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Studying the regulation of expression of genes involved in barley malting quality

Studium regulace exprese genů podmiňujících sladovnickou kvalitu ječmene

Bachelor's thesis

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Declaration

I declare that I created this bachelor thesis independently and only using the cited sources, I also declare that this thesis has not been used to gain any other academic title.

Prague, 03. 08. 2022

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Abstract:

Barley (*Hordeum vulgare L.*) is a grain cultivated as an agricultural crop across many varying climatic areas of the world. It is mainly used for its feed and brewing industry properties. This work will focus on studying the malting process on a molecular level, which mainly includes the genes for degradative enzymes of storage proteins, starch and compounds of endosperm cell walls. We shall observe how all these components interact during malting and other processes of brewing beer. It is known that malting quality parameters are tied to several genes, whose expression is regulated. These regulatory pathways will also be included in this work.

Key words: Barley, Malting quality, QTL, Gibberellic acid, Dormancy, Germination

Abstrakt:

Ječmen setý (*Hordeum vulgare L.*) je obilnina a hojně pěstovaná zemědělská plodina s rozšířením v různých klimatických oblastech světa. Využívá se zejména jako krmivo nebo ve sladovnictví. Tato práce se konkrétně zaměří na studium procesu sladování na molekulární úrovni, což zahrnuje zejména geny pro enzymy účastnící se degradace zásobních proteinů, škrobu a mimo jiné i složek tvořících stěny buněk endospermu. Práce bude sledovat, jak všechny tyto složky interagují během procesu sladování a následujících fází výroby piva. Je známo, že parametry sladovnické kvality jsou podmíněny více geny, jejichž exprese je regulována. Tyto regulační dráhy budou také zahrnuty v této práci.

Klíčová slova: Ječmen, sladovnická kvalita, QTL, kyselina giberelová, dormance, klíčení

Contents

List of Abbreviations

ABA	Abscisic acid
ABI5	ABA Intensive 5
ABRE	ABA response elements
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
bZIP	Basic Leucine Zipper
GA	Gibberellic acid
GA20ox	GA 20-oxidase
GA_3, GA_{12}, GA_{20}, GA_9, GA_{53}	Gibberellin types
GA3ox	GA 3β-hydroxylase
Gb	Gigabases
GGPP	Geranylgeranyl pyrophosphate
Hsp	Heat shock protein
LTP1	Lipid transfer protein 1
LTR	Long terminal repeats
mRNP	Messenger ribonucleoprotein complex
NGS	Next generation sequencing
pI	Isoelectric point
PP2C	Protein phosphatase 2C
PYR	ABA receptor
ROS	Reactive oxygen species
GARE	Gibberellic acid response element
SnRK2	(SNF1-related kinases)

1. Introduction

Malting and feed quality are two widespread factors regarding choosing the correct barley variety – for each purpose, different properties are required. The majority of the quality traits can be traced back to the corresponding genes, whose expression triggers the production of important enzymes and other compounds crucial for the correct outcome – the seeds can either grow into mature plants, which will in turn produce more grain, or find a different use, like the brewing industry. As one of the very few crops planted worldwide, we constantly search for new ways to enhance the production – within the borders of Europe, laws regarding genetic modification are much stricter, and as such, mainly the method of marker assisted breeding is used. In the rest of the world, where the laws are not so strictly regulating, other methods can be used. Transgenic crops are produced through genetical engineering to achieve higher gains, lessen the amounts of water required and acquire higher resistance to pathogens and weather conditions (such as breeding cereals with short stalks to ensure their stability in case of heavy rainfall). Since the neolithic revolution, people have started breeding crops by simple selection – stalks with higher production of seeds and more stable roots were picked out and then bred together (or in case of fruits, those with superior taste or size were chosen). This way, many crops we know these days progressed and differentiated from their wild relatives. However, as population grew, the need for crops with higher gains arose. Along with higher use of pesticides and fertilizers, advancements in the genetical field allowed further scientific discoveries aiding in development of new high yielding varieties of crops – this time, achieved through careful modification of their genetic information. Genome sequencing became an important precursor for such research, and using constantly advancing methods and sharing data worldwide, we are slowly beginning to understand the crops we breed on a molecular level. As with any topic, it’s important to start from the basics – or in this case, the plant itself.

2. Barley (*Hordeum vulgare L.*)

Along with other cereals, barley is a monocot plant of the *Triticeae* tribe, belonging to the *Poaceae* family (Bothmer, *et al.* 2003; Ullrich, 2014). The size of barley genome is 5,1 Gb (Meyers *et al.*, 2012), and it is divided into seven chromosomes labeled 1H to 7H (Linde-Laursen *et al*. 1997). The gene pool of *Hordeum vulgare* includes two subspecies – the wild progenitor, named *H. vulgare ssp. spontaneum*, and the cultivated variety, *H. vulgare ssp. vulgare*. Both varieties are mutually compatible, and as such, by crossbreeding them, the resulting hybrids usually display higher seed production and allow for greater genetic variety within the breeding industry (Asfaw, and Bothmer, 2008).

A relevant factor is the kernel row number – both two-row and six-row barley types (Figure 1) have found their use within the malting industry. In case of two-row barley, only the central of the three florets is fertile (resulting in only one grain per spikelet). As for six-row barley, all the florets within the spikelet are fertile, and therefore, produce more seeds (Bonnet, 1966). However, higher quantity does not always equal quality, and as such, two-row barley oftentimes has plumper and larger seeds. It is also the variety used for the majority of genetic and malting research (Kling, Hayes and Ullrich 2004).

