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Genetic variation in field emergence and related traits in naked and covered spring barley

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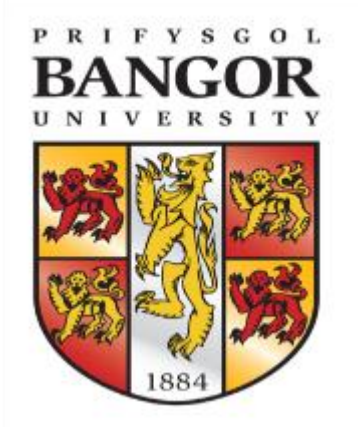
GENETIC VARIATION IN FIELD EMERGENCE AND RELATED TRAITS IN NAKED AND COVERED SPRING BARLEY



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ABSTRACT

Naked, or hull-less, barley is gaining increased interest for human consumption because of its free-threshing habit (lowering processing costs and avoiding loss of bran during pearling) and higher β-glucan (soluble fibre) content, implicated in lowering glycaemic index. The naked seed trait is coded by a single recessive gene, *nud*, where the palea and lemma do not adhere to the caryopsis. However, a persistent problem with naked barley is poor establishment in typical British cool, damp late winter sowing conditions. It was hypothesized that this was due to short coleoptile length in the few European varieties that possess the naked seed trait (most European barleys being hulled). However, the naked trait is widespread in those nations where barley forms a significant component of human diet such as Ethiopia and Central Asia, but where growing conditions are often harsh.

Germination and establishment characteristics of several lines from these areas were tested in the field, cold room and glasshouse. Coleoptile length was found to vary significantly between genotypes and was correlated with successful establishment. However, many exotic genotypes have agronomic traits which are poorly adapted to British growing conditions, such as low disease resistance, unduly short or long duration, lax leaf habit and weak straw. The genotype with the longest coleoptile length, Tibet-37, was crossed with Taiga, a cultivar from Germany which has a short coleoptile and poor field establishment. From this cross a mapping population was produced using the single seed descent method by uni-culm micro plant. 145 recombinant inbred lines (RILs) were produced from which 68 were black, 47 grey and 30 were white seeded. Among these black colour and grey colour lines were true hybrids. However, white testa lines were doubtful; their performance was similar to the Taiga parent. Coleoptile length was found to be heritable in the progeny. Using the rapid micro-plant technique, it was possible to advance to the F_6 generation within two years. F_7 RILs were

phenotyped in the glasshouse as a first stage in genetic mapping, and the F_8 RILs were field tested for coleoptile length and seedling emergence characteristics. Results show that coleoptile length is a continuously variable trait with Tibet-37 and Taiga at the upper and lower ends of the range, respectively. CL showed significant correlation between field and cold room experiment. Seed colour was significantly associated with CL, EP and SE.

The black testa colour RILs showed highest CL length hence, it is concluded that black seed colour might be linked with long coleoptile traits and a good morphological marker for coleoptile length and establishment in this population.

DEDICATION

This thesis is dedicated to my Father, who always inspired me to get higher qualification and position and my family. It is gratification for me that I have attained the dreams of my father and my family.

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Abbreviations

B	Black
C	Centigrade
CL	Coleoptile Length
cm	Centimeter
d	Days
D1	Depth 1
D2	Depth 2
DF	Degree of freedom
DH	Double haploid
DMRT	Duncans` multiple range tests
DNA	Deoxyribonucleic Acid
DRW	Dry root weight
DSW	Dry shoot weight
EP	Emergence percentage
F_2	Filial generation two
F_2B	Filial generation two black
F_2W	Filial generation two white
F_3	Filial generation three
F_3B	Filial generation three black
F_3W	Filial generation three white
F_4	Filial generation four
F_4B	Filial generation four black
F_4W	Filial generation four white
F_5	Filial generation five
F_6	Filial generation six
F_7	Filial generation seven
F_8	Filial generation eight
Fig	Figure
g	gram
G	Grey
GA	Genetic advance
GLM	General linear model
H^2 (BS.)	Heritability broad sense
h^2	Heritability in narrow sense
ICARDA	International Center for Agricultural Research in the Dry Areas
JIC	John Innes Center
K	Selection intensity
L*W*D	Length width and depth
M	Metre
MAS	Marker assisted selection
M_e	Mean square of error
M_g	Mean square of genotype
mm	Millimeters
n	Number

OECD	Organization for Economic Co-operation and Development
OPA	Oligo pool assay
PAK	Pakistan
QTL	Quantitative trait loci
r	Replication/ correlation
RCBD	Randomized Complete Block Design
RILs	Recombinant inbred lines
RL	Root length
SD	Sowing date
SD	Standard deviation
SD1, SD2 and SD3	Sowing date 1, 2 and 3
SE	Speed of emergence
SEM	Standard error of mean
SL	Shoot length
SNP	Single nucleotide polymorphism
SPSS	Statical package for social science
SSD	Single seed descent
TATB	Taiga X Tibet-37
UK	United Kingdom
V_E	Environmental variation
V_G	Genetic variation
V_P	Phenotypic variation
W	White

CHAPTER 1

INTRODUCTION, LITERATURE REVIEW AND OBJECTIVES

1.1 Introduction

Barley (*Hordeum vulgare* L.) is one of the most ancient cereal grain crops and is the world's fourth most important cereal crop after wheat, maize, and rice (Harlan 1995, Salamini *et al*. 2002, Bothmer *et al*. 2003a, Akar *et al.,* 2004, FAO 2009, Mahdi *et al*., 2008 and Durka *et al*., 2010). It can be grown over a wide variety of environments. It is popular because of its broad environmental adjustment, utility as an animal feed and human food and supremacy of barley malt for use in brewing (Poehlman, 1985, Hayes *et al*., 2002, Eticha *et al*., 2008 and Takashi *et al*., 2011). Barley is cultivated in countries with adverse climates for other cereal production, including Tibet, Nepal, Ethiopia, North Africa, Middle East, Afghanistan, Pakistan, Eritrea, and Yemen (Zohary and Hopf 2000 and Bothmer *et al*. 2003b). The Fertile Crescent of the Middle East consisting of Turkey, Iran, Iraq and Lebanon has been proposed as the original area of cultivation and the most likely centre of origin of barley (Harlan, 1979, Lev- Yadun *et al*., 2000 and Ivandic *et al*., 2002). Barley grains have been found in archeological sites in the Fertile Crescent showing that the crop was cultivated there since 10,000 years ago (Badr *et al*., 2000). It has also been proposed that barley has a multicentric domestication (Molina-Cano *et al*., 2002, 2005). Most recently Morrell and Clegg

(2007) proposed that cultivated barley belongs to Indus valley, at the site of Mehargarh of Pakistan about 7,000 B.C.

1.1.1 Economic Importance of Barley

About 40% of the world production occurs in European countries (FAO 2009). Barley is first and foremost a crop utilized as feed, malt and food (70, 20 and 5%), respectively and 5% for other purposes (Wang, 2005). Barley can be used for the manufacture of starch, both for food or chemical industry (OECD, 2004). Moreover, barley has some useful by-products, the most valuable being the straw which is used mainly for livestock bedding in developed countries, but also for animal feed in developing and under-developed countries (Akar *et al.,* 2004).

In Pakistan barley is the second most important winter cereal (Zeeshan *et al.,* 2012). It occupies an area of 107,700 hectares with production of 99,600 t. The average yield is 925 kg per hectare (Govt. of Pakistan, 2004). About one thirds of the area devoted to barley in Pakistan is rain fed and two thirds areis irrigated. Barley offers the best opportunity to bring marginal areas under cultivation where other crops like wheat cannot be grown.

1.1.1.1 Barley used as animal feed

Globally, up to 85% of barley produced is used for feeding animals, including cattle, swine and poultry (Akar *et al*., 2004; OECD, 2004 and Takashi *et al*., 2011). To improve the digestibility barley seed needs to be rolled, ground, and flaked before feeding. Barley is considered to have a poorer nutritive value than wheat for feed because its high fiber content means the energy is not as easily utilized by animals. Although it has higher protein content than maize, the diet of high-performing monogestric animals usually needs to be supplemented with other protein sources because of the low content and quality of protein in the barley grain (OECD, 2004).

1.1.1.2 Barley used as malt

Malting is the second most important use of barley, not only for in beer, but also in hard liquors, malted milk and flavourings in a variety of foods. Brewer's and distiller's grains and sprouts from malting barley have desirable protein contents for animal diets (Akar *et al*., 2004 and Takahashi *et al*., 2011).

Before barley seeds are used for malting they are steeped in water to germinate under controlled conditions. Afterwards they are dried out or heated in an oven, cleaned, and can be stored for long periods. Malt is primarily an intermediary product and needs supplementary processing, such as fermentation in beer and whisky production (OECD, 2004).

1.1.1.3 Barley and human food

Barley used to be one of the leading food grains, but has been replaced by rice and wheat in many countries (Grando and Macpherson 2005, Newman and Newman 2006, 2008, Baik and Ullrich 2008). Nevertheless barley is still a key food grain in several regions of the world, including Morocco, India, China and Ethiopia (OECD, 2004).

In western countries, barley is gradually increasing in popularity as a food grain and can be used in flour for bread making or other specialties such as baby food and health food. The utilization of barley for food in Western countries has increased, because barley contains high dietary soluble fibre, glucan (which helps to reduce the glycogenic index and serum cholesterol) (Grando and Macpherson 2005, McIntosh., *et al* 1991, Newman and Newman 2008 and Wood, 2007). Before it can be eaten the fibrous outer hull must be removed from the barley grain. Pearled barley is dehulled barley that has been pearled or polished further, removing some of the fiber. Pearl barley can be processed into a range of barley products like flour and flakes. Alternatively, hull-less barley varieties, which require minimal processing, have been developed for food applications (US Grains Council, 2007).

1.1.2 Plant morphology of barley.

Barley is characterized by a spike with three single-flowered spikelets at each rachis node (Terzi *et al*., 2001). Barley is an annual plant that stands 60-120 cm tall. It has a seminal and adventitious root system. The depth the roots reach depends on the conditions, texture and structure of the soil, as well as on the temperature and soil moisture content. The roots that are usually deeper are known as seminal and the upper, later developing roots are adventitious. When seeds are planted deeply a 'rhizomatous stem' is formed, which grows leaves when it reaches the surface of soil. One or more internodes that contain adventitious roots are known as the 'rhizome' (Briggs, 1978).

Barley consists of erect stems that are made-up of hollow, cylindrical internodes, alternating with nodes, which bear the leaves (Gomez- Macpherson, 2001). A fully grown barley plant comprises a central stem and typically two to five side stems, called tillers. The spikes appear from the apex of the main stem and each fertile tiller. The lower leaf bases swell to form the crown at the surface of the soil. Barley leaves are 5-15 mm wide and lanceolate in shape and are attached on alternate sides of the main stem. The leaf structure consists of sheath, blade, auricles and ligules. The sheath wraps the stalk entirely. The ligules and auricles differentiate barley from other cereals as they are smooth, envelope the stem and can be pigmented with anthocynanins (Gomez- Macpherson, 2001).

1.1.3 Reproductive morphology of barley

The inflorescence of barley is also known as the ear, head or spike. The flowering elements, the spikelets, are attached to the middle axis/rachis of the section of the stem that supports the spike. There are three spikelets at each node, also known as triplets, arranged alternatively on opposite sides of the spike. The spikelets are made up of two glumes, which are empty bracts, and one floret that includes the lemma and palea. In covered barley, the palea and lemma adhere to the grain. On the other hand, naked barley has a palea and lemma that are not attached and can be separated from the grain during threshing (Kakeda, *et al*., 2011).

In six row barley, spikelets are in a triplet form and all are fertile and able to develop grains (Komatsuda *et al*., 2007). However, the central seeds are round and fat, but the lateral seeds tend to be slightly asymmetrical and in some varieties (intermediate forms), they are also smaller than the central grain. On the other hand, two row barley comprises only the central fertile spikelets. The two outer-most spikelets are minor with condensed stamens and an undeveloped ovary and stigma. A single seed is produced at each node of the spike from two-row barley because the two small outer spikelets are not fertile (Komatsuda *et al.*, 2007). Typically 25-60 grains are produced from a single spike of six-rowed varieties and 15-30 seeds in two-rowed varieties (Briggs, 1978).

1.2 Importance of Naked Barley

Most barley cultivars have caryopses which are attached as hulls at maturity, known as covered (hulled) barley. Harlan (1968) reported that in covered barley, a sticky adhesive material is exuded 10 days after flowering on the caryopsis surface, produced by the caryopsis itself. However, there are various barley cultivars where hulls thresh free from the grains and are known as naked barley (Kakedaet *al*., 2011). The covered or naked characteristic of barley is apparently controlled by a single recessive gene (*nud*), where the covered type is dominant over the naked one (Franckowiack and Konishi 1997, Taketa *et al*., 2008, Kakeda, et *al*., 2011). Naked barley is best for use as human food. The Nud locus was finely mapped on chromosome arm 7HL (Kikuchi *et al.,* 2003, Taketa *et al*., 2004, 2006) and has recently been isolated by positional cloning (Taketa *et al.*, 2008).

Naked barleys are commonly found in East Asia, as human staples, but only rarely found in other parts of the world (Kakeda, *et al*., 2011). However, in recent times, naked barley has attracted significant consideration as a feed and human food, because of its higher feed-value and high level of dietary fibers (Dickin *et al.,* 2010, 2011, 2012). A recent study (Taketa *et al*., 2004) indicates that the naked barley trait has a monophyletic origin, probably in southwestern Iran and all naked barleys are likely to share a common ancestor.

The crude protein content of hull-less barley usually surpasses that of equivalent

hulled types being 1-3% greater (Griffey, 1999). Naked barley also has a benefit above conventional barley in transportation, processing and storage. Eliminating the hull portion boosts the bulk density compared to conventional barley by about 25% (Bhatty, 1999).

