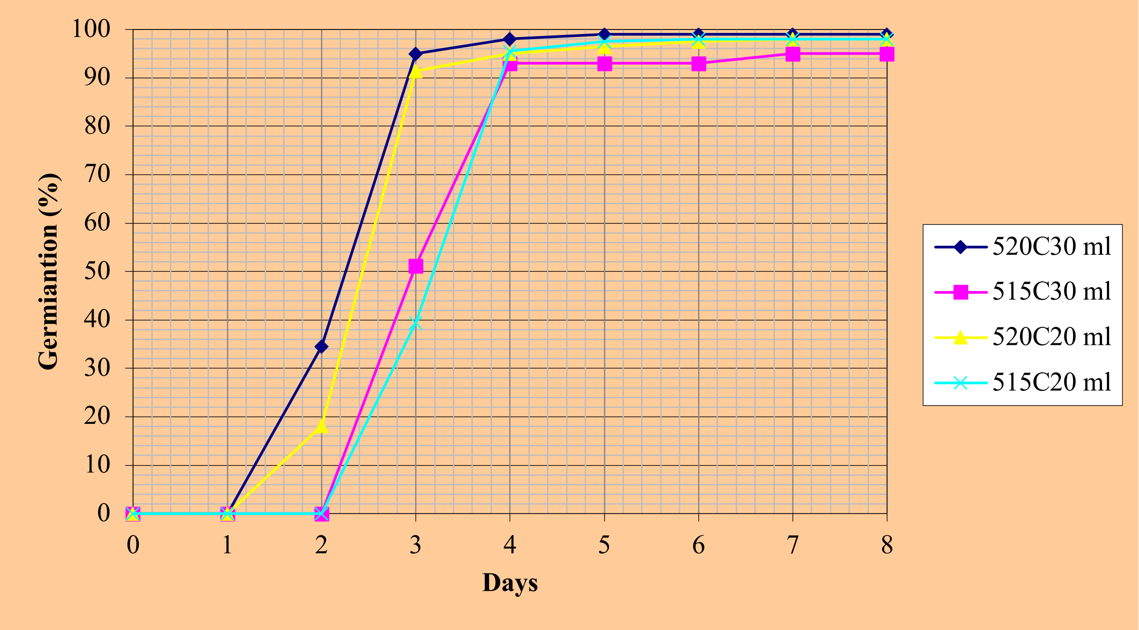

Figure 8: Real values of daily germination in Hyfi

Germination curves present daily germinations of the cultivar, Hyfi. The difference between GE2 observed between conditions 20 °C/30 ml and 20 °C/20 ml was 16%. However, the difference between GE2 between conditions 15 °C/30 ml and 15 °C/20 was 0. The difference between GE3 recorded between conditions 15 °C/30 ml and 15 °C/20 ml was slightly higher. The difference was 12%. The highest GE3 was 95% recorded at 20 °C/30 ml. The least GE3 was 39% recorded at 15 °C/20 ml. The highest total germination was 98% recorded in conditions 20 °C/30 ml, 20 °C/20 ml and 15 °C/20 ml. The least total germination was 95% recorded in 15 °C/30 ml as shown in Fig. 8.

## 5.6 Analysis of germination and coleoptile emergence in Tobak

Table 6 shows the seed quality parameters and recorded average values in bed moisture conditions (30 ml and 20 ml) and incubation temperatures (20 °C and 15 °C) over the germination period. There were statistically significant differences between COL4 between moisture conditions. There was no significant difference between GE2, GE3 and COL5 between moisture conditions. However, there were significant differences between GE2, GE3, COL4 and COL5 between temperatures. There were slight significant differences between total germination in both factors. MGT was significantly shorter at 20 °C than 15 °C.

Table 6: Influence of factors on seed quality parameters in Tobak (values are means for each factor)

| **Factor** | **GE2 (%)** | **GE3 (%)** | **TG (%)** | **MGT (days)** | **COL4 (%)** | **COL5 (%)** |
|---|---|---|---|---|---|---|
| 30 ml | 17.3a | 69.3a | 97.0b | 3.1a | 46.0a | 76.8a |
| 20 ml | 15.8a | 64.8a | 99.0a | 3.2a | 33.5b | 77.8a |
| **HSD** | **5.09** | **7.74** | **1.46** | **0.09** | **8.54** | **4.81** |
| $20^0$C | 33.0a | 95.0a | 97.3b | 2.69b | 66.8a | 97.3a |
| $15^0$C | 0.0b | 39.1b | 98.7a | 3.63a | 12.8b | 57.3b |
| **HSD** | **5.09** | **7.74** | **1.46** | **0.09** | **8.54** | **4.81** |

Means with the same letter are not significantly different. HSD = Tukey's honestly significant difference test (Tukey's HSD) P ≤ 0.05.