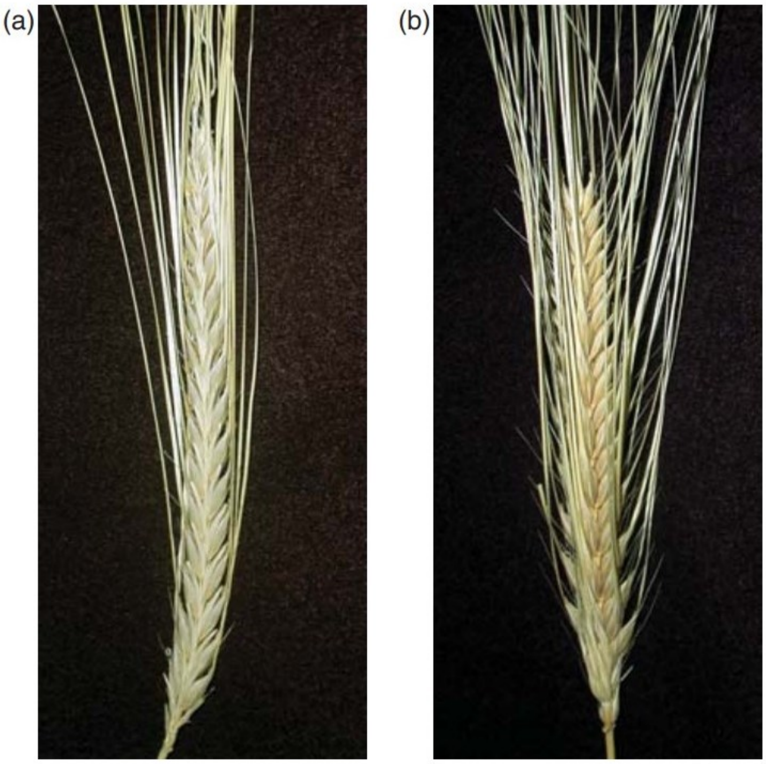


Figure 1: Two-row barley spike (a) and six-row barley spike (b) – from Kling, Hayes and Ullrich (2004)

The main distinctive category or barley type is, however, the season of growth – winter barley makes use of the moisture gained by melting snow or rainfall, giving it a significant growth boost, as opposed to the spring barley, which is oftentimes threatened by droughts. However, winter barley has its own flaws – the most significant one being lacking cold tolerance and being unsuitable for malting purposes. As such, it is mainly used in the feed industry, unlike the spring variety, which has found its use within the malting industry. Therefore, as summarized by Pržulj *et al.* (1998), combining both types could eventually bring about the selection of new genotypes and maximize grain production in both arid and semiarid regions. Currently, attempts at crossbreeding both varieties are being made in order to combine the malting quality traits with the resilience of winter barley (Stockinger, 2021).

2.1 Grain anatomy

While plant morphology is important to the overall viability of the crop, so is the seed anatomy – after all, optimized structure ensures high germination percentage and higher survival rate. The embryo itself makes up only a small portion of the grain (2 – 10%), as indicated in (Figure 2). Molecular communication between it and other grain cells takes place through the scutellum, which provides a crucial link when germination occurs (Rosentrater and Evers, 2018).

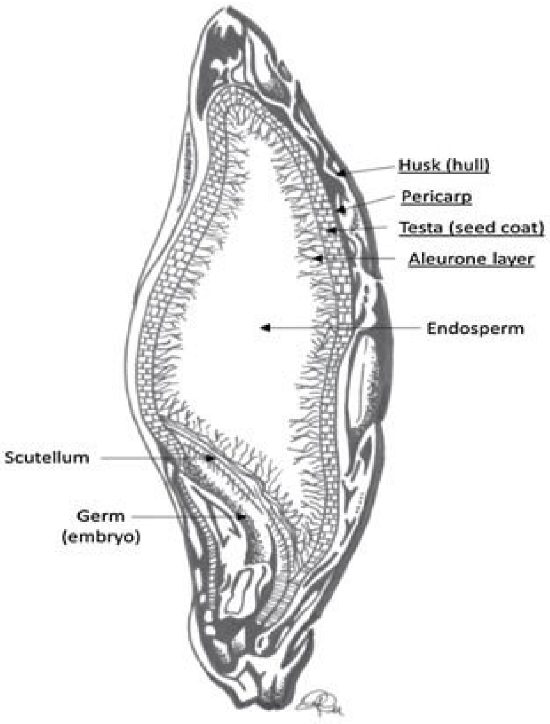


Figure 2: Longitudinal cross-section of a barley seed – from Arendt and Zannini, (2013)

The most prominent tissue within the seed is the endosperm, taking up most space within the kernels. Endosperm cells are further divided into two categories – the so-called starchy endosperm, whose cells can be distinguished by their rather thin cell wall and high granule content, and the aleurone cells, which are cubic in shape and form the outer layers of the tissue. Aleurone cells are living cells producing new endosperm cells by division, but starchy endosperm cells undergo programmed cell death, which simply leaves them as nutrient containers (Rosentrater and Evers, 2018). Unlike other cereal grains, barley aleurone consists of three layers, whereas the others usually have just one (Olsen, Potter and Karra, 1992). As the main energy source for the growing embryo, the endosperm cells serve as storage units and contain starch granules, whereas the aleurone cells store various proteins and lipid bodies necessary for breaking down the starch (Jadhav *et al.*, 1998). Starch degradation occurs only after synthesis of α-amylase within the aleurone cells, and is triggered by GA (gibberellic acid) during germination (Varner, 1964; MacGregor, 1987).

Endosperm is further lined by remaining nucellus, usually in the form of nucellar lysate (Norstog 1974). The true seed coat, also called testa, is derived from the two integuments surrounding the nucellus. It also has a thick cuticle layer, which serves as an efficient water barrier (Evers and Millar 2002). Fused with the testa is the pericarp – within the developing grain, it serves as an energy sink responsible for providing nutrients for the endosperm, and later forms the hull (Weschke and Weber, 2014). The hull serves as a protective layer against stress and helps maintain seed dormancy. The husk itself consists of two morphologically different layers – lemma and palea (Grant, Brennan and Hoad 2021).

3. Malting quality

Malting quality is a set of grain parameters necessary to fulfill certain production needs – in the case of barley, the optimal properties the grain has for malting, and later, beer brewing. The process of malting involves three basic steps – steeping, germination and kilning (for details, see chapter 4). Necessary quality traits include grain protein content, carbohydrate composition, starch type and amount, as well as physiological traits, such as grain size, hardness or dormancy levels (Fox *et al.,* 2003). Barley quality is also influenced greatly by environment – the season, amount of rainfall, row spacing, cultivation of land and of course the amount of sunlight and temperature to name a few (Bishop, 1930).