1.2.1 Naked barley and seedling establishment

Naked barley has smaller seeds compared to hulled barley (Box, *et a.,l* 1999, Dickin, *et al*.,2010). Therefore, the seed rate should be adjusted according to soil moisture conditions and better germination but as well as for seed size to attain optimal plant population.

Poor establishment leading to lower yields can be a problem in covered (hulled) but it is a frequent and more serious problem in naked barley. There are many factors which are considered to be linked with establishment such as seed embryos damaged during harvesting and seed cleaning, resulting in poor viability and germination (Box, *et al.,* 1999). Naked barley has an uncovered embryo, and consequently some of the embryos are damaged by mechanical threshing before the seed is planted with the result that germination percentage is affected (Thomson *et al.,* 2009). Besides damage to the grain, the length of the coleoptile, the sheath which protects the emerging shoot, also has a major bearing on barley establishment.

1.2.2 Factors affecting germination and emergence of naked barley.

When a barley seed germinates, the seedling root, the radicle, emerges first; shortly afterwards, the coleoptile grows underneath the lemma. The coleoptile stops growth soon after it reaches the soil surface and the first true leaf of the seedling then emerges from the tip of the coleoptile. When the seed is sown at greater depth, it is not the coleoptile that emerges from the soil (Simmons, 1987), but rather the first true leaf after it pushes through the tip of the coleoptile.

Temperature also plays a significant role in the elongation of the radicle and shoot and thereafter seedling emergence (Roman *et al*., 1999, 2000). In many species, temperature determines germination percentage and germination rate (Ellis *et al*., 1986, Kebreab and Murdoch, 2000 and Forcella *et al*., 2000).

As a common rule, small seeded species emerge better from shallow soil depths than large seeded species. Actually, small seeded species frequently have a maximum emergence on the soil surface (Grundy *et al*., 1996, 2003). Environmental conditions that directly enclose seeds establish germination success and successive seedling emergence and establishment (Harper, 1977).

Cold or freezing tolerance in temperate plants is a precondition for surviving winter and for continuing growth and development in the spring. Low temperature tolerance in seeds signifies the capability to germinate early in the spring, which

is important for regions with a short growing season. Early emerging seedlings also have aggressive advantages in plant communities (Hou and Romo, 1998) and a higher opportunity of establishment because they can capture the earliest chance in a growing season. Zheng *et al*., (1994), Massardo *et al*., (2000), Fellner and Sawhney, (2001) concluded that low temperatures during germination may possibly reduce the percentage and timing of embryo extension in a number of species.

Mechanisms of cold and freezing tolerance of seed germination and establishment in early stage of plant are poorly understood. Selection for fast germination under stress is useful and considerably improves progeny seed germination rate under cold stress (Foolad *et al*., 2003). The timing of germination plays a significant role in seedling establishment in natural environments and in cropping systems. The highest mortality rate in the plant life cycle is in the seedling phase (Fenner, 1987). Germination and rate of emergence is also affected by sowing depth of seed (Boyd and Acker, 2003).

Barley also shows a late or reduced germination when the soil moisture is very low (Al-Karaki, 1998, Bouaziz, 1990 and Othman, 2005). Better seed germination under a broad range of environments, such as temperature and moisture is important for early seedling establishment (Brar, *et al*, 1991, Huchal 1993 and EL-Hendawy, *et al*., 2005).

Deep crown position is a genotypic character (Webb and Stephens, 1936). It is related with plant resistance to stress like freezing temperatures, wind, grazing and soil moisture limitations, light, seeding depth and genotype (Taylor and McCall, 1936, Webb and Stephens, 1936, Sallans, 1961, Dobrenz, 1967, Ferguson and Boatwright, 1968, McKenzie, 1971, Kail *et al*., 1972, Martin *et al*., 1988 and Loeppky *et al*., 1989).

Small grain species have three root structures. These are the seminal roots, the crown roots, and the sub-crown inter-node (Briggs, 1978, Taylor and Nguyen, 1987). Major diversity for sub-crown internode length has been observed in spring barley (Sallans, 1961). Mostly winter wheat genotypes that were resistant to cold were characterized by deep crowns and short sub-crown internodes (Taylor and McCall 1936 and Webb and Stephens (1936). In winter barley, shallow crown formation has been associated with resistance to winter cold (Dobrenz, 1967). Kail *et al*., (1972) suggested a cultivar by temperature interaction for crown depth in barley. However, in barley (Irvine and Therrien 1985) and in winter wheat (Taylor and McCall 1936) it was found that high temperatures caused crown node to develop near the soil surface. Hunt *et al*., (1983) found no indication of genotype x temperature interaction for crown depth. Sub-crown internode length, coleoptile length and seedling emergence were found to be positively associated in winter wheat (Chowdhry and Allan, 1966, Allan and Pritchett, 1973).

In barley, it can be concluded that cultivars comprising both short sub-crown internodes and short coleoptiles would be undesirable in regions where deep seeding is regularly practiced, because this might result in poor emergence and establishment.

1.2.3 Sowing depth

Availability of soil moisture is a very important factor for seed germination and seedling emergence. In areas where moisture is a limiting factor to germination and seedling development, depth of planting in order to place seed in moist soil often has a limiting effect upon emergence resulting in poor stands (Kolp, *et al*., 1967).

Barley and wheat are normally sown in the field at a soil depth of 5 cm or less if there is sufficient soil moisture to ensure successful seed germination. Topsoil moisture is very low in semi-arid regions, which means it is very important to control sowing depth for both barley and wheat (Takeda and Takahashi 1999). Several studies have signified that emergence from deep sowing depends on coleoptile length of the cultivar. If sowing depth exceeds the maximum coleoptile length seedling emergence is considerably reduced (Allan, 1980, Feather *et al*., 1968, Whan, 1976, McKenzie, 1971), because the coleoptile is unsuccessful in reaching the soil surface. Chambers (1963) reported a 40 percent reduction in emergence when wheat was sown at 10 cm depth (compared to normal depth). Similar results were found by Cornish (1981) and Irvine and Therrien (1985).

Better seedling vigour and larger coleoptile length are key characteristics regarding early establishment and later on high grain yield in a range of dry environments. Long coleoptile length is associated with deep sowing depth

(Paynter and Clarke 2010). During their study, Acevedo *et al*., (1991) observed that deep plantings (7 to 10 cm) had a better crop establishment in those cases where long coleoptile barley genotypes were used. Better seedling emergence was observed in barley with sowing depths of 12 cm compared to normal sowing depth (Takeda and Takahashi 1999 and Keshtkar, *et al*., 2009). The coleoptile and first internode length were significantly correlated with two different doubled haploid (DH) populations of barley when seeds were sown at a depth of 12 cm (Takahashi *et al*., 2001). Varieties with short coleoptile length produced poor germination and abnormal seedlings when sown deep (Shah and Hassan 2006 and Yagmur and Kayan, 2009).

In barley the genetic mechanism that controls coleoptile length and related traits and their relationship to establishment is not fully understood. In the case of wheat, short coleoptile length is linked to dwarfing genes (Matsui *et al*., 2002). Matsui *et al*., (2002) indicated that short coleoptile length and poor emergence are influenced by the presence of Rht-B1b and Rht-D1b dwarfing genes.

There is little else written about coleoptiles in barley, and most research has concentrated upon wheat. Yagmur and Kayan (2009) found that wheat varieties differed in coleoptile lengths. Several older wheat varieties had long coleoptiles therefore these varieties could be sown at more variable and greater depths. Grain yield and its components were positively correlated to coleoptile length, with a clear decline in grain yield and yield components among varieties with

shorter coleoptiles at the deepest sowing (Yagmur and Kayan 2009).

Increasing sowing depths may enhance wheat establishment in dry soils because of the higher soil-moisture content in the immediate seed environment, followed by improved germination and emergence of seedlings (Mahdi *et al*., 1998 and Schillinger *et al*., 1998). Deeper planting also has a role in reducing threat of damage to seeds by birds and mice (Brown *et al*., 2003). However, through deeper sowing, the significant role of coleoptile length on plant emergence must be taken into account (Fick and Qualset, 1976, Whan 1976 and Rebetzke *et al*., 2007). Because coleoptile length varies by genotype and increases only slightly with increases in sowing depth, it is a limiting factor on sowing depth in wheat (Kirby, 1993). Matsui *et al*., (2002) and Rebetzke *et al*., (2007) have confirmed a relationship between coleoptile length and enhancing plant population with deep sowing. Long coleoptile, faster seedling growth and establishment of wheat have been suggested as useful traits for improving yield under arid climates (Rosyara *et al.,* 2008).

Those cultivars having shorter coleoptile length may encounter emergence complications (Allen and Pritchett 1980), particularly when cultivated in soil moisture deficit conditions and seeds are grown greater depths to overcome the soil moisture problem (Pereira *et al.,* 2002). Genotypes having long coleoptiles are preferred for deep planting. A very little genetic difference has been noticed for these seedling traits in wheat (Richards and Lukacs 2002 and Matsui *et al*., 2002).

1.3 Breeding and genetics of naked barley

1.3.1 Genetics of barley

Barley holds a key position as a crop plant as well as a model species for genetic studies within the *Triticeae* (Bolouri-Moghadam., *et al*., 2011 and Takashi *et al*., 2011) the full draft genome sequence of barley has now been published (Anonymous, 2012). For the last 100 years, classical barley breeding has greatly improved yield and disease resistance as well as malting quality.

Barley is in the genus *Hordeum*, in the *Triticeae* tribe and Poaceae family. Poaceae is considered to be monophyletic; therefore all grasses belonging to this family may have evolved from a single ancestor (Devos, 2005). The genus *Hordeum* consists of 32 species and 45 taxa including diploid (2n = 2x = 14), tetraploid (2n = 4x = 28) and hexaploid (2n= 6x= 42) cytotypes (Bothmer *et al*., 1995). Most *Hordeum* species are perennials and different species have different reproductive systems (Bothmer *et al*., 2003). Cultivated barley (*Hordeum vulgare* ssp. *vulgare* L.) and its wild progenitor (*Hordeum*. *vulgare* ssp. *spontaneum* C. Koch.) belong to a single biological species, which is an annual and is diploid.

1.3.2 Breeding methods of barley

Plant breeding is the science and art of improving crop plants for yield, nutritional quality, environmental stress tolerance and improved plant structure (Mackill *et al*., 1999, Slafer *et al*., 2005 and Trethowan *et al*. 2005). The basic principles of plant breeding are the selection of specific plants with useful traits; useful genes and crossing them to bring these together in one genotype. To achieve these goals the analysis of genetic composition of a plant population plays an important role in breeding programmes.

Hybridization is one of the most important tools to produce genetic variability. Selection presents an outlook for carrying out any breeding program efficiently. In population breeding schemes large populations of individual plants are grown to maximise recombination (Witcombe & Virk 2001). Classical plant breeding depends on phenotypic selection and has traditionally been an efficient method. Nevertheless very little progress has been made for some traits by phenotypic selection because of problem of identifying individuals with high breeding value. Environmental and genotype x environment interactions also contribute to observed differences. Assessment of genotypes in several environments with replicated designs permits better estimation of breeding values, however, much extra time and cost is required.

Many important agronomic traits are complex (quantitative) traits that are controlled by more than one gene. The recorded levels of quantitative traits are often highly influenced by the environment. To analyze the great amount of genes transmitted in a population, a population of plants is required to accurately classify gene combinations. Molecular markers are relatively new tools in plant breeding; bringing resolution in crop improvement in 21st century. They can be used to identify genomic regions underlying quantitative traits, and then the markers can be used for indirect selection of the trait. The selection of DNA markers in plant breeding is called marker-assisted selection (MAS) and is a component of the new direction of molecular breeding. Utilization of molecular markers has provided the basis of molecular plant breeding (Lörz and Wenzel 2005, Varshney *et al*., 2006, Eathington *et al*., 2007). Recently molecular markers have received increased emphasis in genome sequencing efforts and have considerably increased the ability to differentiate genetic diversity in the germplasm of any crop species. Additionally this knowledge may guide the selection of better parents for improvement of populations (Dudley *et al*., 1992 and Collard and Mackill, 2008).

Recent reviews considered the effectiveness of molecular marker-assisted selection combined with different breeding methods (Holland 2004, Varshney *et al*., 2006 and Collard and Mackill, 2008). Marker-assisted selection has been used in cereals to improve single gene traits more commonly than for complex traits which are influenced by environmental conditions. It is a valuable tool to

select superior genotypes and eliminate inferior genotypes in early stages of plant growth. Marker-assisted selection (MAS) may significantly enhance the proficiency and efficiency of breeding over conventional breeding.

The principal advantages of MAS compared to conventional phenotypic selection are: in conventional breeding, screening for desirable traits requires a large population of plants grown and single plant selection is not reliable because of environmental influences. However, in MAS, single plants can be selected and selection might be carried out at seedling stage. MAS is a reliable method and homozygous and heterozygous plants can be screened which are not possible to identify using conventional breeding methods. Marker assisted selection is very helpful in the assessment of genetic diversity, back-cross breeding, purifying cultivars and gene pyramiding (Hittalmani *et al*., 2000, Castro *et al*., 2003, Holland 2004, Xu *et al*., 2004 and Reif *et al.,* 2005.)

Barley is a self-pollinated crop, so breeding methods are similar to wheat. General breeding methods for barley improvement are: (Acquaah, 2007, 2012).

- Mass selection
- Pure line selection
- Pedigree selection
- Bulk population
- Single seed descent

1.3.3 Heritability

Heritability is an important index for breeders (Acquaah, 2007, 2012). It provides information about inheritance of characters from their parents. The concept of heritability is to assign the relative influence of heredity and environment. Heritability provides appropriate information concerning the characters which are transmitted from parents to offspring as well as subsequent generations. This kind of knowledge helps plant breeders to forecast a successful cross with high heritability transmission to the offspring and therefore is useful in the assimilation of characters into the progeny. The wider the range of inherited variation the more efficient will be the selection. Heritability measures the phenotypic variance, which is attributable to genetic basis. Heritability specifies the constitution of population and the degree to which a particular character would be transmitted to the next generation. The information on heritability helps the plant breeder to forecast the performance of the following generation and making desirable selections. The greater the heritability the simpler will be the selection procedure and larger will be the response to selection.