The difference in incubation temperature had more effect on the germination of Tobak as demonstrated in Table 6. GE2 was 0% at 15 °C and 33% at 20 °C. There was a statistically significant difference between the influence of temperatures on seed germination. GE3 was significantly higher at 20 °C with an average of 95.0% versus 39.1% at 15 °C. Therefore, there was a significant difference between GE between temperatures as clearly demonstrated throughout the germination period. There was a significant difference between MGT between temperature conditions. It was shorter at 20 °C than 15 °C. There was no significant difference between MGT between moisture conditions. There was a significant difference between COL5 between temperatures but there was no significant difference in emergence when moisture was lower than the standard amount. On day 5, the coleoptile emergence was 97.3% at 20 °C compared to 57.3% at 15 °C.

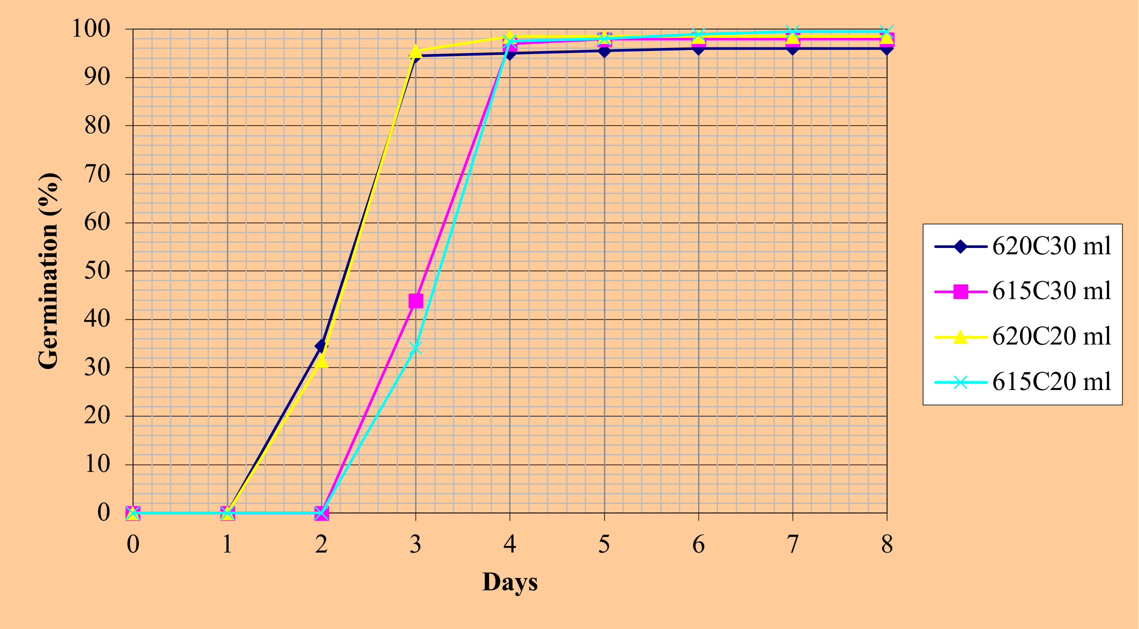

Figure 9: Real values of daily germination in Tobak

Germination curves present daily germination of the cultivar, Tobak. The difference between GE2 recorded between conditions 20 °C/30 ml and 20 °C/20 ml was 3%. However, the difference between GE2 between conditions 15 °C/30 ml and 15 °C/20 ml was 0. The difference between GE3 between conditions 15 °C/30 ml and 15 °C/20 ml was higher than the previous day. The difference was 9%. The highest GE3 was 95% recorded under the influence of 20 °C/20 ml. The least GE3 was 34% recorded in conditions 15 °C/20 ml. The highest total germination was 99% recorded at 15 °C/20 ml. The least total germination was 96% recorded at 20 °C/30 ml as shown in Fig. 9.

## 5.7 Analysis of germination and coleoptile emergence in Bohemia

Table 7 shows the seed quality parameters and recorded average values in bed moisture conditions (30 ml and 20 ml) and incubation temperatures (20 °C and 15 °C) over the germination period. Statistically, there was a slightly significant difference between total germination and COL4 between moisture conditions. There was no significant difference between GE2, GE3 and COL5 between moisture conditions. However, there were visible significant differences between GE2, GE3, TG, COL4 and COL5 between temperatures. Although there was a significant difference between total germination between temperatures, the margin was very small. MGT was significantly shorter at 20 °C than 15 °C but moisture differences had no significant influence on MGT.