3.1 Protein content

While the names of protein types present within cereal seeds have changed over time, it can be said what we know today is historically based on what is nowadays called the 'Osborne fractionation', organized by T. B. Osborne (1907). His methods research into various compounds present within the grains brought us four main categories – albumins (water soluble), globulins (soluble in dilute salt solution), prolamins (alcohol solution) and glutelins (detergents, or dilute alkali/ acid solution), as well as several others which will not be discussed in detail (Osborne, 1924; Finnie and Svensson, 2014).

Albumin and globulin form the soluble segment of plant proteins and are often grouped together as cytoplasmic or metabolically active proteins, whereas prolamins (or hordeins, in context of barley) and glutelins classify as storage proteins (Lásztity, 1985).

Certain albumins also have an amylase-inhibitory function, however, said function was observed only in wheat and rye grains. Its function was identified as inhibition of animal species' amylases, not ones of the plant itself (Kneen and Sandstedt, 1943; Lásztity, 1985).

As investigated by Danielsson (1949), globulins are further divided into four groups (α, β, γ and δ), each having a different localization and amount present within the barley seed. Altogether, they rarely cross 6 % of dry weight and it is also suspected they function as an early resource available for the embryo.

Hordeins create a substantial amount of grain protein, found mainly in starchy endosperm, and their alternative name (prolamins) also indicates high proline and glutamine content (Shewry, 1996). These polypeptides are also designated into four fractions labeled A – D (Shewry 1993), A – C being labeled according to lysine present, with decreasing lysine content (Køie *et al.*, 1976). Fraction A corresponds to α, B to β + γ and C to δ + ε. A hordeins are characteristically high in lysine (however, compared to other protein fractions within the seeds, they still have the lowest lysine content) and low molecular weight, whereas B hordeins typically have high molecular weight, partially aided by disulfide bonds forming between cysteine residues (Shewry *et al.*, 1977).

Along with prolamins, glutelins form what is commonly known as gluten – a compound widely used within the food industry for its special properties and ability to influence texture (Sharma and Rallabhandi, 2015).

3.2 Carbohydrates

As with any cereal crop, the most abundant carbohydrate within the kernel is the starch – taking up to two thirds of the grain dry weight. Starch granules include two polysaccharides, amylose and amylopectin, both polymers of glucose. As illustrated in Figure 3, amylose is a linear chain linked with α-(1,4) bond, whereas amylopectin branches, and the bond is α-(1,6) in the branching points (Hough. 1985).

3.2.1 Degradation enzymes

Cleaving starch polymers is done by several enzymes, mainly by amylases, glucanases, glucosidases and limit dextrinase, which cause the chains to eventually be separated back into glucose units. Said units can then be used by the growing embryo – the regulatory pathways controlling the triggering of both α-amylase and β-amylase are rather complex, and while they are an important piece of the puzzle, they are not the focal point of this work. But in short, α-amylase is a heterogenous group of enzymes, which randomly cleaves the α-(1,4) glycosidic bonds (MacGregor, 1987), while β-amylase cleaves maltose (two bonded glucose units) from within the polymer chain. (Fox 2018; Ziegler, 1999). There is, however, a dichotomy in the opinion of direction from which β-amylase cleaves – Fox (2018) as the only one presents the opinion the direction is from the reducing end, while Hough (1985) and Ziegler (1999) claim β-amylase cleaves from the nonreducing end. β-amylase can often be found within a hetero-dimer with a protein Z, which is an albumin protein (Hejgaard, 1978; Guerin, Lance and Wallace, 1992).

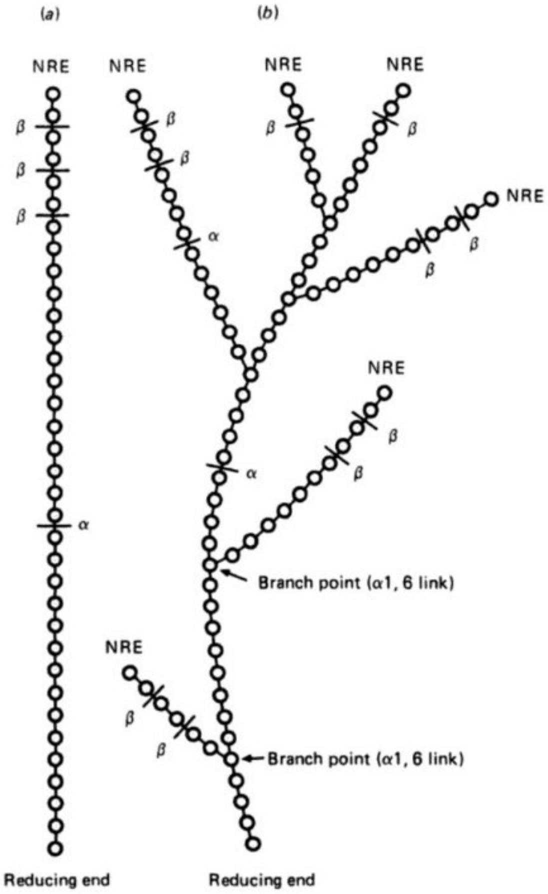


Figure 3: Chain structure of amylose (a) and amylopectin (b) along with possible amylase cleaving sites – from Hough (1985)

Another very important enzyme involved in starch degradation is limit dextrinase, which has the ability to cleave the α-(1,6) bonds of amylopectin. A such, it creates linear oligosaccharide chains, which are then further hydrolyzed by α-amylase (Manners and Yellowlees, 1973; Fox *et al.*, 2003).

Another link in the starch hydrolysis chain would be α-glucosidase, which causes splitting of glucose from maltose and other, higher sugars, but preferably chooses oligosaccharide substrates rather than complex polymers (Agu and Palmer, 1997; Osman, de Jersey and Inkerman, 1996a). Barley seeds contain β-glucosidase as well, and unlike α-glucosidase, which can cleave only α-(1,6) bonds, it can cleave β-(1,2), β-(1,3) and β-(1,4) linkages (Leah *et al.*, 1995). Compared to α- and β-amylases, limit dextrinase and both glucosidases can only be found in very small amounts within the matured grain (Osman, 2002).