The genetic variance of the trait for selection and a higher heritability are essential for improvement of that trait (Falconer and Maccay, 1996). Successful breeding schemes depend on the knowledge of key traits, genetic structure controlling their inheritance, genetic and environmental natural issues that influence their expression (Kahrizi and Mohammadi, 2009 and Mohammadi *et al*., 2010).

Analysis of variability among the characters and the correlation of a particular trait in relation to other characters contributing to the yield of a crop would be of great importance in forecasting a successful breeding programme (Mary and Gopalan, 2006).

Heritability is defined as the proportion of phenotypic variance attributable to genetic variance in a population. Heritability is of two types a broad sense (H^2) and narrow sense (h^2). Broad-sense heritability (H^2) expresses the degree to which difference in the phenotypes among individuals in population is determined by differences in their genotypes. Narrow-sense heritability (h^2) provides a measure of the degree to which the genetic constitution of individuals determines the phenotypes of their offspring. Most of the traits in crop plants are quantitatively controlled. Therefore, quantitative genetic methods have proved to be a powerful tool and provide the basis for both population improvement and methods of selecting and stabilizing desirable genotypes (Hallauer, 2007).

Quantitative genetics uses the hypothetical concept of heritability to quantify the proportion of phenotypic variation that is controlled by genotype. Practically, heritability is greatly influenced by the genetic architecture of the trait of interest, which is described by the number of genes, the magnitude of their effects, and the type of gene action associated with phenotypes.

Better understanding of gene action and genetic architecture frequently has significant impact on improving genetic gain. In crop plants, selection of desirable plants starts in early generations for traits of higher heritability using pedigree breeding method. Conversely, for traits with low heritability, selection is often delayed until later generations (F_5 or F_6). The selection of superior plants/lines takes a long time, about 8-10 years, and this process is also expensive.

Heritability estimate itself does not give an idea regarding the expected gain in the subsequent generation (Shukla *et al*., 2006). However it is to be considered in combination with estimates of genetic advance and average value among generations. Numerous researchers have reported that high heritability alone is not sufficient for selection in advanced generations. It must be combined with considerable amount of predicted genetic advance (Memon *et al*., 2007 and Mangi *et al.,* 2008).

High heritability (broad sense) related with high genetic advance makes strong contributions of additive genetic variance for expression of the traits. Selection based on these traits could play a vital role in improving grain yield (Iqbal & Khan, 2003). The characters controlled by non-additive genes suggest high heritability and low genetic advance, whereas the characters controlled by additive genes, provides high heritability and genetic advance (Ahmed *et al*., 2007).

One of the most appropriate methods of genetic analysis is the generation mean analysis. In this method, epistatic effects as well as additive and dominance effects can be estimated. Besides gene effects, breeders would also like to know how much of the variation in a crop is genetic and to what extent this variation is heritable. Because efficiency of selection mainly depends on additive genetic variance, influence of the environment and interaction between genotype and environment.

1.4 Objectives of research

The literature review showed that although there is increasing interest in naked barley for food in Europe and North America, constraints to production exist, particularly early seedling vigour and establishment in a cool, maritime climate. Accordingly, a general objective was to study heritability and some breeding value of traits of interest in naked barley crosses. In particular the project considered factors influencing field emergence in cool, wet late winter conditions in Wales.

1.5 Hypotheses tested:

- Coleoptile length is strongly heritable and influences germination.
- Depth of sowing affects naked more than covered barley genotypes in emergence.
- Poor growing conditions affect naked more than covered barley genotypes in emergence.

1.6 The plan of thesis

The thesis contains two results chapters to reflect two phases of the work. The first of these (Chapter 2) starts with a short introduction and description of the plant material used (Table 2.1) and a plan of the investigation to screen existing genotypes to develop subsequently (phase 2) a mapping population for seedling

emergence and field establishment of spring naked barley under varying growth conditions (Table 2.2).

In the second phase (Chapter 3) the genotype that gave best seedling establishment (in phase one) was crossed with a genotype having poor seedling establishment. A single seed descent procedure (Fig 3.2) was used to develop recombinant inbred lines (RILs) to F_8, These were tested for seedling establishment (Table 3.2).

CHAPTER 2

Performance of spring barley genotypes for early seedling establishment and yield traits under UK field conditions.

2.1 Introduction

Naked barley establishment is often a major problem in arid or cool regions worldwide. There are several environmental factors that are involved in seedling establishment although the availability of soil moisture is the major factor in germination and seedling emergence. At the same time as there may be sufficient sub-soil moisture, the soil surface may be dry at sowing time. When faced with these conditions growers have attempted deeper sowing to drop seed in to moist soil. Consequently, germination may occur but many seedlings do not emerge successfully, leading to poor establishment. The main cause of problems with emergence in Britain is cool, damp climates. The cold and damp seem to affect naked barley more adversely than hulled barley.

In cereals numerous studies have revealed that poor emergence under unfavorable soil moisture and planting depth conditions is caused by failure of coleoptiles to emerge. Reduction of coleoptile emergence has been attributed to several issues including sowing depth, temperature, emergence rate, and length of coleoptile. It is therefore necessary to increase knowledge regarding genotypic and environmental effects involving coleoptile length and related parameters in

naked spring barley. The information that will be acquired will be helpful for barley breeding and agronomic management to improve stand establishment.

Coleoptile length is genetically controlled but is also affected by environments, particularly sowing depth (Paynter and Clarke 2010). Varieties with short coleoptile length exhibit poor germination when sown deep (Shah and Hassan 2006). Better seedling vigour and greater coleoptile length are key characteristics regarding early establishment, leading on to high grain yield in a range of adverse environments.

It is believed that genotypes of cereals having longer coleoptiles, rapid seedling growth and establishment have an advantage in adverse environments. Very little genetic variation has been noticed for these seedling traits in wheat (Richards and Lukacs, 2002, Matsui *et al*., 2002). Matsui *et al*. (2002) indicated that short coleoptile length and poor emergence are influenced by the presence of Rht-B1b and Rht-D1b dwarfing genes.

Successful crop production requires the utilization of excellent quality of seeds to achieve superior seedling establishment and higher crop yield. The use of high quality seed is the final stage use to improve highest seedling emergence and stand establishment in the field (Tekrony, 2006). Numerous scientists such as Larson and Andreason, (2004) Turk and Tawaha, (2002), have reported that seed size and germination percentage were found to be closely associated to

each other. Definitely this response will be varied according to environment condition and genotype (Hakizimamna *et al*., 2000).

Research has also shown that early growth and vigour of many other species are affected by seed size and weight (Hampton, 1981; Khan *et al*., 2002). Better crop establishment and early plant growth are thought to be a significant aspect of crop yield (Acevedo *et al*., 1991). Awan *et al*., (2005) reported that germination percentage, seedling dry weight and seedling length are considered as best indicators of seedling vigour. Khan *et al*., (2002) reported that seedling emergence percentage, emergence rate index, root shoot dry weight and length of root and shoot are under genetic control.

2.2 Objectives

- To screen naked barley genotype for seedling establishment under varying environmental conditions.
- To determine the relationship between coleoptile length and emergence of barley seedlings.
- To select parental material for developing recombinant inbred lines (RILs) for seedling establishment.

2.3 Plant Material

Nine naked and one covered spring barley genotypes were used in this study from stock already held by Bangor University (School of Environment, Natural Resources & Geography). The cultivars were selected because of their diverse origin. They also represent a wide range of cultivation and genetic variability. Details of genotypes are presented in Table 2.1 and detail of experiments and treatments used in this chapter are presented in Table 2.2. Seeds of all the genotypes were not treated with any fungicide or other chemicals. All seeds were hand threshed and thus suffered no embryo damage.

Table 2.1 Genotypes of barley used, their characteristics and origin.

Name	Hull type	Row type	Origin	JIC accession	Comments
Taiga	Naked	2	German		White grain
Hiproly	Naked	2	Ethiopia	7979	White grain
Tibet-37	Naked	2	Tibet	4218	Black grain
Static	Covered	2	British		White grain
IC-93-747	Naked	6	ICARDA		White grain
IC-93-855	Naked	6	ICARDA		White grain
Pak-23	Naked	6	Pakistan	6439	Grey grain
Nepal-92	Naked	6	Nepal	4777	Hooded
Men Jun	Naked	6	China	19406	Grey grain
Huang Yen	Naked	6	China	19395	Purple grain

Table 2.2 Summary of experiments and the number of genotypes tested in each experiment.

Title	No. of genotypes tested	Treatment	Location	Starting date
Screening of spring barley genotypes for coleoptile length, emergence percentage and seedling establishment (Exp 2.1).	4	Genotypes	Glasshouse	12th August 2008
Effect of sowing depth on coleoptile length, emergence percentage and other seedling traits in spring naked barley genotypes (Exp 2.2).	4	Genotypes & Sowing depths	Glasshouse	9th Sep 2008
Effect of sowing depth on coleoptile length, emergence percentage and other seedling traits in spring naked barley genotypes (Exp 2.3).	10	Genotypes & Sowing depths	Glasshouse	5th January 2009
Effect of different temperature (sowing dates) on coleoptile length and seedling establishment in spring naked barley genotypes under field conditions (Exp 2.4).	10	Genotypes & Sowing dates	Field	27th February 2009
Effect of low temperature on coleoptile length and seedling establishment in spring barley genotypes (Exp 2.5).	10	Genotypes	Cold room	15th Sep 2009
Effect of sowing depth and low temperature on coleoptile length and seedling establishment in spring barley genotypes (Exp 2.6).	5	Genotypes & Sowing depths	Cold room	20th May 2010

2.4 Materials and Methods

2.4.1 Growth conditions glasshouse

Three genotypes of spring naked barley varieties (Taiga, Hiproly, Tibet-37) and one UK covered variety (Static) were selected for the first experiment. John Innes No 3 compost was dried in the oven at 30^0C before use. One kg of dry compost was filled in each seedling tray ((22.5 cm (L) x 16.5 cm (W) x 5.5 cm (H)) size then 1.5 L of tap water was added to each tray (excess drained through holes). Eight seeds were placed at 15 mm depth at 1 cm distance between seed and 10 cm between rows.

Subsequently, the effects of sowing depth (20 & 25 mm) on the performance of the same varieties were carried out. Small pots of 0.5 L size were used (8 seeds per pot). Seeds were covered with 20 mm or 25 mm depth with John Innes No 3 compost. Both experiments were laid out in a Randomized Complete Block Design (RCBD) with four replications in the glasshouse at Henfaes Research Centre, Bangor University. The germination started from the 4^{th} day after sowing and completed on the 8^{th} day of sowing.

Seedling emergence was counted daily; up to the stage when all the seeds were emerged, then the experiments were terminated. Plants were harvested on same day, washed with tap water to separate from soil and coleoptile length (CL) (mm) was measured with a ruler from the point of emergence of the coleoptile from the

radicle to its tip (Fig 2.2). Shoot length (SL) and root length (RL) were also measured. To measure dry root weight (DRW) and dry shoot weight (DSW), roots and shoots were separated and each variety was placed in separate paper bags and dried at 70^0C for 48 h.

After the success of the first two experiments, six additional spring naked barley genotypes (Table 2.1) were included in the next experiment. Five seeds of each genotype per replication were placed in 1 L pots containing John Innes No 3 potting soil and field soil at 1:1 ratio[1]. The seeds were sown at two sowing depths (SD1= 25 and SD2=50 mm). The experiment was conducted using RCBD with three replications in glasshouse at Henfaes Research Centre, Bangor University. The first seeds emerged after 4^{th} day of sowing and were complete within five more days. Plants were harvested on the same day, washed with tap water to separate from soil and coleoptile length (CL) (mm) was measured and EP and SE were calculated. Shoot and root length, and shoot and root dry weights were no longer recorded because they were dependent on stage of sampling and these were difficult to interpret when comparing different experiments.

2.4.2 Field growth conditions

The ten spring naked barley genotypes were sown in field at the Henfaes Research Centre Bangor University during 2009. Three sowings were done in the

[1] This reflects a control experiment to investigate the effect of soil type on emergence. No difference between JI No3 compost and the mixture was found. The soils were, therefore, considered equivalent in this work. These data are not reported here.

field on 28th February, 15th March and 28th March 2009. Each genotype was planted in three rows 2 m long, 12 cm distance between rows. 100 seed per row were placed by hand at 25 mm depth. Two solid state temperature sensors (I-buttons, iButton® Devices) per replication were placed at 25 mm depth in the soil to record temperature every 3 hours until harvesting. The experiment was set out as a RCBD with three replications. Daily seedling emergence was counted to the end of the germination. Once germination was completed (as defined by two consecutive days without any new seedlings), 10 plants were carefully excavated from the field per genotype per replication from middle row to avoid border effects. Coleoptile length (CL) (mm) was measured and EP and SE were calculated. Time to complete germination shortened with later (and warmer) plantings. It ranged from 21 to 12 days.

2.4.3 Cold room growth conditions

Two experiments were sown in cold room during 2009 and 2010 years in the cold room at Memorial Building Bangor University. In 2009 year twenty five seeds of ten spring barley varieties were sown in trays (36 cm x 23 cm x 9 cm) size at 25 mm depth. John Innes No 3 potting soil was used in both experiments. In the year 2010 number of varieties was reduced but was conducted with two sowing depths. Sowing was done at 25 and 50 mm depth. The experiments were sown using a RCBD with three replications. The mean temperature for the room was 4^0C in year 2009 but 13^0C in year 2010 due to a fault in cooling system. It did not invalidate comparisons between varieties. In 2009, the first seed emerged after

20 days and germination was complete after sixteen more days. When the seeds were emerged lights were switched on for 16 h per day photoperiod. The experiment was harvested 16th day after initial seedling emergence.