Table 7: Influence of factors on seed quality parameters in Bohemia (values are means for each factor)

| **Factor** | **GE2 (%)** | **GE3 (%)** | **TG (%)** | **MGT (days)** | **COL4 (%)** | **COL5 (%)** |
|---|---|---|---|---|---|---|
| 30 ml | 27.3a | 73.1a | 100.0a | 3.03a | 52.5a | 81.8a |
| 20 ml | 24.9a | 74.0a | 98.3b | 3.01a | 38.8b | 77.0a |
| **HSD** | **9.71** | **8.01** | **0.83** | **0.10** | **8.21** | **9.31** |
| 20$^0$C | 45.9a | 97.0a | 99.8a | 2.58b | 79.0a | 98.0a |
| 15$^0$C | 6.3b | 50.1b | 98.5b | 3.5a | 12.3b | 60.8b |
| **HSD** | **9.71** | **8.01** | **0.83** | **0.10** | **8.21** | **9.31** |

Means with the same letter are not significantly different. HSD = Tukey's honestly significant difference test (Tukey's HSD) P ≤ 0.05.

The differences between temperature conditions influenced the rate of germination in Bohemia as clearly demonstrated in Table 7. GE2 was an impressive 6.3% at 15 °C and 45.9% at 20 °C. Even under low-temperature conditions, Bohemia quickly germinated. In all other cultivars, germination energy was only measurable on the third day, at lower temperatures (15

°C). Statistically, there was a significant difference between the influence of temperatures on the rate of seed germination. GE3 was significantly higher at 20 °C with an average of 97% versus 50.1% at 15 °C. Thus, a significant difference between the GE between temperatures was demonstrated throughout the germination period. There was a significant difference between MGT between temperatures and it was shorter at 20 °C. However, moisture difference had no significant influence on MGT differences. There was a significant difference between coleoptile emergence between temperatures. On day 5, average coleoptile emergence was 98% at 20 °C compared to 60.8% at 15 °C.

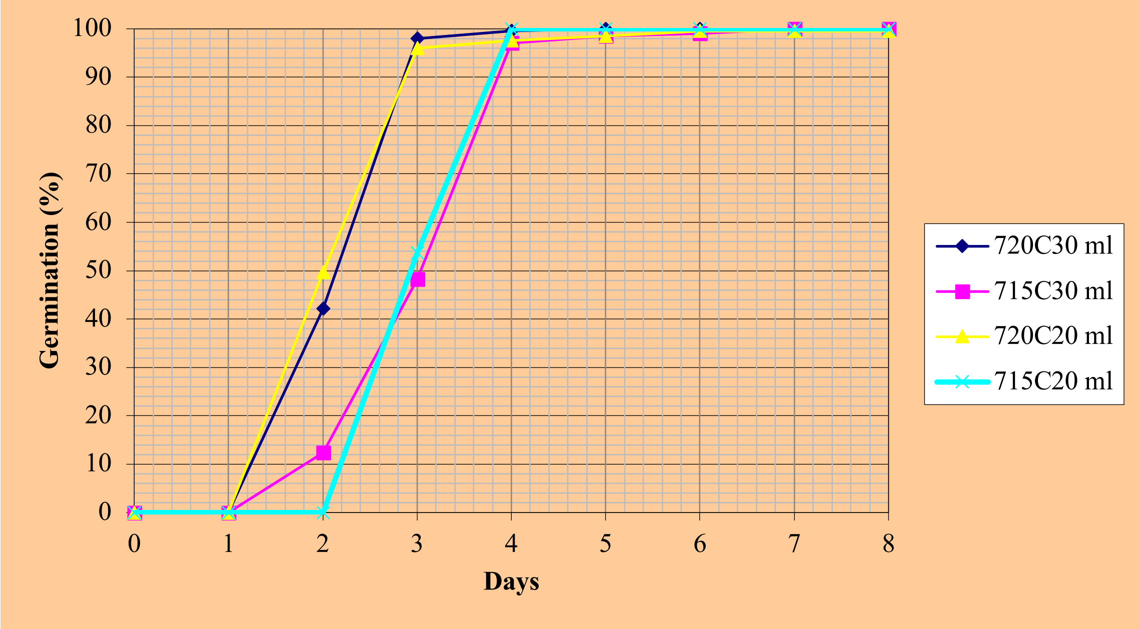

Figure 10: Real values of daily germination in Bohemia

Germination curves present daily germination of the cultivar, Bohemia. The difference between GE2 observed between conditions 20 °C/30 ml and 20 °C/20 ml was 7%. However, the difference between GE2 between 15 °C/30 ml and 15 °C/20 ml was 10%. The difference between GE3 between 15 °C/30 ml and 15 °C/20 ml was slightly lower. The difference was 5%. The highest GE3 was 98% recorded at 20 °C/30 ml. The least GE3 was 48% recorded at 15 °C/30 ml. The total germination was an impressive 100% recorded in all conditions 20 °C/30 ml, 15 °C/30 ml, 20 °C/20 ml and 15 °C/20 ml as shown in Fig. 10.