It is important to note that starch is not the only carbohydrate within barley kernels – both fructans (very diverse group, ranging from trisaccharides to large oligosaccharides) and raffinose can be found within the growing embryo, as well as endosperm reserves (MacLeod and Preece, 1954), as well as many other monosaccharides, such as glucose, fructose, and maltose or sucrose among the disaccharides (Henry, 1988).

Another noteworthy group would be the cell wall polysaccharides, which are responsible for the unique structure of endosperm cell wall and ultimately, it's timely breakdown during germination. Cell wall is composed of cellulose, which is a glucose polymer. Unlike starch, however, the glucose units are joined with a β-(1,4) bond, and the chains are organized into microfibrils (Brigham, 2018). Together with β-glucans, they form a considerable part of the cell wall. As opposed to cellulose, β-glucan bonds are a combination of β-(1,3) and β-(1,4), the latter being a lot more common. β-glucans are hydrolyzed by β-glucanase during germination. The last main component are the arabinoxylans, which are branched heteroglycans of a xylan backbone, arabinose and pentose sugars (Izydorczyk and Edney, 2003).

3.3 Lipids

Lipids take up approx. 4 % of barley dry weight – the majority of fatty acids within the grains can be found in the form of triglycerides, or free long fatty acid chains (Anness, 1984). Lipid components change during the various malting stages, and their amount also significantly falls during malting. The main fats present within the kernels are oleic and linoleic acid, taking up to 80 % of all lipids present (MacLeod and White1961). Together with amylose, they form starch granules and by the amount of fatty acids present, we are able to distinguish what is commonly called waxy and non-waxy starch (Cozzolino and Degner, 2016). A noteworthy protein responsible for binding lipid and starch within the endosperm is LTP1 (lipid transfer protein 1), and as the lipids themselves, it is crucial in beer foam formation and retention (Lusk, Goldstein and Ryder 1995).

3.4 Physiological grain traits

Aside from biochemical components within the barley grain, there are several physiological factors directly reflecting the quality. Grain size correlates with amounts of starch present – the larger the grain, the bigger the starch reserve. Kernel size and weight is directly influenced by both the environment and genetic predisposition (Coventry *et al.*, 2003).

3.4.1 Seed dormancy

The level of grain dormancy is an important trait of malting quality barley – the grain's ability to germinate is absolutely crucial for the malting process, and the germination level should always be above 95 % (Fox *et al.*, 2003). Grain dormancy is induced and maintained by ABA (abscisic acid), which holds an inhibitory effect, whereas GA (gibberellic acid) works antagonistically and triggers seed germination (Gómez-Cadenas *et al.*, 2001).

While the main function of seed dormancy is to ensure the embryo remains 'asleep' before it reaches full maturity, it is also the means of protection from the environment or excess damage the seed could sustain while germinating too early (Baskin and Baskin, 1998). There is more than one type of dormancy – namely morphological, physical and physiological (Fenner and Thompson, 2005). Morphological dormancy is caused when the embryo is underdeveloped, in which case it gives it time to fully differentiate and grow. Physical dormancy is a consequence of impenetrable seed coat, which prevents even water from seeping in and can be broken only by and outside influence, or in this case, damage (Baskin and Baskin, 1998). Physiological dormancy is the most common type, and is caused by chemical influences, mainly the presence of phytohormones (Baskin and Baskin, 2004).

3.4.1.1 Abscisic acid

ABA is a sesquiterpenoid found universally in plants, but may be accumulated within the chloroplasts. While acting as a general inhibitor, its influence varies depending on the tissue of effect (Srivastava, 2002). As with the majority of organic compounds present, even ABA has more than one configuration, which may impede its ability to bind active sites (Milborrow, 1986). Aside from the now obvious role in seed dormancy, ABA serves as a universal stress signal (Bulgakov and Koren, 2022), and promotes lateral shoot growth and emergence of adventitious roots (Abou-Mandour and Hartung, 1980), and is also involved in the closure of stomata (Daszkowska-Golec and Szarejko, 2013).

ABA accumulates within the grain during seed development, but its absolute content does not seem to have a direct effect on maintaining seed dormancy (Jacobsen *et al.*, 2002). The mechanism of effect is rather complex – the phytohormone itself is synthesized from C_{40} carotenoids (Koornneef, 1986; Sano and Marion-Poll, 2021). The regulatory pathway itself starts with a PYR receptor, which enables the inhibition of PP2C (protein phosphatase 2C) in the presence of ABA – this in turn allows the phosphorylation of SnRK2 (SNF1-related kinases) and further of bZIP transcription factors of the ABI5 family. These transcription factors bind themselves to ABRE (ABA response elements) in promoter sequences of genes responsible for ABA gene induction (Cutler, *et al.*, 2010).

3.4.1.2 Gibberellic acid

Gibberellins are a group of acids, the most used of which is GA_3. This gibberellic acid is a pentacyclic diterpene (Mbaveng, Hamm and Kuete, 2014), which works antagonistically to ABA (Sano and Marion-Poll, 2021). Gibberellins in general are mainly involved in breaking seed dormancy and inducing germination, but they are also responsible for cell elongation, reproductive growth and senescence (Rodrigues *et al.*, 2011; Bhattacharya, 2019). Gibberellic acid is highly active in influencing synthesis of α-amylase within the aleurone layer. It is spreading from the growing embryo after germination commences, and stimulates the still living cells into triggering gene expression (Jacobsen and Chandler, 1987).

The metabolic pathway of gibberellins is very complex, for it includes many transformations from one type to several others. As such, the first piece of the puzzle is GA_{12}-aldehyde, synthesized from GGPP (trans-geranylgeranyl diphosphate) originating either from mevalonate, or carotenoids and chlorophyll residues. GA_{12}-aldehyde is then further processed by cytochrome-P450-dependent mono-oxygenases, which creates both GA_{12} and GA_{53}. Both these gibberellins are then further oxidated by GA20ox (GA 20-oxidase) and converted to GA_9 and GA_{20}. The activity of GA3ox (GA 3β-hydroxylase) then turns GA_9 into 2,3-didehydroGA_9, which eventually brings about GA_3 (Hedden and Phillips, 2000).