Speed of emergence (as expressed as a seedling emergence index - SE) was calculated according to the formula (Saeidi *et al*., 2008):

$$\mathrm{SE} = \frac{\mathrm{S1}}{\mathrm{D1}} + \frac{\mathrm{S2}}{\mathrm{D2}} + \frac{\mathrm{S3}}{\mathrm{D3}} \ldots + \frac{\mathrm{Sn}}{\mathrm{Dn}}$$

Where, S = number of seeds germinating per day,
D = number of days and
n = number of seeds on final observation.

In this chapter, only parental data was available, therefore heritability estimates for these traits were carried out based on the variance components obtained from an analysis of variance procedure (general heritability analysis. Cf. generation mean analysis used in section 3.3) (Singh and Chaudhary 1985, Acquaah, 2007 and 2012).

Heritability H^2 (B.S) = $(V_G/V_P)*100$
Genetic variance $V_G = (M_g-M_e)/r$
Phenotypic variance $V_P = V_G+V_E$
M_g and M_e are mean squares of genotypes and error respectively.
r= replication

Data observed from all the experiments were analyzed using statistical software SPSS ver.16 using GLM multivariate model. Analysis included descriptive, analysis of variance and simple correlations. The means were separated with Duncan's multiple range tests to determine any significant difference between two means. The graphs were made using Sigma Plot ver.8 and Excel 2010.

Fig 2.1 Seeds of contrasting spring barley genotypes showing colour of the testa. For this work, the standard descriptions black, grey and white (together with golden) were used. Four examples are illustrated; Static golden, Tibet-37 black, Pak-23 grey and Taiga white. Note that the illustrated example of "white" is towards the more pigmented range of a "white" spectrum. Details of these and other varieties are given in Table 2.1.

Fig 2.2 Coleoptile length of naked and covered genotypes was measured using a ruler. The arrows indicate where the emergent leaves have burst through the tip of each coleoptile.

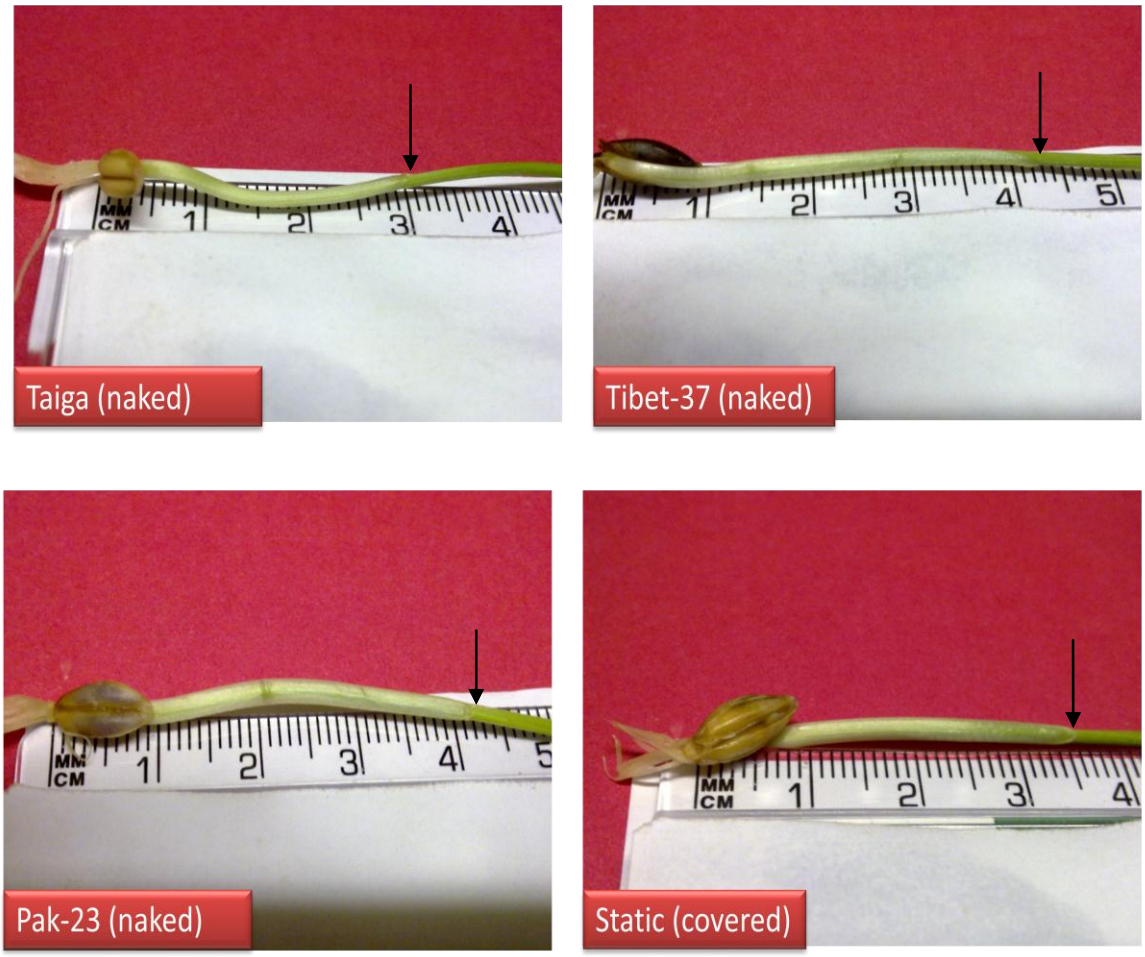


Fig 2.3 Seedling structure of germinating barley (Tibet-37). The first leaf has just emerged from the coleoptile.

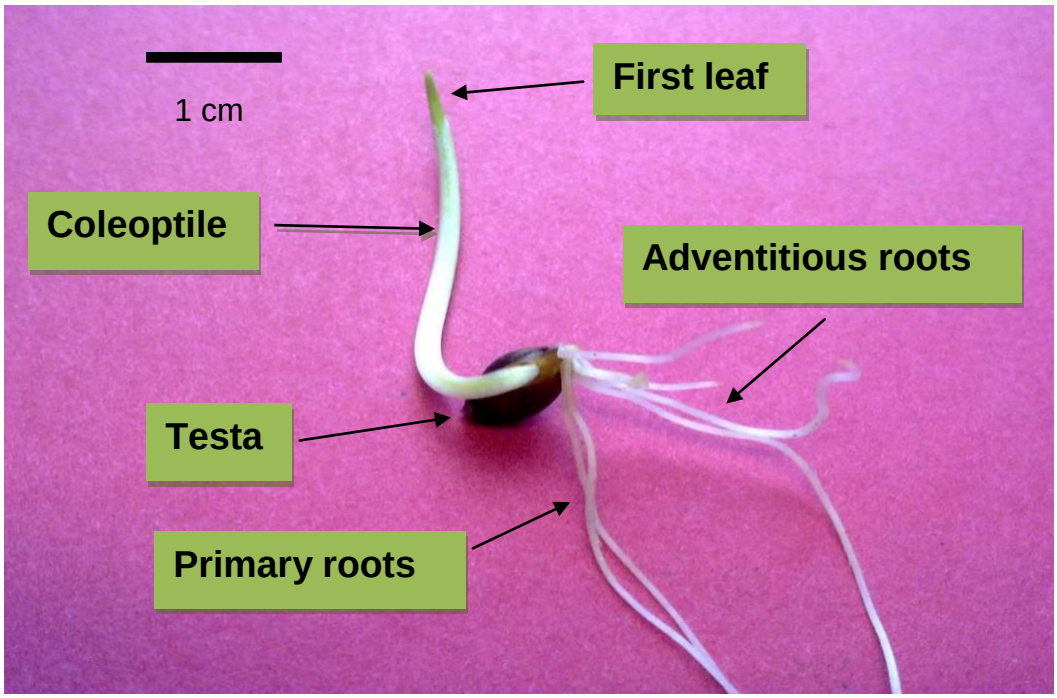
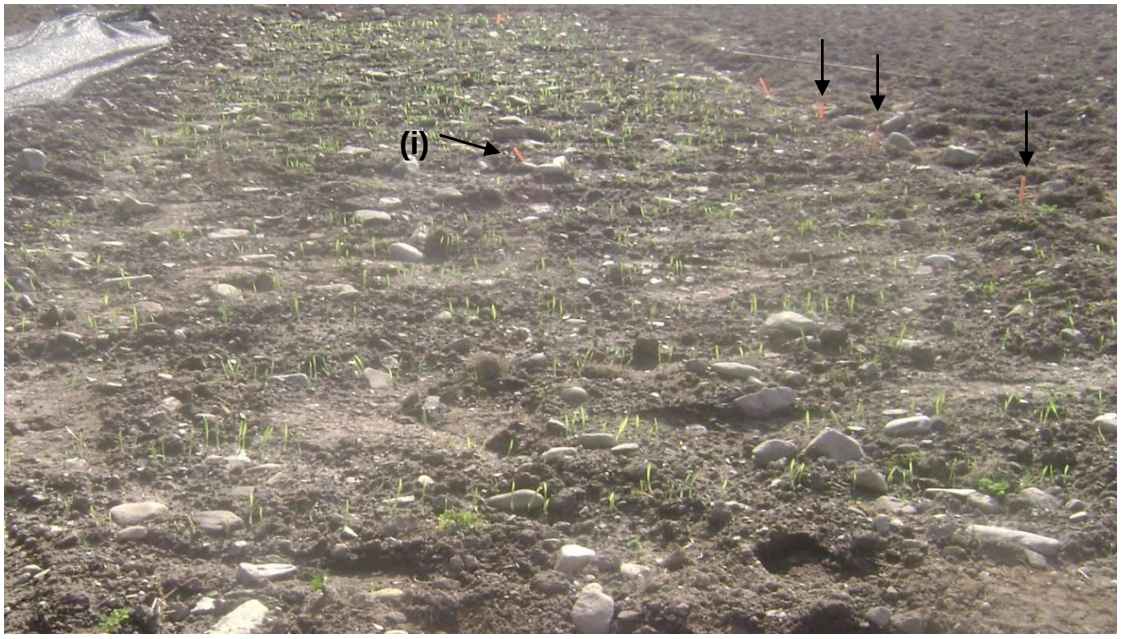


Fig 2.4 Sowing of barley genotypes for seedling establishment in the field at Henfaes Research Centre (Experiment 2.4). Details are given in section 2.4.2.

Fig 2.5 Emergence of 10 spring barley genotypes (indicated by red pegs – examples arrowed) in the field in the year 2009**.** This illustrates a plot during the germination period – before the end of germination as defined in section 2.4.2. Position of i-button indicated (i).



2.5 Results

Screening of spring barley genotypes for coleoptile length, emergence percentage and seedling establishment at glasshouse (Experiment 2.1).

The varieties differed significantly in coleoptile length ($P<0.01$) (Table 2.1.1). The longest coleoptile length was observed in Tibet-37 followed by Hiproly (31 and 23 mm respectively), and the shortest coleoptile length was displayed by variety Taiga (21 mm) (Table 2.1.2). A statistical difference was found between Tibet-37 and Taiga barley varieties (Table 2.1.2)

Highly significant variation was found for the trait emergence percentage (Table 2.1.1). Genotype Tibet-37 was significantly differing from other three genotypes according to DMRT (Table 2.1.2). Tibet-37 showed 100% emergence percentage followed by Static (75%) and lowest emergence percentage was observed in variety Taiga (56%) (Table 2.1.2).

In the case of shoot length (SL), highly significant variation was noticed between the genotypes. Tibet-37 showed longest SL (249 mm) followed by Taiga (198 mm). Lowest shoot length was observed in covered variety Static (136 mm). Results showed that root length (RL) exhibited highly significant variation between genotypes. Longest root length was obtained in genotype Tibet-37 (179 mm) followed by Taiga (141 mm). Variety Static had lowest root length (101 mm).

Regarding dry shoot weight (DSW), it was revealed that varieties were significantly different P<0.01 (Table 2.1.1). Highest DSW was obtained by variety Tibet-37 (0.37 g), Hiproly was in 2nd position with (0.28g) and lowest DSW was recorded in Static (0.17 g). Similarly for the trait DRW, highly significant difference was observed for the genotypes. Highest DRW was discovered by genotype Tibet-37 (0.13 g) and Taiga was on 2nd position with average DRW (0.11g). All the traits showed high broad sense heritability ranged between (56-100%) (Table 2.1.1).

The simple correlation results presented in Table 2.1.3 show that coleoptile length is significantly correlated with emergence percentage (r=0.703; p<0.01), shoot length (r=0.709; p<0.01), root length (r=0.585; p<0.05), dry shoot weight (r=0.651; p<0.01) and dry root weight (r=0.581; p<0.05).

Table 2.1.1 A comparison of a range of seedling characteristics between four genotypes of spring barley (Static, Hiproly, Tibet-37 & Taiga) grown in the glasshouse (Experiment 2.1). ANOVA (mean squares) and heritability values for Coleoptile length (CL), emergence percentage (EP), shoot length (SL), root length (RL), dry shoot length (DSW) and dry root length (DRW). ** Significant at p<0.01 level

Source	DF	CL (mm)	EP (%)	SL (mm)	RL (mm)	DSW (g)	DRW (g)
Genotype	3	76.417**	1497.396**	86.604**	4558.854**	0.027**	0.002**
Error	12	6.875	91.146	2.927	744.531	0.002	0.000
V_G		17.386	351.563	20.919	953.581	0.006	0.001
V_E		6.875	91.146	2.927	744.531	0.002	0.000
V_P		24.261	442.709	23.846	1698.112	0.008	0.001
H^2 (B.S) %		72	79	88	56	76	100

Table 2.1.2 A comparison of a range of seedling characteristics between four genotypes of spring barley (Static, Hiproly, Tibet-37 & Taiga) grown in the glasshouse (Experiment 2.1). Mean values for Coleoptile length (CL), emergence percentage (EP), shoot length (SL), root length (RL), dry shoot length (DSW) and dry root length (DRW). The means followed by similar letters are not significantly different at p<0.05 levels according to Duncan's Multiple Range Test (DMRT).