## 5.8 Analysis of germination and coleoptile emergence in Julie

Table 8 shows the seed quality parameters and recorded average values in bed moisture conditions (30 ml and 20 ml) and incubation temperatures (20 °C and 15 °C) over the germination period. There were statistically significant differences between GE2 and COL4 between moisture conditions. There were no significant differences between GE3 and COL5 between moisture conditions. There were visible significant differences between GE2, GE3, COL4 and COL5 between temperatures. However, there was no significant difference between total germination in both factors. MGT was significantly shorter at 20 °C than 15 °C. The difference between moisture levels had no significant influence on MGT.

Table 8: Influence of factors on seed quality parameters in Julie (values are means for each factor)

| **Factor** | **GE2 (%)** | **GE3 (%)** | **TG (%)** | **MGT (days)** | **COL4 (%)** | **COL5 (%)** |
|---|---|---|---|---|---|---|
| 30 ml | 21.3a | 72.8a | 96.4a | 3.0a | 49.0a | 77.7a |
| 20 ml | 16.8b | 71.0a | 98.8a | 3.1a | 36.3b | 75.0a |
| **HSD** | **3.25** | **8.88** | **2.93** | **0.10** | **7.77** | **6.14** |
| $20^0$C | 38.1a | 96.5a | 99.0a | 2.6b | 74.3a | 97.3a |
| $15^0$C | 0.0b | 47.3b | 96.2a | 3.5a | 11.0b | 55.5b |
| **HSD** | **3.25** | **8.88** | **2.93** | **0.09** | **7.77** | **6.14** |

Means with the same letter are not significantly different. HSD = Tukey's honestly significant difference test (Tukey's HSD) $P \leq 0.05$.

The differences between temperature had more influence on performance and the rate of germination than those differences between moisture levels in Julie, as clearly demonstrated in Table 8. GE2 was 0% at 15 °C and 38.1% at 20 °C. There was a statistically significant difference between the influence of both temperatures on seed germination. GE3 was significantly higher at 20 °C with an average of 96.5% versus 47.3% at 15 °C. Therefore, a significant difference between GE between temperatures was demonstrated throughout the germination period. There was a statistically significant difference between MGT between temperatures and it was shorter at 20 °C. However, the difference between moisture conditions had no significant influence on MGT variance. There was a significant difference between coleoptile emergence between temperatures. On day 5, the average coleoptile emergence was 97.3% at 20 °C compared to 55.5% at 15 °C.