Seed dormancy is also influenced by the presence of light (as such, the grains will not germinate unless submitted to darkness). Modern barley cultivars, however, usually reach only low dormancy levels, mainly due to selective pressure during breeding, which in turn causes early sprouting (Gubler *et al.*, 2008).

3.4.2 Environmental effects

While genetical predisposition and regulatory pathways can influence much, the environmental effect cannot be ignored. As such, droughts are one of the main factors presenting a risk to the growing crops, which of course leads to further breeding for drought resistance (González, Bermejo, and Gimeno, 2010). In opposition, flooding can cause accumulation of ROS (reactive oxygen species) and hypoxia, which then leads to cell death (Shalygo *et al.*, 2012). As with any plant, sufficient soil nutrients must be present – this is oftentimes achieved by adding specialized fertilizers to the soil, which both raise the plant's viability, as well as its yields (Ladha *et al.*, 2005). Finally, factor that correlates with sufficient temperatures – sunlight. It is crucial during plant, and later, seed development, because photosynthesis cannot sufficiently take place during darkness. As opposed to its lack, too much sunlight can in turn cause damage to the photosystems, which could have catastrophic effects on the whole crop (Živčák *et al.*, 2014). Barley, as the majority of grasses, is a C4 plant, meaning it is more efficient when it comes to carbon fixation. Not all cells are capable of photosynthesis, which raises the amount of availably stored CO_2, which allows for maximal efficiency (Furbank and Kelly, 2021).

4. Malting

Beer brewing has a rich history tightly interconnected with growing barley as an agricultural crop. The cultivation probably began around 10000 BC, but its direct origin is debatable. The speculated areas are in Egypt or China – both these nations cultivated barley and later, brewed beer in large quantities. The main purpose of the grain was, however, production of bread, which sustained large populations of Greeks, Romans and Hebrews, as well as the majority of Europe during the 16th century (Zhou, 2009). Barley production today is much higher, due to greater population and its requirements, as well as the use of higher-yield cultivars, the use of modernized equipment and fertilizers. Worldwide barley production estimate for the year 2022 is 145526000 tons (ipad.fas.usda.gov).

4.1 Malting process

In order to fully process the genetics and add context to them within the malting quality range, it is also important to list the process itself. What exactly is malting, what happens during this time and how the quality traits reflect in the end product (or in this case, malt and later, beer)? While beer brewing itself is a long and complex process, each step holds varying importance and takes a different amount of time. These, of course, also vary depending on the type of beer, or even the brewers themselves – our focus in this segment will be on the malting process.

Malting consists of three steps – steeping, germination and kilning (Figure 4). Steeping is a term used for grain hydration, in which the barley kernels rapidly take up water (to rise from cca 10 % to below 50 % of water content, with temperatures of 14 – 16 °C) and leave their dormant state. This can be further aided by addition of GA (Briggs, 1998). After sufficient soaking of the kernels, the endosperm cells soften, which allows mobilization of the varying compounds aiding the embryo in its growth. During germination (with the necessary temperature between 16 – 20 °C), both shoot and root apical meristems start cell division while receiving ‘nutrition’ from the endosperm (Gorzolka *et al.*, 2016). The creation of so-called ‘green malt’ is then furthered by enzyme production within the aleurone layer and scutellum, and is then left alone for 3 – 5 days, until endosperm cell walls degrade completely and at least a half of present proteins is solubilized (MacLeod and Evans, 2016). Kilning the germinated seeds not only serves as the means to remove excess water, drying them in the process, but also halts starch degrading enzymes, thus stopping the germination process altogether. The drying time tends to vary, but takes at least 21 h to complete and the temperatures oscillate in range of 50 – 85 °C. Depending on the temperature used, the malt gains color and flavor, which in turn impacts the final product (Poutanen, 2020).

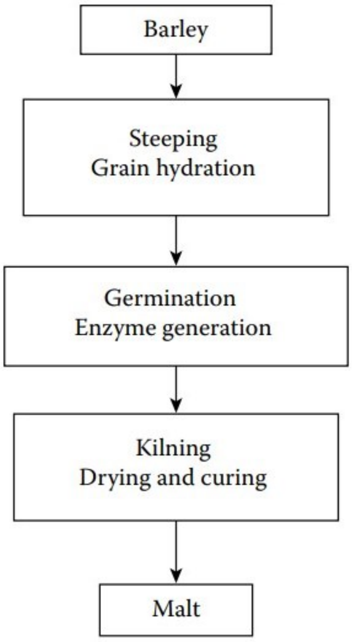


Figure 4: Steps of the malting process – from Eaton (2017)

4.2 Germination and enzymatic activity during malting

While the process of malting has been known for millennia, the exact mechanism was unknown until a few decades back. Steeping ensures the kernels have enough moisture to start germinating, which is a result of several processes happening within. During the rapid water uptake, the previously dried out cells start leaking low molecular solutes into the apoplast, but only until the cell membrane structure stabilizes. The germination process starts with imbibition of water, which in turn starts the enzymatic activity and swelling of cells. Germination concludes with root radicle emergence, and then continues with seedling growth (Figure 5). Like many other cell processes, it is irreversible (Fenner and Thompson, 2005).