Genotypes	CL (mm)	EP (%)	SL (mm)	RL (mm)	DSW (g)	DRW (g)
Taiga	21^{a}	56^{a}	198^{b}	141^{ab}	0.27^{b}	0.11^{bc}
Hiproly	23^{a}	63^{ab}	180^{b}	118^{a}	0.28^{b}	0.09^{ab}
Tibet-37	31^{b}	100^{c}	249^{c}	179^{b}	0.37^{c}	0.13^{c}
Static	22^{b}	75^{b}	136^{a}	101^{a}	0.17^{a}	0.08^{a}

Table 2.1.3 A comparison of a range of seedling characteristics between four genotypes of spring barley (Static, Hiproly, Tibet-37 & Taiga) grown in the glasshouse (Experiment 2.1). Correlations of Coleoptile length (CL), emergence percentage (EP), shoot length (SL), root length (RL), dry shoot length (DSW) and dry root length (DRW). ** Correlation is significant at p< 0.01 level (2-tailed). * Correlation is significant at p< 0.05 level (2-tailed).

	CL (mm)	EP (%)	SL (mm)	RL (mm)	DSW (g)	DRW (g)
CL	1	0.703**	0.709**	0.585*	0.651**	0.581*
EP		1	0.41	0.294	0.381	0.306
SL			1	0.813**	0.811**	0.763**
RL				1	0.744**	0.910**
DSW					1	0.696**
DRW						1

Effect of sowing depth on coleoptile length, emergence percentage and other seedling traits in spring naked barley genotypes (Experiment 2. 2).

Sowing depth had highly significant effect on the traits emergence percentage, speed of emergence, shoot length and dry shoot weight (Table 2.2.1). On the other hand, the result suggested that there was no significant effect of sowing depths upon coleoptile length, root length and dry root weight[2].

There were highly significant different genotype effects on all the traits (Table 2.2.1), suggesting that there is a great variability for these traits in these genotypes. Means were separated according to (DMRT) to determine the significance between genotypes.

Heritability in these traits ranged between 50 to 94 %, the highest heritability was recorded in the trait emergence percentage (94%) followed by speed of emergence (93%). Coleoptile length exhibited (83%) heritability. These results suggest that these traits are highly heritable and can be useful in future breeding program aimed to improve seedling establishment in barley.

Longest coleoptile length (31 mm) was obtained by Tibet-37 followed by Hiproly (26 mm). However, shortest was recorded in Taiga (23 mm) (Table 2.2.2).

[2] Note that in several later experiments with larger ranges of sowing depths (Exp 2.3 and 2.6; Exp 3.1 and 3.3) a significant influence of sowing depth on coleoptile length was found. This led to the conclusion that the result in this section is due to the difference in depths (20 and 25 mm) being insufficient to observe the effect.

Emergence percentage was highest in genotype Tibet-37 (100%) followed by Static (86%). Lowest emergence percentage was observed in genotype Taiga (72%). In the case of speed of emergence it was noticed from the data presented in Table 2.2.2 that highest speed of emergence was observed in genotype Tibet-37 (47 seeds day^{-1}). Genotype Taiga showed lowest speed of emergence (18 seeds day^{-1}).

Tibet-37 had the longest SL (304 mm) followed by Hiproly (261 mm). Static had the shortest shoot length (228 mm). The mean value of root length was highest in Tibet-37 followed by Hiproly. The shortest root length was displayed by Static. Data presented in Table 2.2.2 reveals that the variety Tibet-37 displayed highest DSW and DRW followed by Hiproly for DSW and Taiga for DRW.

Data presented in Table 2.2.3 showed that all the traits displayed highly significant positive correlations ($p<0.01$). Coleoptile length showed strong highly significant correlation with emergence percentage ($r=0.752$) and speed of emergence ($r=0.738$). Emergence percentage has strong highly significant association with speed of emergence ($r=0.844$). Speed of emergence showed strong positive highly significant correlation with SL, RL and DSW ($r= 0.629$, 0.641 and 0.520 respectively). Shoot length showed strong highly significant positive e correlation with RL, DSW and DRW ($r=0.860$, 0.819 and 0.682 respectively). Root length had strong highly significant positive correlation with DSW ($r=0.799$) and DRW ($r=0.702$). Finally dry shoot weight showed strong highly significant positive correlation with DRW ($r=0.729$).

Table 2.2.1 A comparison of a range of seedling characteristics between four genotypes of spring barley (Static, Hiproly, Tibet-37 & Taiga) grown at two sowing depths (20 or 25 mm) in the glasshouse (Experiment 2.2). ANOVA (mean squares) and heritability values for Coleoptile length (CL), emergence percentage (EP), speed of emergence (SE), shoot length (SL), root length (RL), dry shoot length (DSW) and dry root length (DRW). ** Significant at p<0.01 level

Source	DF	CL (mm)	EP (%)	SE (seeds day^{-1})	SL (mm)	RL (mm)	DSW (g)	DRW (g)
Sowing depth	1	1.24	122.07**	317.82**	11940.41**	4521.8	0.17**	0.01
Genotype	3	105.21**	1176.76**	1433.54**	9291.22**	14807.27**	0.17**	0.03**
Depth * genotypes	3	2.56	17.9	8.72	562.09	2514.3	0.03	0.01
Error	24	5.2	17.9	24.49	1269.2	2297.8	0.02	0.01
V_G		25.00	289.71	352.26	2005.52	3127.36	0.04	0.01
V_E		5.20	17.90	24.49	1269.15	2297.82	0.02	0.01
V_P		30.20	307.62	376.76	3274.66	5425.19	0.05	0.01
H^2 (B.S) %		83	94	93	61	58	69	50

Table 2.2.2 A comparison of a range of seedling characteristics between four genotypes of spring barley (data from sowing depths 20 or 25 mm combined) (Static, Hiproly, Tibet-37 & Taiga) grown in the glasshouse (Experiment 2.2). Mean values for Coleoptile length (CL), emergence percentage (EP), speed of emergence (SE), shoot length (SL), root length (RL), dry shoot length (DSW) and dry root length (DRW). The means followed by similar letters are not significantly different at $p<0.05$ levels according to Duncan's Multiple Range Test (DMRT).

Genotype	CL (mm)	EP (%)	SE (seeds day^{-1})	SL (mm)	RL (mm)	DSW (g)	DRW (g)
Taiga	23^{a}	72^{a}	18^{a}	236^{a}	154^{a}	0.45^{b}	0.14^{ab}
Hiproly	26^{b}	78^{b}	21^{a}	261^{a}	161^{a}	0.48^{b}	0.13^{a}
Tibet-37	31^{b}	100^{d}	47^{b}	304^{b}	235^{b}	0.62^{c}	0.21^{b}
Static	26^{c}	86^{c}	22^{a}	228^{a}	138^{a}	0.27^{a}	0.07^{a}

Table 2.2.3 A comparison of a range of seedling characteristics between four genotypes of spring barley (data from sowing depths 20 or 25 mm combined) (Static, Hiproly, Tibet-37 & Taiga) grown in the glasshouse (Experiment 2.2). Correlations of Coleoptile length (CL), emergence percentage (EP), speed of emergence (SE), shoot length (SL), root length (RL), dry shoot length (DSW) and dry root length (DRW). ** Correlation is significant at $p< 0.01$ level (2-tailed). * Correlation is significant at $p< 0.05$ level (2-tailed).

	EP (%)	SE (seeds day^{-1})	SL (mm)	RL (mm)	DSW (g)	DRW (g)
CL (mm)	0.752**	0.738**	0.595**	0.459**	0.393*	0.357*
EP (%)	1	0.844**	0.477**	0.477**	0.331	0.254
SE (seeds day^{-1})		1	0.629**	0.641**	0.520**	0.437*
SL (mm)			1	0.860**	0.819**	0.682**
RL (mm)				1	0.799**	0.702**
DSW (g)					1	0.729**
DRW (g)						1

Effects of different temperature on coleoptile length, emergence percentage and speed of emergence of ten spring barley genotypes (Experiments 2.3, 2.4, 2.5 and 2.6).

Significant differences were observed for temperature and sowing depths for all the traits studied in glasshouse, field and cold room growth conditions (Table 2.3.1). Genotypes also showed highly significant variation $p<0.001$ for all the traits. The temperature x sowing depth interaction showed highly significant variation only for the trait speed of emergence. Temperature x genotypes interaction showed highly significant difference for all the traits studied. Sowing depth x genotype interactions as well as temperature x sowing depth x genotypes interaction showed non-significant variation for all the traits.

The estimates of genotypic variance for CL, EP and SE were (24.82, 57.80 and 15.24 respectively), which was more than phenotypic variance, indicating the presence of greater variability for these traits (Table 2.3.1). Phenotypic variance for CL was (130.15), EP (638.73) and SE (131.79). These traits showed highest heritability CL (97%), EP (93%) and SE (83%).

Longest CL was obtained in genotype Tibet-37 (51 mm) followed by PAK-23 (46 mm) (Fig 2.3.1). Shortest CL was observed in genotypes Taiga and IC-93-747 (34 mm). Highest emergence percentage was observed in genotype Tibet-37 (90%) followed by Pak-23 (86%) and genotype Men Jun was in third position (80%) (Fig 2.3.2). Lowest emergence percentage was noticed in genotypes Taiga

and IC-93-747 (60%). Similarly for the trait speed of emergence it was noticed that genotype Tibet-37 showed highest SE (33 seeds day^{-1}), genotypes PAK-23 was on 2^{nd} position (32 seeds day^{-1}) and Men Jun was on 3^{rd} position (29 seeds day^{-1}) (Fig 2.3.3). Genotypes Taiga and IC-93-747 showed lowest SE (21 seeds day^{-1}).

The highest mean CL (56 mm) was observed at temperature $13^{O}C$ grown in "cold" room during 2010 season followed by temperature $4^{O}C$ in cold room for the year 2009 (Fig 2.3.4). The lowest mean CL was observed in temperature $7^{O}C$ and $12^{O}C$ grown in 2^{nd} and 3^{rd} sowing date in field conditions during year 2009.

Highest EP (82 and 80%) were observed from the temperature $8^{O}C$ and $13^{O}C$ respectively (Fig 2.3.5). Lowest EP (60%) was recorded in 3^{rd} sowing date in field at temperature $12^{O}C$ (Fig 2.3.5). Regarding the trait SE highest SE (35 seeds day^{-1}) was observed at $15^{O}C$ grown in glasshouse followed by $8^{O}C$ grown in 2^{nd} sowing date in field (30 seeds day^{-1}) (Fig 2.3.6). Lowest SE (18 seeds day^{-1}) was observed at lowest temperature $4^{O}C$ and $7^{O}C$ cold room and 1^{st} sowing date in field respectively.

The deep sowing at 50 mm showed highest CL (48 mm) compared to 25 mm depth (36 mm) (Fig 2.3.7). Highest EP (76%) was observed at 50 mm depth and lowest EP (74%) was in 25 mm depth (Fig 2.3.8). Consequently for the trait SE it was revealed that highest SE (27 seeds day^{-1}) was noticed in 25 mm depth compared to 50 mm dept with average EP (24 seeds day^{-1}) (Fig 2.3.9).

There was highly significant positive correlation between coleoptile length with emergence percentage (r=0.443) (Table 2. 3.2). Emergence percentage showed highly significant positive correlation with speed of emergence (r=0.559).

Table 2.3.1 A comparison of a range of seedling characteristics between ten genotypes of spring barley (Table 2.1) grown at two sowing depths (25 or 50 mm) in the glasshouse (Experiment 2.3), 3 sowing dates in field (Experiment 2.4) and 2 sowing dates in cold room (Experiment 2.5 & 2.6). (Data from all experiments combined.) ANOVA (mean squares) and heritability values for Coleoptile length (CL), emergence percentage (EP), speed of emergence (SE). ** Significant at p<0.01 level

Source	DF	CL (mm)	EP (%)	SE (seeds day^{-1})
Experiment (Temperature)	5	1188.40**	1840.54**	2536.85**
Sowing depth	1	1576.06**	525.50**	3197.09**
Genotype	9	383.43**	1827.23**	350.55**
Expt * sowing depth	1	2.57	107.63	380.15**
Expt * genotype	39	20.76**	148.68**	40.66**
Sowing depth * genotype	9	1.59	61.12	12.44
Expt * SD * genotype	3	1.13	94.52	16.75
Error	136	3.52	44.49	22.41
V_G		24.82	57.80	15.24
V_E		3.52	44.49	22.41
V_P		130.15	638.73	131.79
h^2 (B.S) %		97	93	83

Table 2.3.2 A comparison of a range of seedling characteristics between 10 genotypes of spring barley. Details of experiments and genotypes as in Table 2.3.1. Correlations of Coleoptile length (CL), emergence percentage (EP). ** Correlation is significant at p< 0.01 level (2-tailed).