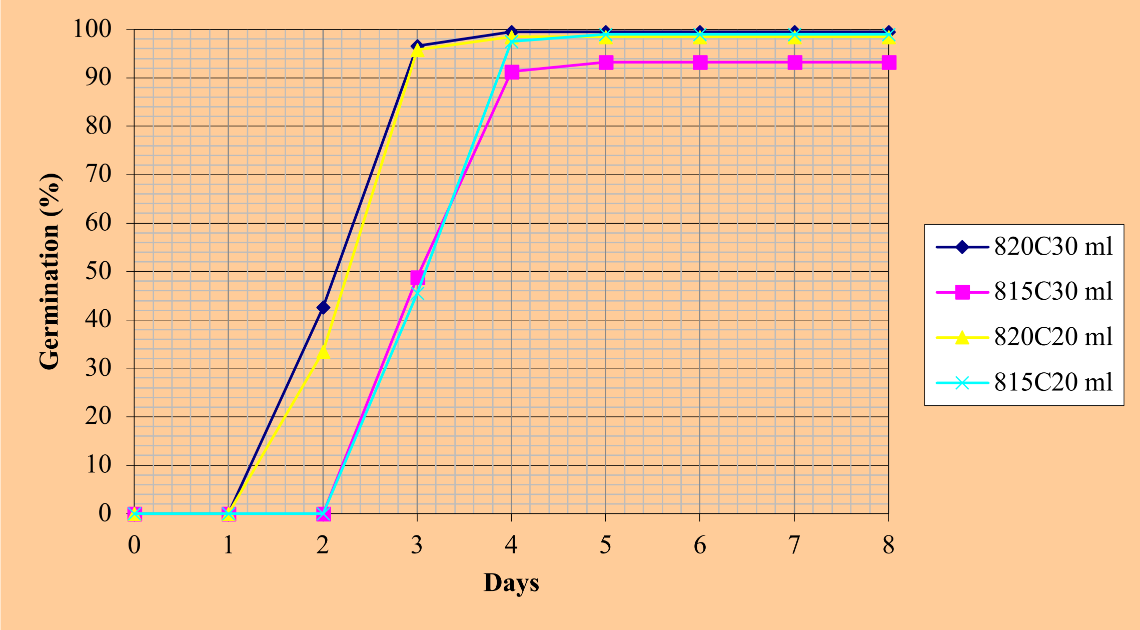

Figure 11: Real values of daily germination in Julie

Germination curves present daily germination of the cultivar, Julie. The difference between GE2 observed between conditions 20 °C/30 ml and 20 °C/20 ml was 9%. However, the difference between GE2 between conditions 15 °C/30 ml and 15 °C/20 ml was 0. The difference between GE3 between 15 °C/30 ml and 15 °C/20 ml was quite lower. The difference was only 3%. The highest GE3 was 95% recorded under the conditions 20 °C/30 ml. The least GE3 was 45% recorded under conditions 15 °C/20 ml. The highest total germination was 99% recorded under conditions 20 °C/30 ml, 15 °C/20 ml, 20 °C/20 ml. The least total germination was 93% recorded at 15 °C/30 ml.

## 5.9 Analysis of germination in moisture doses 30 ml and 20 ml

Early seedling establishment was realized in 30 ml and delayed germination took place in 20 ml as shown in Fig. 12. At day 8, the average germination percentage of all cultivars was 98% in both moisture levels. Germination in 20 ml was slower than in 30 ml until day 5 when cultivars attained average germination of 96%. Fig. 12 shows that the difference between the higher dose of moisture and the stressed variant had no great influence on seedling establishment in all cultivars.

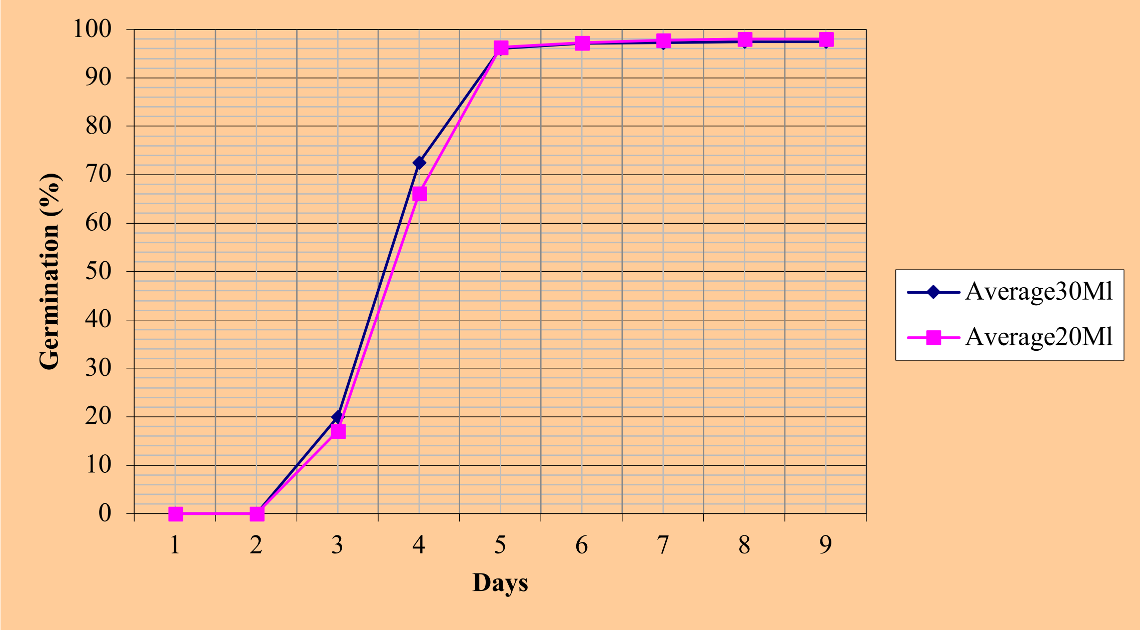

Figure 12: Average values of daily germination of cultivars in 20 ml and 30 ml

### 5.10 Analysis of germination at temperatures 20 °C and 15 °C

Early seedling establishment was realized at 20 °C and delayed germination took place at 15 °C as shown in Fig. 13. At day 8, the average germination for all cultivars at 20 °C was 98%. Whereas, the average germination for all cultivars at 15 °C was 97%. Germination at 20 °C was quicker than at 15 °C. Fig. 13 shows that the difference between temperatures had a great influence on early germination and seedling establishment in all cultivars.