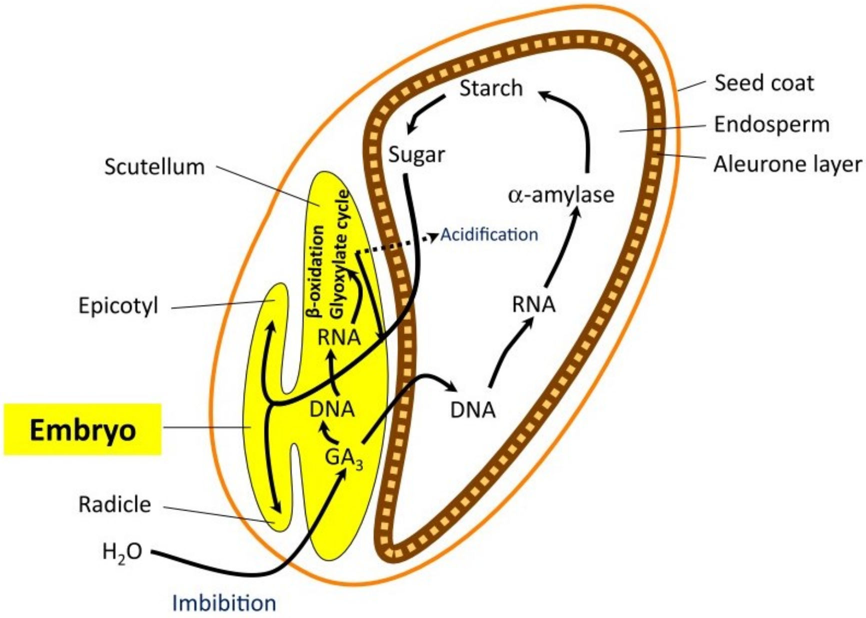


Figure 5: Metabolic processes within the embryo and endosperm during seed germination – from Ma, Bykova and Igamberdiev (2017)

The imbibition itself is the starting signal necessary for resumption of metabolic processes (Bewley, 1997). During this time, respiration activity resumes and both oxygen and CO_2 uptake increases significantly. The oxygen is processed during oxidative phosphorylation, while carbon from CO_2 finds its use in the tricarboxylic acid cycle, which is a part of the Krebs cycle (Botha, Potgieter and Botha, 1992). Sufficient water uptake ensures DNA reparations by DNA ligase (some sequences get damaged during seed dehydration), as well as reparations of damaged mitochondria, which restarts the production of ATP (Tuan *et al.*, 2019). In the so-called phase II, further reparations proceed, as well as synthesis of new mitochondria and proteins from freshy transcribed mRNA (Weitbrecht, Müller and Leubner-Metzger, 2011). Over 12 000 mRNA species remain within the desiccated grain, and fill in before fresh mRNA can be fully transcribed (Sreenivasulu *et al.*, 2008). As mRNAs have a short lifespan, they must be properly stored in order to prevent degradation. While it is not yet exactly known where, there is some speculation they could be part of the mRNPs (messenger ribonucleoprotein complexes) in the cytoplasm (Pramanik and Bewley, 1996). Eventually, the embryo starts expanding and the radicle eventually protrudes through the seed coats, which concludes germination itself (Bewley, 1997).

During post-germination stage (or alternatively, seedling development), storage reserves are mobilized. This is where the degradation enzymes come in – the activity of β-glucanase hydrolyses β-glucans, and subsequently causes overall relaxation of endosperm cell wall (Habte-Tsion and Kumar, 2018). The degradation enzymes, namely α-amylase, β-amylase and limit dextrinase, start degrading the present starch reserves into fermentable sugars (for details, see chapter 3.2.1). The presence of too many β-glucans within the malt is undesirable, because it creates gels, which later prevent separation of malt extract and impurities. At the same time, optimal amount of amino acids is required in order to properly aid the process of fermentation (Hughes, 2003).

4.3 Brewing process

This part of beer production process is much more complicated than malting, with plenty of steps and their variations. There is a distinct difference between Czech & German and American beer brewing, for simplification, the American system will be described on these pages. These steps include milling, mashing, wort separation (lautering) and boiling, trub removal, yeast handling, fermentation and removal, aging and finally, clarification. During milling, the malt is crushed into smaller particles, thus enlarging the reactive area, which aids with mashing. Said step requires mixing hot water with the malt, along with some adjuncts, should they be required (in this case, other cereals, which aid processing or flavor). This eventually yields wort, also known as malt extract – gained by enzymatic activity and degradation of both proteins and starch. As part of the process, it is important to separate the mash and the wort, which is then boiled to sterilize the liquid, while adding hops to the mix. After sufficient boil down, any solids (or trub) are removed to ensure beer stability. Next, the mixture is cooled to fermentation temperature (which ranges from 8 – 22° C, depending on beverage type) and both yeast and oxygen are added to aid the fermentation process. During fermentation, sugars (or in this case, glucose, fructose maltose and saccharose; Huang, Carragher and Cozzolino, 2015) are metabolically transformed into ethanol, and the beer flavor is established. After fermentation is completed, the so-called ‘green beer’ must be aged at low temperatures in order to stabilize it. After the final filtration, the close to sterile beer is created (Pires and Brányik, 2015; Eaton, 2017).

5. Genetic basis of malting quality

Cultivated barley, in its haploid state, has 7 chromosomes labeled 1H – 7H, with an overall genome size of 5,1 Gb (gigabases). Bearing between 38 000 and 48 000 of genes depending on the variety (Mayer *et al.*, 2009), over 80 % of said genome consists of repeat content (Mascher *et al.*, 2017). LTR (long terminal repeat) retrotransposons constitute about 99,6 % or all mobile structures present (Mayer *et al.*, 2012).

Malting quality traits are controlled by a number of genes with strong ties to the growth environment. Several genes have been identified and mapped as QTL (quantitative trait loci), which are specific DNA regions on the chromosomes, found to be associated with phenotypic traits (Miles and Wayne, 2008).