	EP (%)	SE (seeds day^{-1})
CL (mm)	0.443**	-0.067
EP (%)	1	0.559**

Fig 2.3.1 Mean coleoptile length of the 10 genotypes (Table 2.1). Data pooled from several experiments as described in Table 2.3.1. Note that Taiga and Tibet-37 represent the two extreme cases. These were chosen as the basis of the construction of a mapping population (chapter 3). Mean ± se (n = 48)

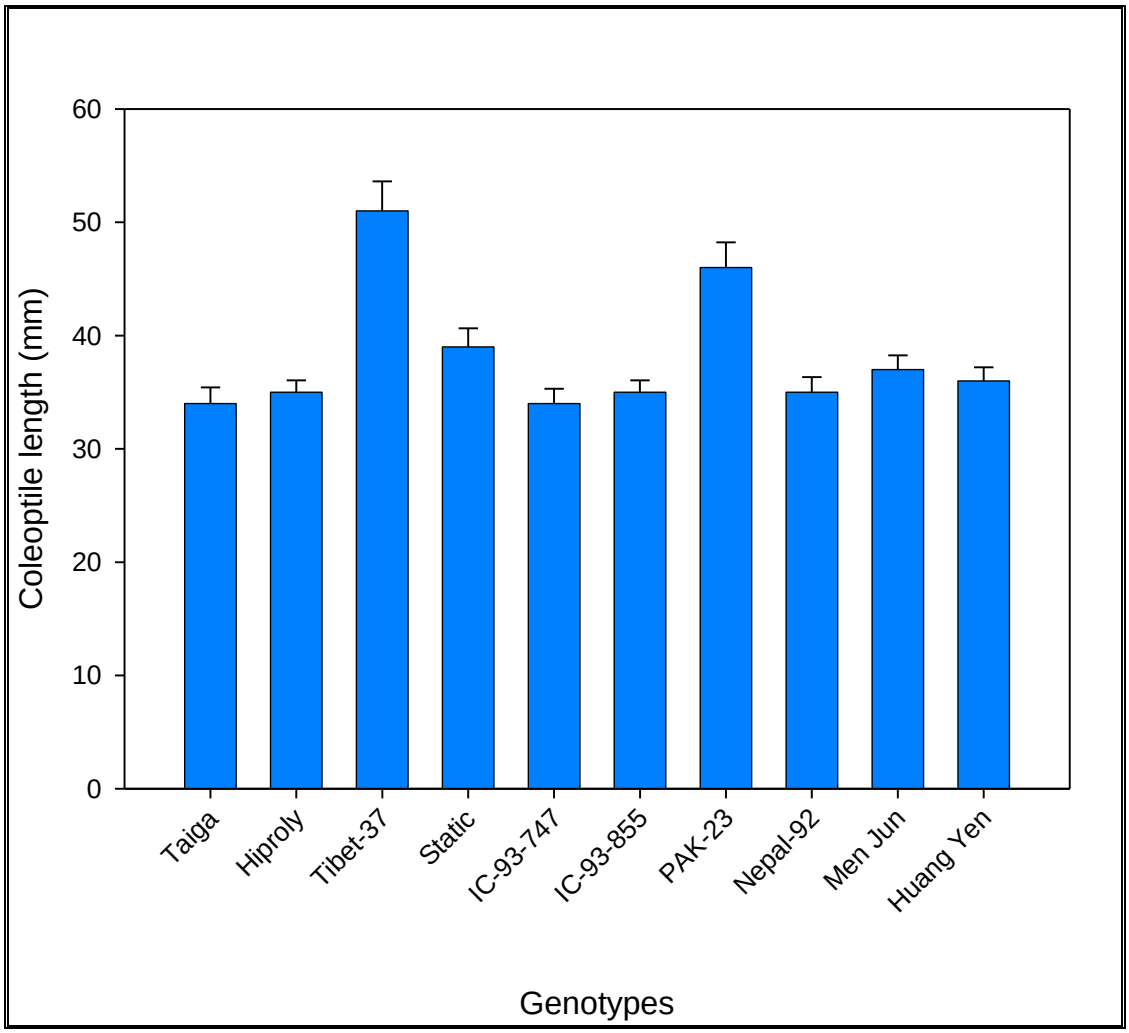


Fig 2.3.2 Mean emergence percentage of the 10 genotypes (Table 2.1). Data pooled from several experiments as described in Table 2.3.1. Mean ± se (n = 48)

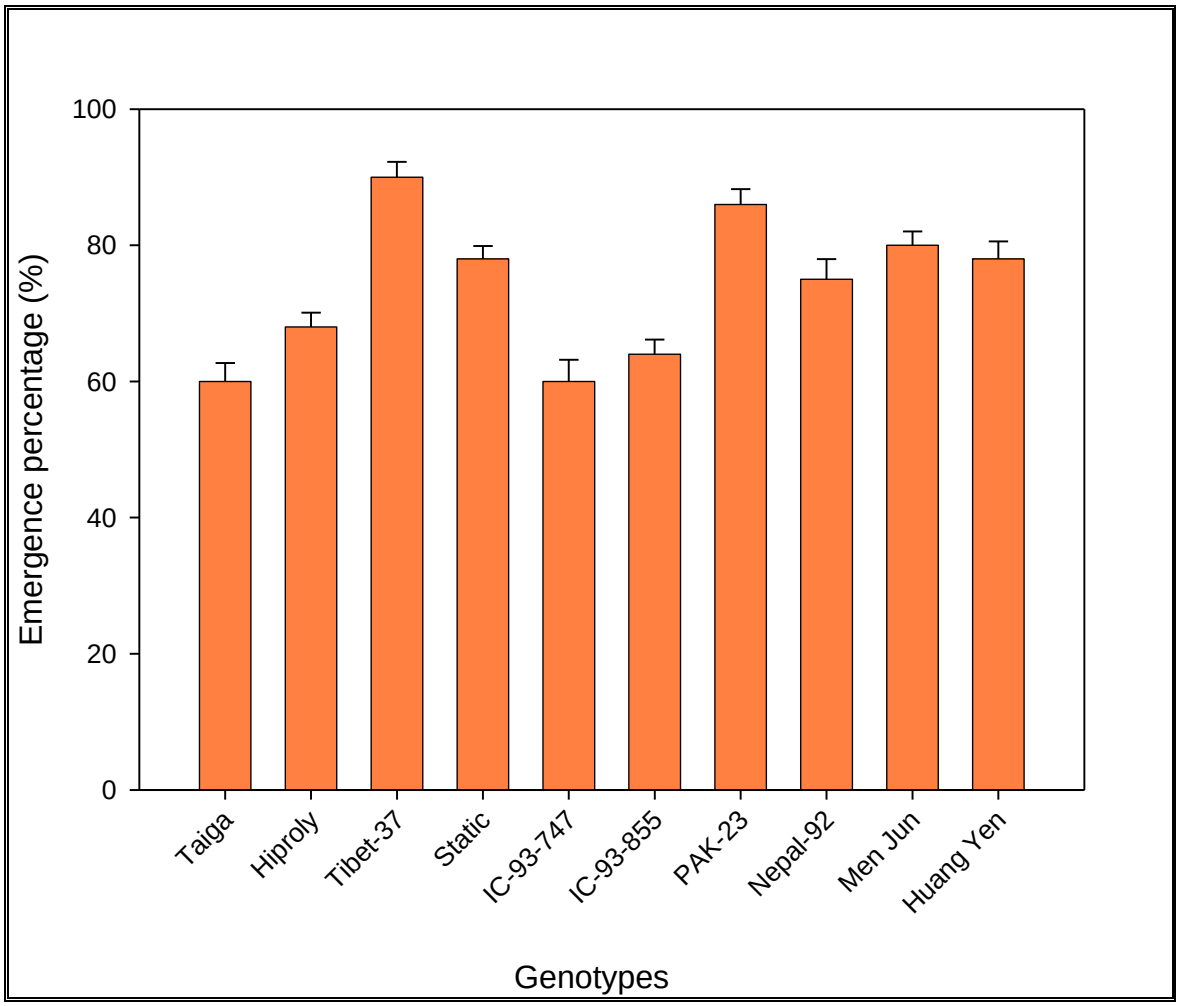


Fig 2.3.3 Mean speed of emergence of the 10 genotypes (Table 2.1). Data pooled from several experiments as described in Table 2.3.1. Mean ± se (n = 48)

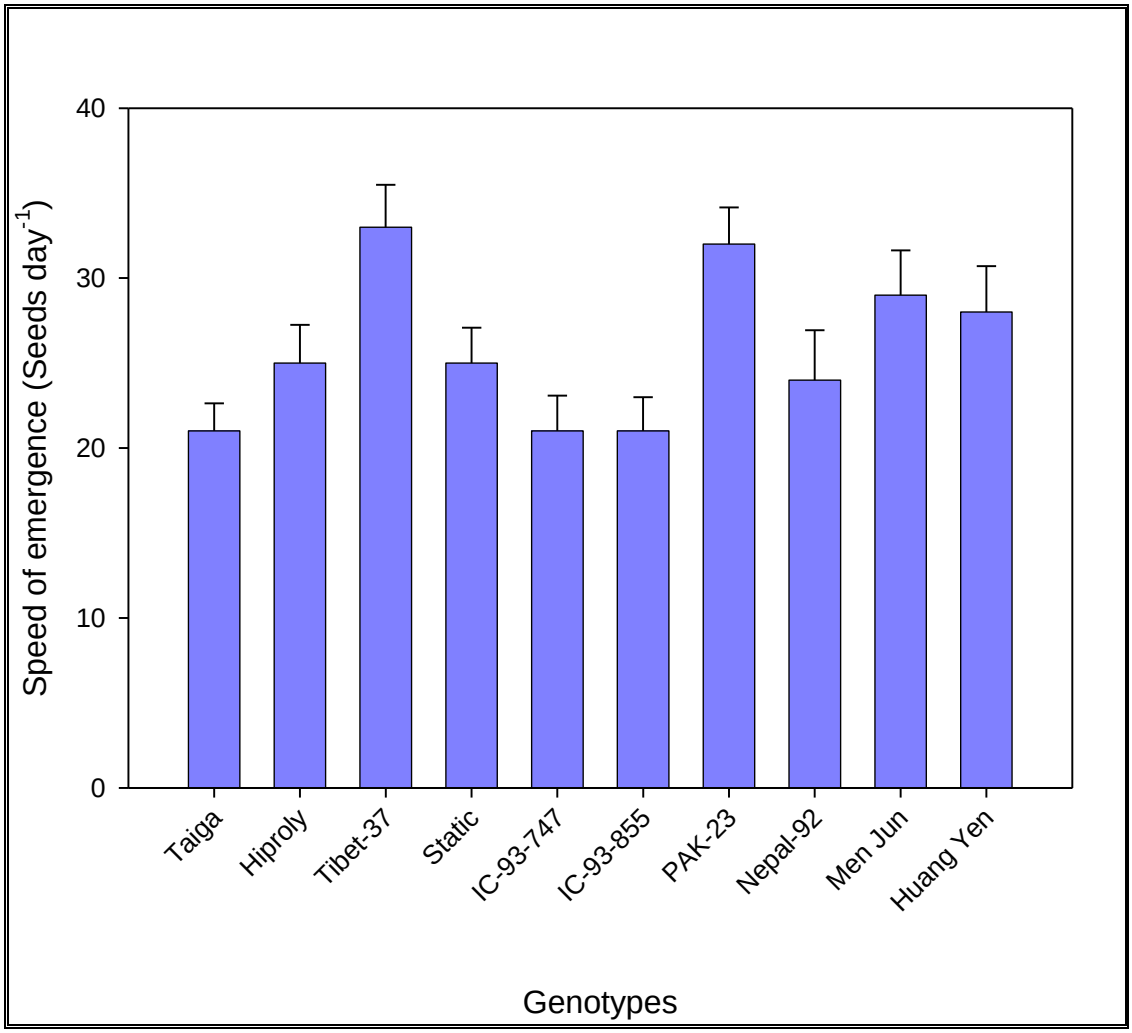


Fig 2.3.4 The effect of temperature (and light) on coleoptile length of the 10 genotypes on spring barley (Table 2.1). Data pooled from several experiments as described in Table 2.3.1. Mean ± se (n = 48; where error bars are not visible, they are smaller than the symbols). In these experiments, temperature was only one of several environmental variables – see details for experiments 2.3 – 2.6. In summary, blue diamonds represent seedlings in field (7-12°C) or glasshouse (15°C) (both with a day/night rhythm) while red squares represent data from dark "cold" room. Most of these data are means of considerable environmental variation (Fig 2.3.10 & 2.3.11). These data show not only a major light dependence of CL, but also, possibly, a temperature dependence (that may be light dependent).

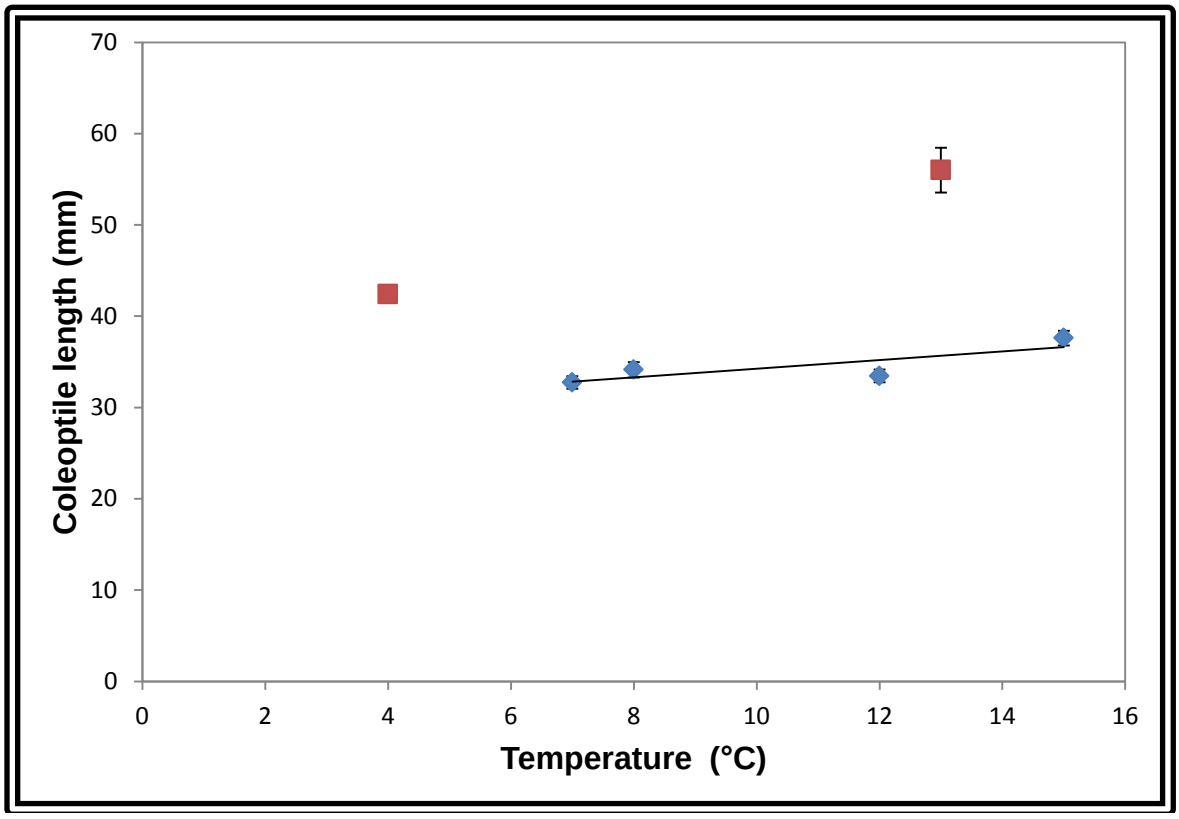


Fig 2.3.5 The effect of temperature (and light) on emergence percentage of the 10 genotypes on spring barley (Table 2.1). Data pooled from several experiments as described in Table 2.3.1. Mean ± se (n = 48; where bars represents error bars). In these experiments, temperature was only one of several environmental variables – see details for experiments 2.3 – 2.6. In summary, temperature in field (7-12°C) or glasshouse (15°C) and "cold" room (4 & 13 °C). Most of these data are means of considerable environmental variation (Fig 2.3.10 & 2.3.11).