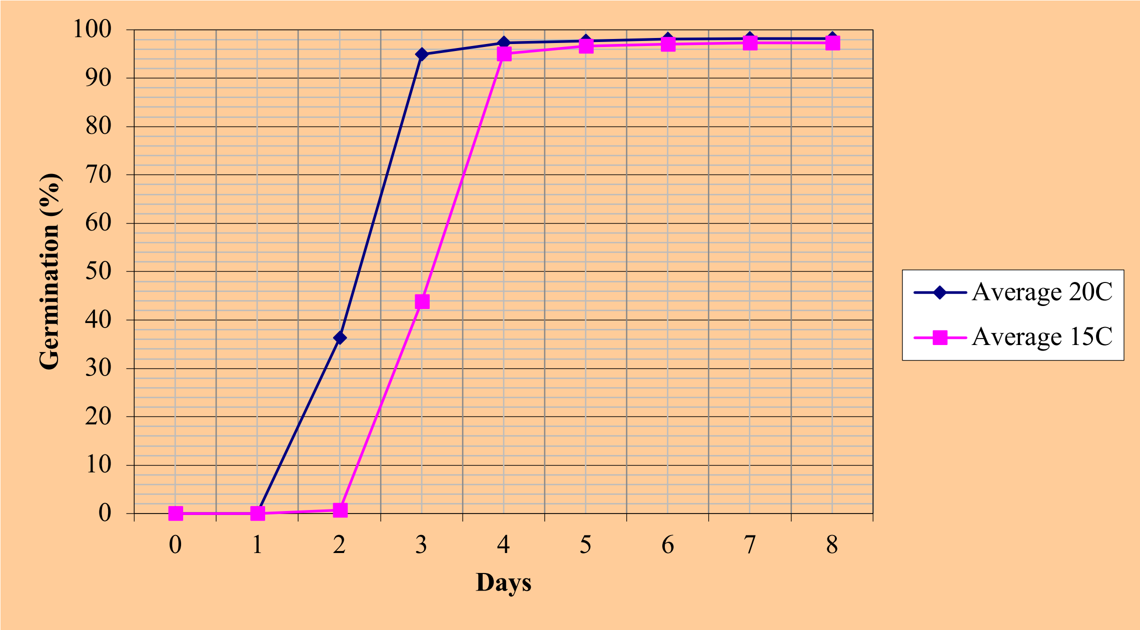

Figure 13: Average values of daily germination of cultivars at 20 °C and 15 °C

# 6 Discussion

This study aimed to evaluate the tolerance of wheat seed lots to water stress conditions using the International Rules for Seed Testing by the International Seed Testing Association (ISTA). During the laboratory test, seed lots germination was evaluated under the influence of two moisture levels; higher dose 30 ml (standard/optimal) and lower dose 20 ml (stressed variant) of tap water. Seed lots were incubated at two temperatures of 20 °C and 15 °C interacting with bed moisture to determine variation in stress tolerance of cultivars. Based on this study, experiments confirmed the hypothesis that “It is possible to select stress tolerant seed lots by the detailed seed testing in stress conditions.”. The results will provide a theoretical basis for selecting seed lots with tolerant abilities, for quick and uniform seedling establishment in water stress conditions.

The difference between a higher dose of moisture and the lower variant did not have a significant influence on germination percentage in 5 out of the 8 seed lots assessed. This was observed in Genius, Annie, Turandot, Hyfi, and Julie. Mean germination time is an important seed quality parameter. It denotes early or late germination of cultivars. In 6 out of 8 seed lots assessed it was found that there were no significant differences between mean germination time based on the differences between bed moisture levels. These cultivars were Genius, Annie, Hyfi, Tobak, Bohemia and Julie. Thus, a higher dose and stressed variant of moisture levels used particularly for this test had no major significance on the differences in the time it took for seed lots to germinate. However, total germination was high in the following cultivars: Genius, Annie, Turandot Hyfi, Bohemia and Julie even under the influence of moisture stress. Slight significant differences were observed in Matchball and Tobak. Thus, moisture stress may affect seed germination of these two seed lots.

Bohemia showed maximum values of germination (100%) in all interactions of bed moisture and incubation temperature. Whereas, under the influence of 15 ° C and 20 ml, Annie showed the average germination percentage (92%). 6 out of 8 cultivars realized total germination above 95% under the influence of 20 °C and 30 ml. Although germination energy and mean germination time varied between cultivars, average germination in all cultivars was above 90%.