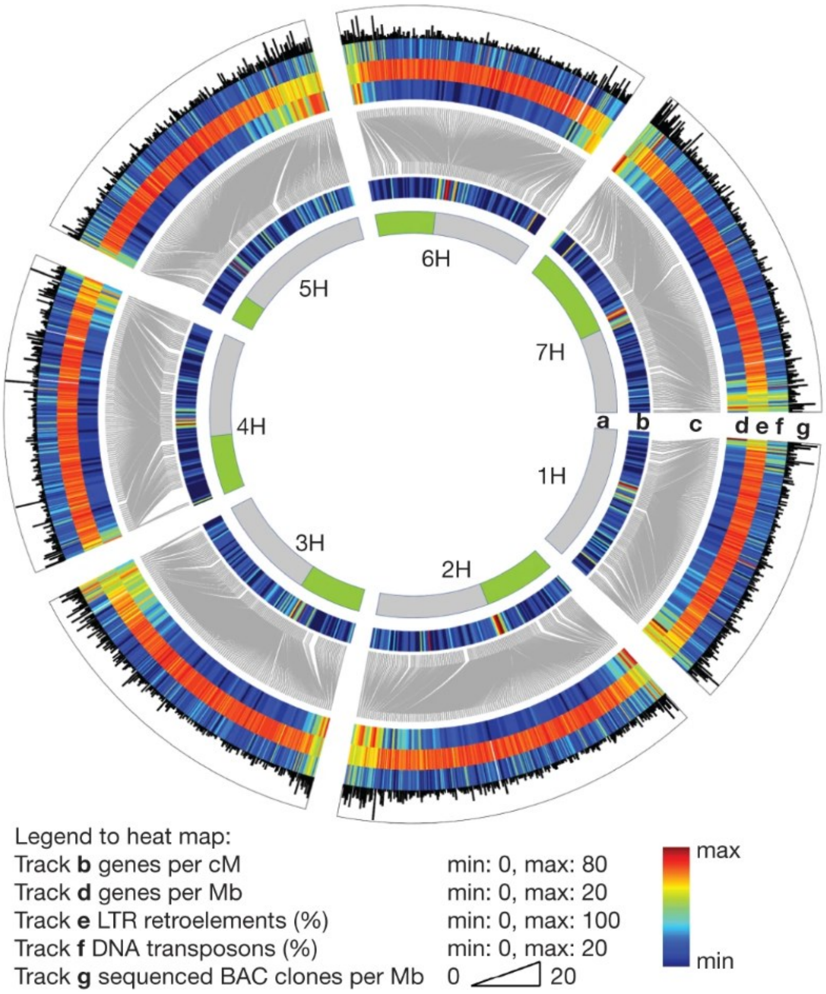
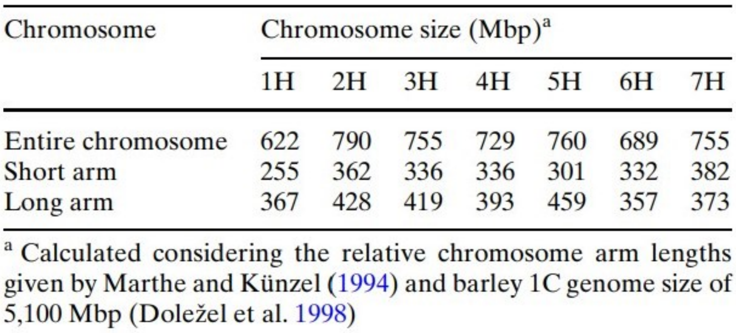


Figure 6: Barley gene landscape – from Mayer *et al.* (2012)

As displayed in Figure 6, barley genome has been meticulously studied, which largely helps and enables further research into breeding and selection of superior breeding lines. Nowadays, the method largely used is so-called ‘shotgun sequencing’, which consists of breaking up the DNA into small fragments, sequencing it and lastly, reconstructing it back, depending on overlaps within the sequences. In order to properly assemble the genetic map of barley, homologous sequences from other cereals are used, which significantly helps fill in the gaps (Mayer *et al.*, 2009). Recently, the already third version of Morex sequence reference has been released (Yao *et al.*, 2022).

5.1 Chromosomes

Chromosome 1H is considerably smaller than the others, with its 622 Mb of genetic information and an estimated number of genes in range between 4600 - 5800 (Mayer *et al.*, 2009). This chromosome takes up approximately 12 % of barley genome (Suchánková *et al.*, 2006). Several QTLs connected to malting quality traits are localized here – namely endosperm hardness, malt extract, grain volume and length (Walker *et al.*, 2013). Malting quality can be influenced even negatively – the presence of *Hsp* alleles near the centromere significantly lowers it, primarily by raising the protein content and wort viscosity, while decreasing friability and Kolbach index (soluble/ insoluble nitrogen ratio; March *et al.* 2012).



Chromosome	Chromosome size (Mbp)[a]						
	1H	2H	3H	4H	5H	6H	7H
Entire chromosome	622	790	755	729	760	689	755
Short arm	255	362	336	336	301	332	382
Long arm	367	428	419	393	459	357	373

[a] Calculated considering the relative chromosome arm lengths given by Marthe and Künzel (1994) and barley 1C genome size of 5,100 Mbp (Doležel et al. 1998)

Table 1: Size of barley chromosomes – from Suchánková *et al.* (2006)

Chromosome 2H has the size of 790 Mb, making it the largest (as seen in Table 1). While the number of genes present remains unknown, there are QTLs for grain density, volume, length, weight and flowering time – their presence, however, depends on the barley variety used for their detection (Walker *et al.*, 2013). This chromosome also holds the genes responsible for the number of kernel rows present (Karakousis *et al.*, 2003). Unlike with chromosome 1H, the others are not so closely described – we know of some genes present, but the majority still remains uncovered.

Chromosome 3H, with its 755 Mb, contains QTLs for grain length and volume (much like 1H). It is also the location of many genes for β-glucanase, as well as xylanase and GA_3 induced gene (Li *et al.*, 1996; Li, 1997; Banik *et al.*, 1997).

Chromosome 4H has the size of 729 Mb, and three genomic regions with QTLs for malt extract. These regions contain QTLs for endosperm hardness, flowering date, vernalization, grain yield, as well as grain weight and size. It houses the gene for manganese response. Mangan is an important micronutrient necessary for correct function of the plant, as the lack of it causes issues with photosynthesis (Pallotta *et al.*, 2000).

Chromosome 5H (760 Mb) houses QTLs with similar traits as the previous – endosperm hardness, grain weight, length and volume, as well as flowering date. Some of the genes present code cellulose synthase 3, which is a component of a protein complex responsible for the construction of the cell wall (Karakousis *et al.*, 2003).

Chromosome 6H (689 Mb) has a QTL responsible for variation in grain density and QTLs connected to grain weight, length and volume (much like 1H, 2H and 5H). The genes responsible for enzymatic activity are those for ADP phosporylase and β-D-xylosidase (Li, 1997; Karakousis *et al.*, 2003).

Chromosome 7H, with the size of 755 Mb contains QTLs associated with endosperm hardness, grain length, yield, flowering date and protein content present (Walker *et al.*, 2013). As well as chromosome 4, it includes the manganese response gene (Pallotta *et al.*, 2000).