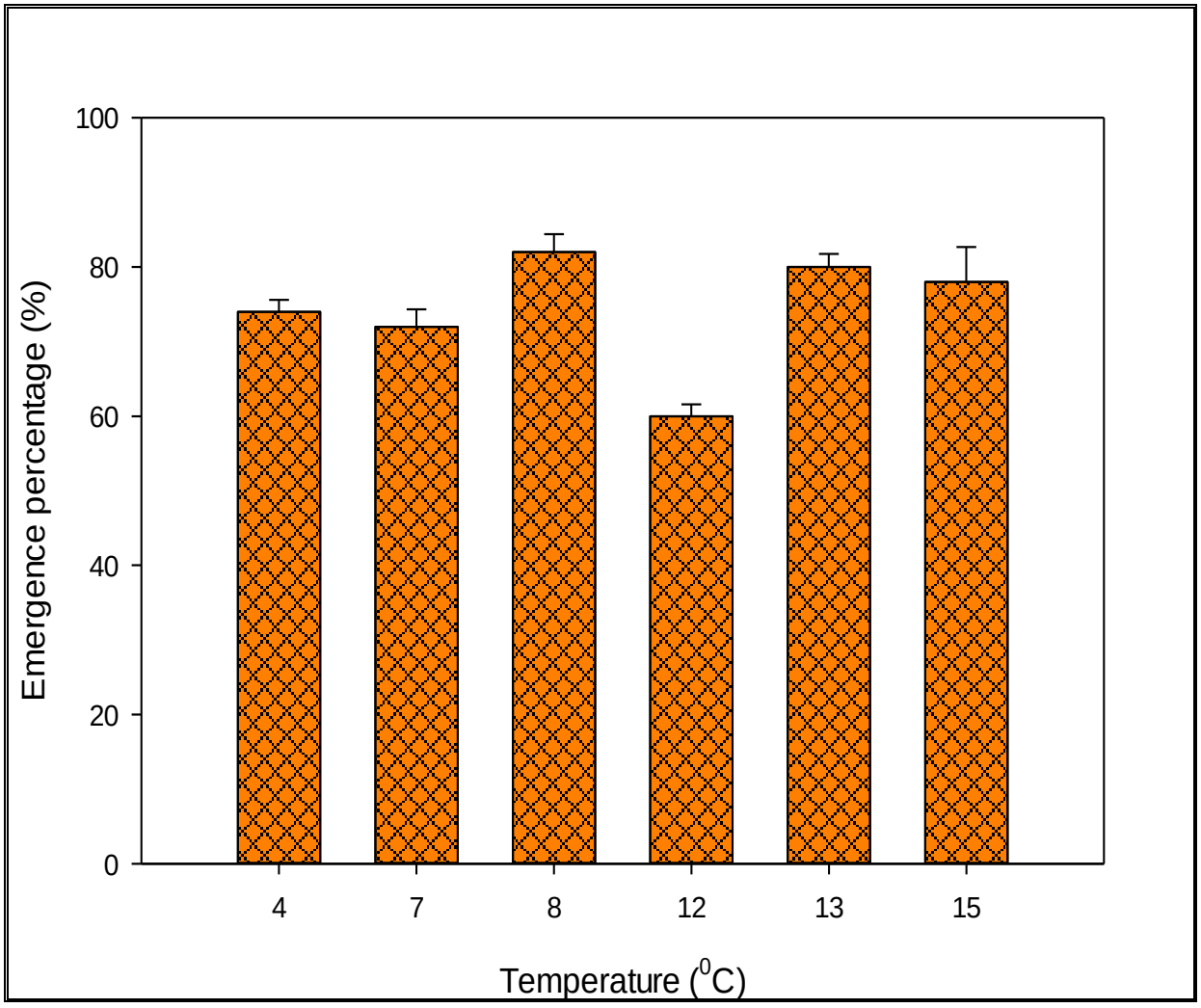


Fig 2.3.6 The effect of temperature on speed of emergence of the 10 genotypes on spring barley (Table 2.1). Data pooled from several experiments as described in Table 2.3.1. Mean ± se (n = 48). In these experiments, temperature was only one of several environmental variables – see details for experiments 2.3 – 2.6. In summary, temperature in field (7-12°C) or glasshouse (15°C) and "cold" room (4 & 13 °C). Most of these data are means of considerable environmental variation (Fig 2.3.10 & 2.3.11).

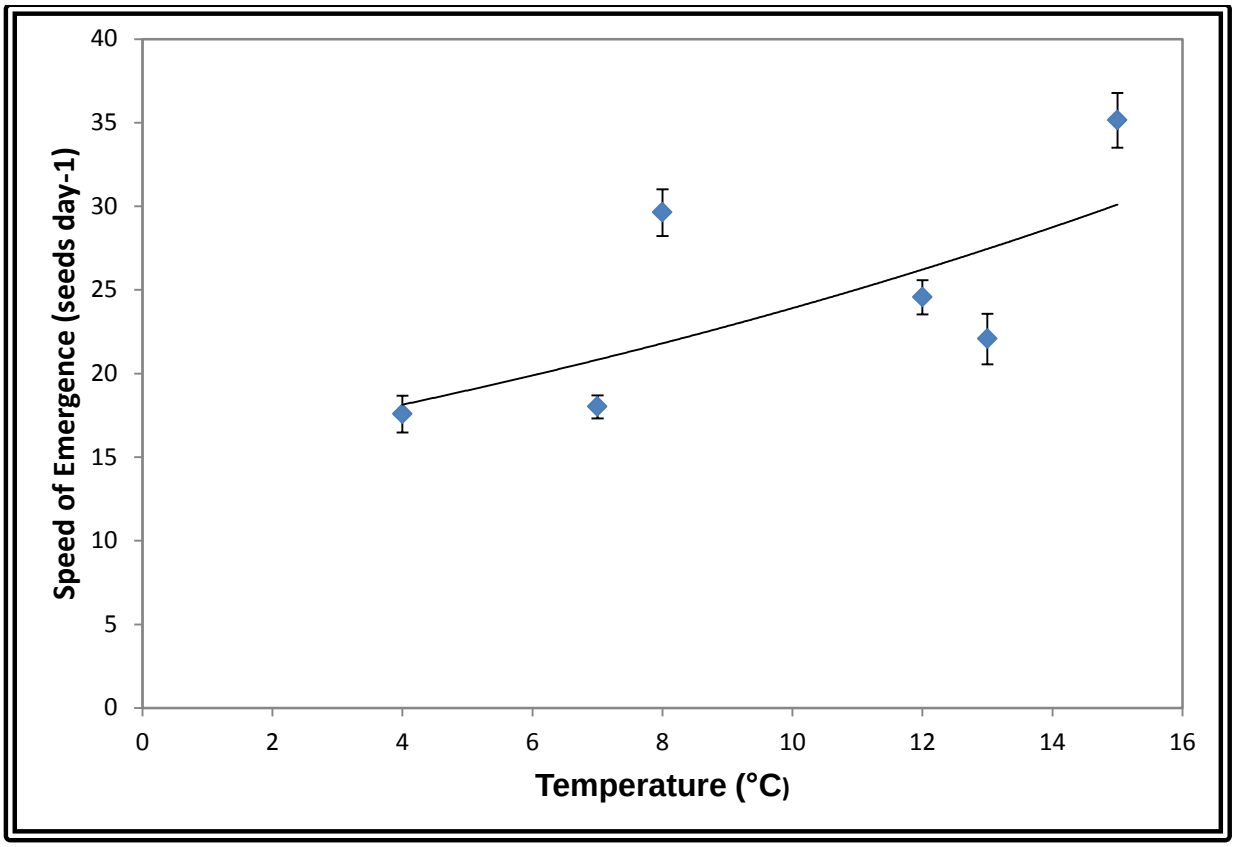


Fig 2.3.7 The effect of sowing depth on coleoptile length of the 10 genotypes on spring barley (Table 2.1). Data pooled from several experiments as described in Table 2.3.1. Mean ± se (n = 48).

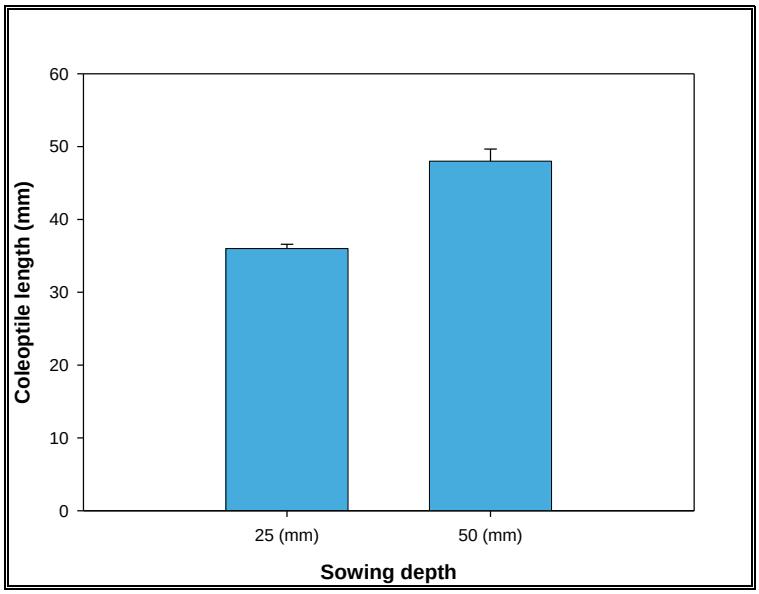


Fig 2.3.8 The effect of sowing depth on emergence percentage of the 10 genotypes on spring barley (Table 2.1). Data pooled from several experiments as described in Table 2.3.1. Mean ± se (n = 48).

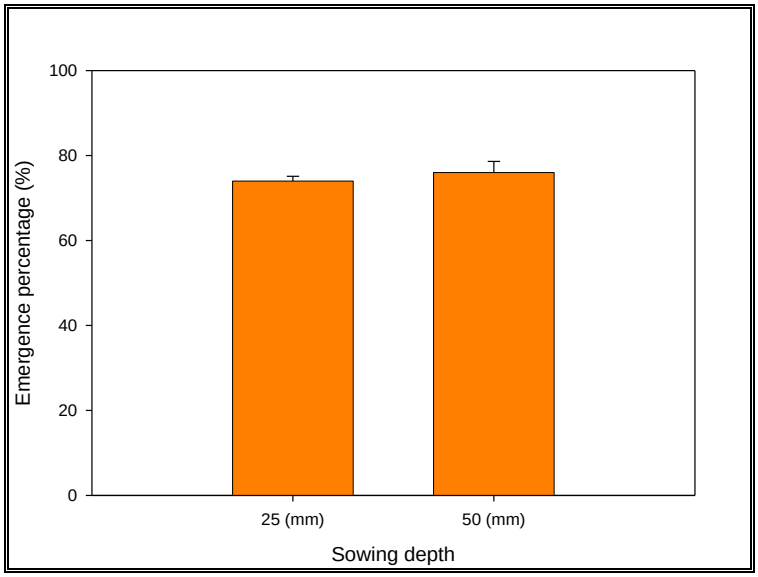


Fig 2.3.9 The effect of sowing depth on speed of emergence of the 10 genotypes on spring barley (Table 2.1). Data pooled from several experiments as described in Table 2.3.1. Mean ± se (n = 48).

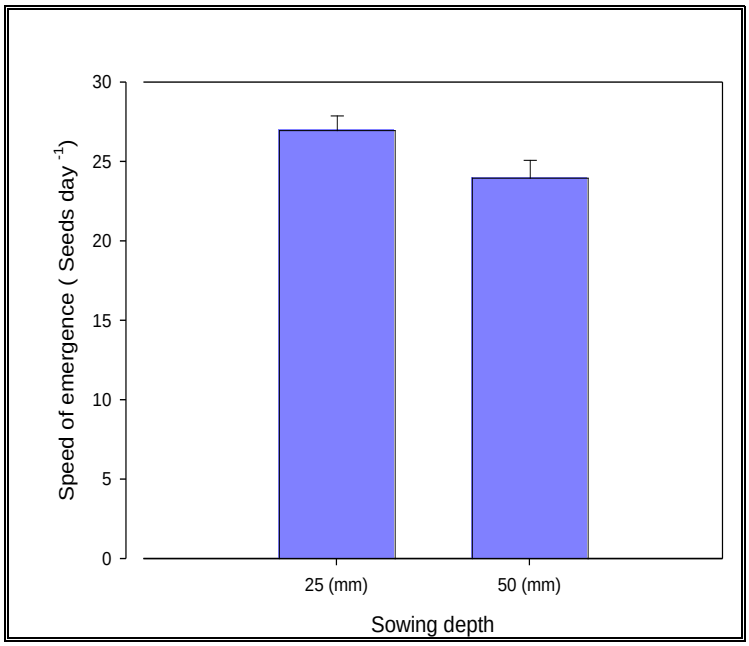


Fig 2.3.10 Daily average soil temperature for three sowing dates in field experiment at Henfaes Research Centre 2009. (Measured with i-buttons.)