Under the influence of 20 °C, Bohemia’s mean germination time was 2.58 days. This was the quickest in all cultivars assessed. However, under the influence of 15 °C, the mean germination time of Bohemia was 3.5 days. This is a clear indication that germination of Bohemia is significantly influenced by temperature i.e., lower temperatures mean a longer time to germinate. This was also true in all seed lots that the lower the incubation temperature, the longer it took for seed lots to germinate.

According to Delcour and Hoseney (2010), the severest form of moisture stress that affects wheat seeds at germination stage is when rainfall during the growing season is negligible; i.e., the seed has access to residual water available only at germination and emergence, such that the soil water is depleted. This situation happens under rainfed wheat and affects major wheat-growing areas in India and Australia, where average yields of wheat are only 1 t/ha. It has now become evident that in Central Europe, climate change probably increases the frequency of droughts, severity and duration. Adaptive measures will determine the resilience of wheat production. Selecting cultivars that are tolerant to abiotic stress will ensure higher yields because cultivars can thrive under moisture stress conditions and quickly develop seminal roots that supply nutrients to the tillers.

Various research papers have documented the performance of cultivars, their performance in germination and seedling parameters due to moisture stress. Abido and Zsombik (2018) noted that Hungarian wheat landraces significantly differed between them in their behaviours under abiotic stresses and the behaviour of the tested was genotype-dependent. They concluded that some landrace varieties, when tested under the influence of moisture stress conditions, had better germination percentage, germination speed, relative water content, shoot and root length, shoot and root fresh, shoot and root dry weight and drought tolerance than others. Soufflet Agro (2019) outlines some agronomic and technological aspects of wheat cultivars registered in the Czech Republic. In the catalogue, there are no specific details about the germination of cultivars but it mentions agronomic aspects of different cultivars common in the Czech Republic.

Overall, temperature had a greater significant influence on seed germination parameters. The differences among cultivars were well expressed by temperature variation more than the differences exhibited between bed moisture levels. Low temperatures did affect germination energy in all 8 cultivars. Evans et al. (1975): Abdul et al. (2011) stated that the speed of germination is faster at higher moisture contents and the optimal temperature for germination of non-dormant seeds is 20 °C–25 °C. There is a linear decrease in germination percentage when seed lots are subjected to further moisture stress conditions and temperatures below 20 °C. According to Lafond and Baker (1986), the interaction of moisture and temperature plays a significant role in successful wheat seed germination. Mean germination time is much shorter with the increase in temperatures. This was confirmed in all 8 seed lots that the mean germination time was shorter under the influence of 20 °C and much longer under the influence of 15 °C.

The time between seeding and germination varies among plant biotypes or cultivars. According to Lafond and Baker (1986); Jame and Cutforth (2004), temperature, moisture stress, and seed size may influence germination of specific wheat cultivars. They concluded that seed germination may also be influenced by other but not so obvious factors such as kernel texture, seed size and colour. However, they maintained that the main factors influencing germination were temperature and moisture.

From this study, we also discovered the influence of temperature on coleoptile emergence. Coleoptile emergence is when the first leaf becomes visible above the soil surface (White and Edwards, 2008). In 7 out of 8 cultivars assessed, except in Hyfi, differences in bed moisture did not have a significant influence on coleoptile emergence. However, at an incubation temperature of 15 °C, there was a significant difference between coleoptile emergence in seed lots. Thus, coleoptile emergence may tolerate moisture deficit stress within cultivars, but they may be more susceptible to low temperatures at germination. This greatly affects the uniform establishment and overall seed yield.

In areas susceptible to moisture stress, selecting wheat cultivars with long coleoptile is an important component of improving emergence, weed suppression and grain yield. Singh and Khanna-Chopra (2010) stated that coleoptile length plays a major role in seedlings emergence, early plant vigour and successful establishment of wheat. The wheat seeds can be planted at a depth between 8 and 12 cm. However, optimum moisture conditions that enable smooth and uniform coleoptile germination are between 2 to 4 cm. Low depth makes non-uniform emergence of seed plants. Therefore, coleoptile length together with seed size has tremendous effects on seed vigour and less on germination (Alaei et al., 2010).

Table 9 presents the influence of cultivar, water and temperature on germination parameters, F values and statistical evidence of evaluated factors. Temperature had the most significant influence on germination energy as shown in the table. However, its influence on total germination was not so high as F-value was not so different from the F-value of the cultivar. Thus, cultivar and temperature had the most significant influence on germination. The influence of moisture conditions on germination energy was significantly high but less significant on total germination. The influence of moisture differences and the interaction between water and temperature was non-significant. Non-significance of factors in the table are when $P>0.05$.