5.2 Genes of interest

Barley chromosomes contain many genes and QTLs with great importance to malting quality (Muñoz-Amatriaín *et al.*, 2009). While significant differences can be found within the varieties, attempts were previously made to create a universal genetic map (Qi *et al.*, 1996). Said consensus revealed the general locations of QTL types, including those for α-amylase, starch granules, β-glucan and β-glucanase, fermentability, grain protein, malt extract, seed dormancy and many others (Fox *et al.*, 2003). Localization is ensured by molecular markers, which are sequences of DNA with a given location within the genome (Pierce, 2010). While the position of markers is strictly given within the genome, their position is verified by the use of NGS (next generation sequencing) and the newly established Morex3 reference sequence. NGS is much faster compared to previous techniques used. Millions of DNA fragments are sequenced at the same time, which greatly cuts the amount of time necessary to acquire the entire genomic sequence (Behjati and Tarpey, 2013). In the next paragraph, some of mention-worthy gene families will be described.

Important to mention would be the α-amylase gene families, namely the subfamilies of gene loci *Amy1* and *Amy2*, coding α-amylase I and II respectively, and *Amy3* and *Amy4*. These subfamilies include at least 12 genes. *Amy1* subfamily has 6 of these genes, four of which can be found on chromosome 6H, while the other two belong to the unsorted chromosome. Subfamily *amy2* consists of three genes, all on chromosome 7H. *Amy3* has one gene on chromosome 3H, while *amy4* has two, on 2H and 3H respectively (Zhang and Li, 2017). All these isozymes can be separated into two groups depending on their isoelectric point – namely high pI and low pI (Callis and Ho, 1983). The high pI gene (Amy pHV19) promoter region is responsive to both GA and ABA, which regulates its expression during dormancy and later, germination, and can be found downstream of -174 (Jacobsen and Close, 1991). Attempts to identify GARE (gibberellic acid response elements) within the sequence of -174 and +53 have been made (Gubler, 1992). The *α-Amy1* gene, which is high pI, contains two introns, while *α-Amy2* (low pI) has three introns (Knox *et al.*, 1987).

Compared to α-amylase gene family, that of β-amylase is much smaller, with at least two copies within a haploid genome. This gene family is highly regulated, mainly through the presence and amount of its mRNA (Kreis *et al.*, 1987). β-amylase is coded by three varying loci, two of which can be found on chromosome 4H (*Bmy1* and *Bamy3*) and the last on chromosome 2H (*Bmy2*). *Bmy1* contains six introns (Yoshigi, *et al.*, 1995), and while there is a promoter sequence present somewhere in the 5' upstream region, its exact position is still unknown (Okada *et al.*, 2000).

Ldx, found on chromosome 7H, is a single gene responsible for coding limit dextrinase (Li *et al.*, 1999a). Unlike the others mentioned, it is much larger and contains 26 introns, with a promotor in the 5' -142 upstream region (Burton *et al.*, 1999).

The gene family responsible for coding β-glucanase contains seven genes, all of which are found on chromosome 3H. In case of the GVII, there is only one intron present (Li *et al.*, 1996). The location of the promoter for this gene is not yet known – however, the promoter of GIII is fused with the *gus* (β -glucurodinase) reporter gene. While its whole sequence is known, its exact position is not (Li, Zhu and Xu, 2005).

There are many loci present for hordeins in general (A hordeins forming a separate group removed to chromosome 4H, while the rest remains on 1H) – *Hor1* is responsible for C hordeins, *Hor2* for B hordeins and *Ho*r3 codes D hordeins (Fox *et al.*, 2003).

5.3 Pseudogenes

Aside from regular genetic sequences, the barley genome includes pseudogenes – remnants of DNA that lost their coding abilities by evolution (Mighell *et al.*, 2000). A pseudogene can come to be in more than one way – unitary pseudogenes arise by a mutation, causing inability to transcribe and translate the sequence (Zhang, *et al.*, 2010). Duplicated pseudogenes arise by uneven crossing over or tandem duplication (Mighell *et al.*, 2000). The third category is called processed pseudogenes, they are caused by retrotransposition and lack promotors and introns (Maestre, J. *et al.*, 1995). While it was formerly thought that pseudogenes remain completely inactive, recent studies show that it may not be so, and they may retain gene expression or regulation (Pink *et al.*, 2012; Tutar, 2012). As reported by Prade *et al.* (2018), 89 440 potential pseudogenes were discovered, of which slightly over 11 000 equal full-length gene copies (hence why they're called high coverage pseudogenes). This is almost twice as much as the number of high-confidence gene loci within the genome. They also reported that these pseudogenes had a higher probability of being found close to their 'parent' gene. Both duplicated and processed pseudogenes were preferentially located on the same chromosome as their parent gene, which is most likely caused by the Rabl configuration chromosomes adapt during anaphase (Dong and Jiang, 1998). As such, pseudogenes present a way to better understand plant genome dynamics, as well as the mechanisms with which evolution takes place (Prade *et al.*, 2018).

6. Conclusion

Barley grain has found many uses over the centuries, the most important of which to today's society would be the production of beer and animal feed, or alternatively, the baking industry. In order to produce beer, however, optimal grain attributes are necessary – said attributes are gained and improved upon by breeding. Historically, crop breeding was done by simple selection according to physical attributes, such as grain size or number, or crop viability. Nowadays, breeding is done mainly on the molecular level, and while crossbreeding of varieties is the most common way to gain new varieties, genetic editing is also starting to spread. The main goal of barley breeding for the standards of malting quality is to ensure the prevailing quality, as well as higher resistance to environmental factors, as well as pathogens. Full genome sequence is not yet known, which complicates any gene related work – we may know the desired traits, but we don't know for sure which genes cause them. As such, extensive research is required in order to reach further and speed up the progress, which will certainly aid greater production and quality.

The goal for further research is combining both spring and winter barley types, which would allow for greater environmental resistance, while retaining desired malting quality. The goal of this work was to introduce grain parameters necessary for what we consider malting quality, as well what causes it and the malting process itself.

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