Table 9: The effects of genotype, water and temperature on wheat germination parameters (ANOVA, Fisher´s F-values)

| | **GE2** | **GE3** | **GE4** | **TG** |
|---|---|---|---|---|
| **Cultivar (C)** | 7.75** | 2.72* | 3.49** | 4.95** |
| **Water (W)** | 8.37** | 31.76** | 0.03 $^{NS}$ | 0.61 $^{NS}$ |
| **C x W** | 1.21$^{NS}$ | 1.82* | 1.28 $^{NS}$ | 3.24** |
| **Temperature (T)** | 1264.30** | 1997.78** | 16.82** | 7.03** |
| **C x T** | 3.47** | 1.05 $^{NS}$ | 1.79 $^{NS}$ | 2.57* |
| **W x T** | 1.77 $^{NS}$ | 22.60** | 1.97 $^{NS}$ | 0.19 $^{NS}$ |
| **C x W x T** | 3.85** | 2.06 $^{NS}$ | 0.95 $^{NS}$ | 2.38* |

NS, $P < 0.05$*; $P < 0.01$**

Higher F-values mean higher influence of evaluated factor on germination after 8 days.

# 7 Conclusions

- The hypothesis was accepted "It is possible to select stress tolerant seed lots by detailed seed testing in stress conditions"
- Performances in germination percentage, germination energy, mean germination time and coleoptile emergence varied between cultivars
- The difference between a higher dose of moisture and the lower variant did not have a significant effect on total germination in 5 out of the 8 seed lots assessed: Genius, Annie, Turandot Hyfi and Julie.
- There were slightly significant differences between germination percentage in Bohemia, Tobak and Matchball.
- Under the influence of 15 °C and 20 ml, Annie showed the least germination values of 92%. This is an indication that Annie might be affected by stress variants even in field conditions
- The best response was in Bohemia which had a 100% total germination under the influence of 20 °C/30 ml, 15 °C/30 ml, 20 °C/20 ml and 15 °C/20 ml
- Germination energy was fastest in Bohemia than any other cultivar
- At an optimal temperature of 20 °C, Bohemia had the shortest mean germination time
- The germination percentage was generally high under conditions 20 °C and 30 ml
- Germination parameters, mostly germination energy, were affected under conditions 15 °C and 20 ml
- Coleoptile emergence was significantly affected by aberrant temperature rather than moisture differences
- Total germination in all varieties was above 92%
- Based on ISTA Rules, germination tests can provide critical data on seed quality.
- It is recommended to note that seed lots with high laboratory germination may be low in vigour. This is manifested as poor seedling emergence under unfavourable conditions. Thus, emergence percentage may deviate sharply from germination percentage (ISTA Vigour Test Committee, 1995)

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# 9 List of Figures

# 10 List of Tables

# 11 List of Abbreviations and Symbols

| | |
|---|---|
| ABA | Abscisic acid |
| BP | between filter paper |
| CELSS | Controlled (or closed) ecological life-support systems |
| CIMMYT | International Maize and Wheat Improvement Center |
| CISTA | Central Institute for Supervising and Testing in Agriculture |
| COL4 | Coleoptile emergence at day 5 |
| COL5 | Coleoptile emergence at day 6 |
| CRD | Completely Randomized Design |
| DNA | Deoxyribonucleic acid |
| dS m-1 | deciSiemens per metre |
| ETH | Ethylene |
| FAO | Food and Agriculture Organization of the United Nations |
| FGRMS | Alberta Forest Genetic Resource Management and Conservation Standards |
| GA | Gibberellic acid |
| GE | Germination energy |
| GE2 | Germination Energy on the second day |
| GE3 | Germination Energy on the third day |
| $G_{st}$ | Standard germination test |
| HSD | The Tukey HSD "honest significant difference" |
| ISTA | International Seed Testing Association |
| LD | Long Day |

| | |
|---|---|
| LED | light-emitting diode |
| MGT | Mean Germination Time |
| MPa | megapascal |
| NaCl | Sodium Chloride |
| PAF | photoperiod after flowering |
| PBF | photoperiod after flowering |
| PHS | Preharvest Sprouting |
| PEG | Polyethylene glycol |
| TG | Total Germination |
| TP | top of filter paper |
| WEF | World Economic Forum |
| Ψ | Water potential |
| Ψcell | Cell or seed water potential |
| Ψm | Matric Potential |
| Ψp | Pressure potential |
| Ψπ | Osmotic potential |