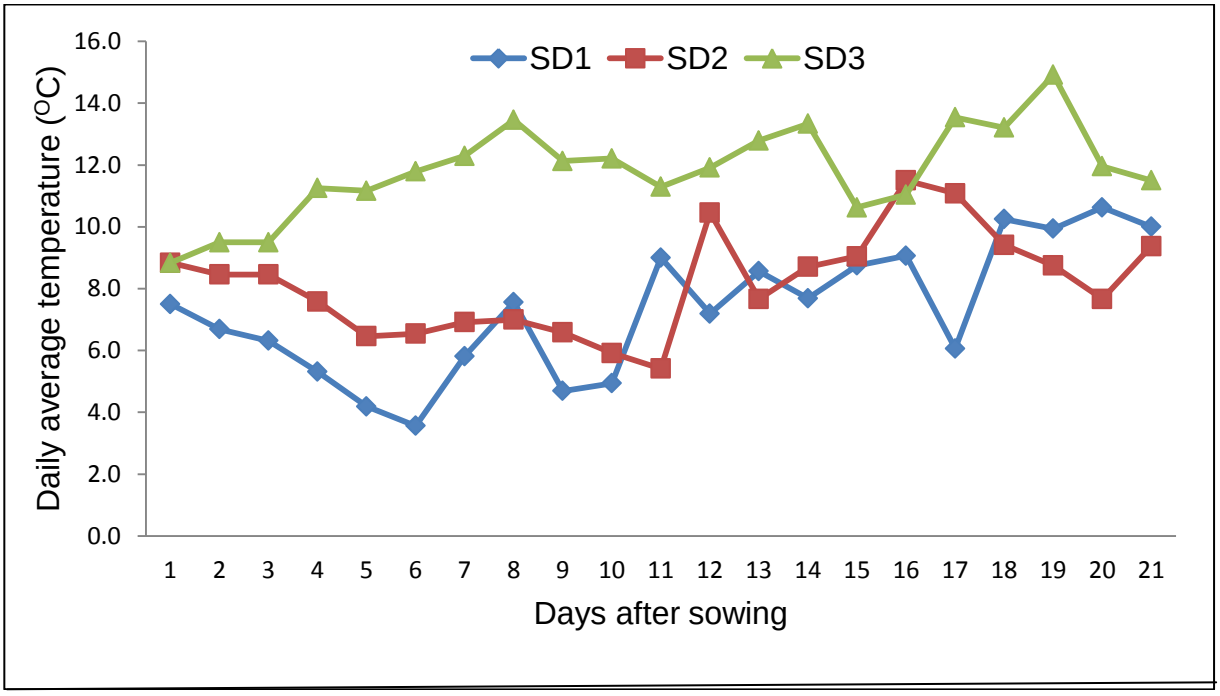
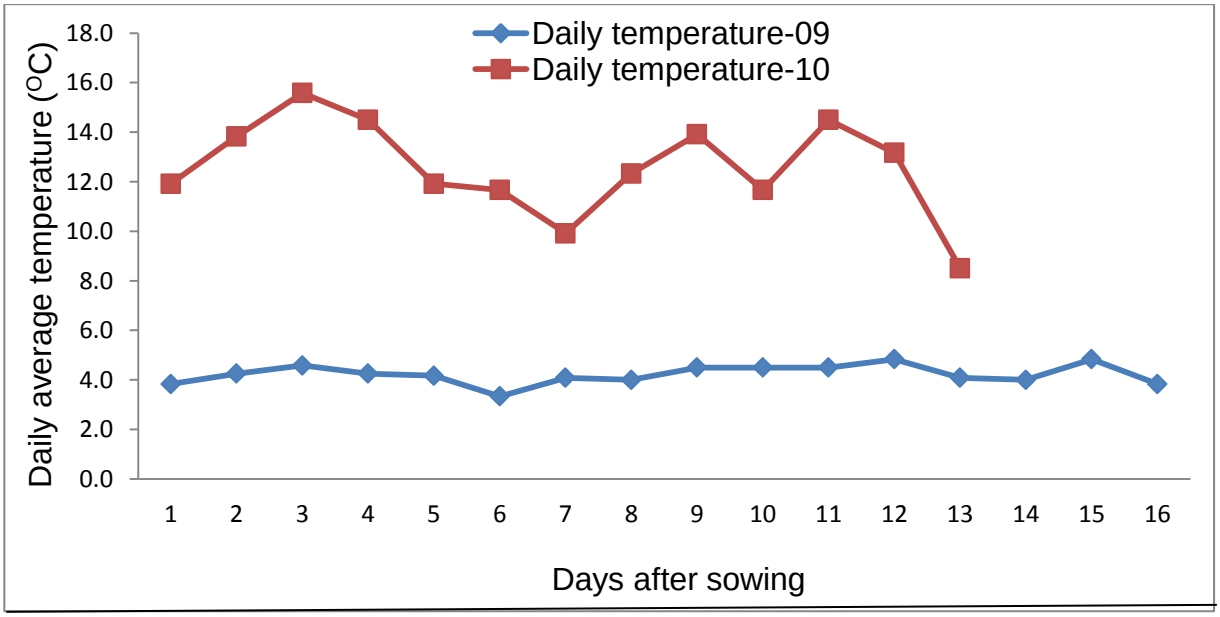


Fig 2.3.11 Daily average soil temperature for barley genotypes in cold room experiment 2009 and 2010. (Measured with i-buttons.)



2.6 Discussion

Poor seedling emergence is a crucial problem in naked barley all over the world including Europe. There are many factors that are responsible for the poor emergence like seed viability, damage of seed during threshing, soil moisture and temperature. To overcome the soil moisture deficiency the seed need to be sown sufficiently deep to reach adequate soil moisture for germination. This also confers some other some advantages such as reduction of seed damage by birds and mice (Brown *et al*., 2003).

Sowing too deep however, has some disadvantages including slower emergence and abnormal seedlings (Hadjichristodoulou *et al*., 1977 and Kirby 1993). Less dry matter production is also incompatible with low temperature (Loeppky *et al*., 1989). In this situation coleoptile length is one of the key traits that improve the seedling emergence.

Plant breeders are always interested to overcome to solve these problems genetically, therefore this study was undertaken to find out better solution of seedling establishment in naked spring barley. Results from the initial experiment (2.1) showed that there was wide variation between genotypes for coleoptile length and other seedling traits. It was noticed that coleoptile length was highly heritable trait. In experiment (2.2) sowing depth was used to determine the sowing depths effect on coleoptile length but due to only 5 mm difference between sowing depth there was no significant effect.

More genotypes were included in subsequent experiments study to select from a wider range of genotypes to create a mapping population for better seedling establishment of spring naked barley. Combined analysis of four experiments was carried out and results showed that CL was significantly affected by sowing depth when the difference between two depths was increased from 25 to 50 mm. Coleoptile lengths of all cultivars significantly increased with increasing planting depths.

Temperature had a small effect on coleoptile length (Fig 2.3.4), but this effect appeared to be considerably magnified under dark growth conditions ("cold" room). It is not known how far daylight permeated into the soil under field and glasshouse conditions. The light inhibition of coleoptile length found is expected from classical observations of coleoptile growth. A cultivar x temperature interaction was found to be significant. Effect of light on coleoptile length was also observed in barley, dark light increased the coleoptile length compared to red (Uematsu, *et al*., 1981 and Burstin, 2000).

Cultivars with longer coleoptile under greenhouse conditions also tended to have longer coleoptile length under deep planting in the field. Current results also showed that there was a significant correlation between coleoptile length and other seedling traits which proved the importance of coleoptile length for improving field establishment.

These results are in support of earlier workers who also reported similar results for example in numerous barley studies, who reported that there was a genetic variation in coleoptile length (Grando and Ceccarelli 1995, Portmann *et al.,* 1996, Box *et al*., 1999, Platz *et al*., 1999, Takahashi and Takeda 1999, Takeda and Takahashi 1999 and Takahashi *et al.*, 2001, 2008). Cultivars having shorter coleoptile length do not have the ability to emerge well from deep sowing and may cause poor seedling emergence, which may affect crop yield later on.

In the initial experiments dry root /shoot weight also significantly varied between genotypes. It was noticed that deep sowing reduced the dry weight. It was demonstrated from the results that shoot and root dry weights were significantly affected by sowing depth. These results are in agreement with the results reported earlier that dry seedling weight was affected by deep sowing and reduced the dry shoot weight (Molatudi and Mariga 2009). In case of shoot length present results suggested that planting depth significantly affected shoot length. Current results are in agreement with other findings that planting depth showed significant effect on shoot length of seedlings (Molatudi and Mariga, 2009).

Simple traits such as seedling or plant height and seedling dry weight (Acevedo *et al*., 1991, Regan *et al*., 1992 and Trachsel *et al*., 2010), rapid shoot growth,

shoot dry weight and shoot length have been identified as good indicators of a good seedling and early vigour in barley. Kaydan and Yagmur 2005 reported that bigger size seed displayed high shoot and root length. Awan *et al*., (2005) reported that seedling dry weight and seedling length and are considered as best example of seedling vigour.

Regarding emergence percentage it was noticed that there were significant effects upon sowing depth and genotypes for this trait in all the experiments under this study. For the speed of emergence trait significant difference was observed in sowing depth as well as genotypes. These results are in agreement with earlier research where depth of planting showed significant differences on seedling emergence (Awan *et al*., 2005, Tavakoli *et al*., 2008 and Molatudi and Mariga, 2009). In deep sowing coleoptile length play a significant role in germination as well as uniform seedling emergence therefore, this trait may be given full attention while breeding varieties for better seedling establishment in cereals (Fick and Qualset, 1976, Whan, 1976, Rebetzke *et al*., 2007).

In these results cultivars with comparatively longer coleoptiles tended to have higher speed of emergence at all temperatures. Variation in SE among planting depths was highly significant in all temperature experiments, deeper planting resulting in lower emergence rates.

Overall in view of the present results, variety Tibet-37 having long coleoptile

length showed superior performance in all the traits under this study. Genotype Pak-23 was ranked in second position and variety Taiga having short coleoptile length generally performed poorly for all the traits in these experiments. Hence, it was concluded that genotypes having long coleoptile length are preferable for early seedling establishment especially in water deficit regions of the world. It was also concluded from the present as well as previous results that to achieve better crop yield in barley, seedling establishment plays significant role. It is concluded that regarding better seedling establishment, traits like coleoptile length, early root shoot length and root shoot dry weight should be taken into consideration in future breeding programs. In particular, Tibet-37 displayed superior germination and early growth characteristics, so this makes it a promising parent in a crossing programme to improve establishment.

Chapter 3

Improving field establishment of naked barley using exotic genotypes.

3.1 Introduction

The main problem with emergence of spring barley in Britain is the cool and damp conditions at sowing which seem to affect naked barley more adversely than hulled varieties. Naked barley lines with short coleoptile length (CL) exhibit poor emergence when sown deep (Shah & Hassan, 2006). Previous work with genotypes with longer coleoptiles showed rapid emergence and early seedling growth, the consequent establishment benefits having an advantage in adverse environments. That is, better establishment leads to a greater chance of optimum population density and higher grain yields. However, the genetics of control of CL, related traits and their relationship to establishment are not fully understood. Various researchers have implied that both major and minor genes are involved in the heritability of these traits. For example, CL was found to be an important characteristic that played a significant role in seedling emergence of cereals, especially when sown deep to avoid moisture stress (Takahashi *et al*., 2001, 2008; Paynter & Clarke, 2010).

In numerous other barley studies it has been reported that there are genotypic variations in CL (Box *et al*., 1999, Platz *et al*., 1999 and Takahashi *et al*., 2001,

2008). Hybridization between phenotypes that exhibit variation in the traits of interest is one of the most important tools to produce genetic variability, allowing scope for subsequent selection.

In earlier experiments reported in this thesis, germination and establishment characteristics of several naked barley lines from many regions were tested in the field, cold-room and glasshouse, and CL was found to vary significantly between genotypes and was correlated with successful establishment. Initial experiments demonstrated that the genotype with the longest CL across a wide range of environments was Tibet 37, whereas Taiga, a naked seeded cultivar from Germany had a short CL and poor field establishment. Therefore, these were chosen as parents to determine factors contributing to the heritability of CL and other factors influencing germination.

3.2 The objectives of this study

To assess emergence traits of seedlings using spring naked barley recombinant inbred lines (RILs) arising from hybridization of Taiga x Tibet-37. Specifically, the aim was to estimate the inheritance of coleoptile length and other seedling traits.

A mapping population developed by crossing Static x Skardu was also examined for seedling establishment, but insufficient variation was observed in traits of interest, thus data are not presented.

Table 3.1 Summary of experiments to investigate the behavior of different generations during the generation of F_9 recombinant inbred lines (RILs). The initial cross was of the two varieties of naked spring barley with the shortest and longest coleoptile length (Taiga x Tibet-37) (Chapter 2).

Title	No. of genotypes tested	Treatments	Location	Starting date
Experiment 3.1 Effect of sowing depth on coleoptile and shoot length in early generations of two naked spring barley Taiga and Tibet-37 cross.	F_2, F_3 & F_4	Generation & Sowing depth	Glasshouse	24th June 2010 1st July 2010
Exp 3.2 Effect of sowing dates on coleoptile length and emergence of F_9 recombinant inbred lines of naked spring barley.	104	RILs & Temperature	Field	20th January, 10th February and 4th March 2011
Exp 3.3 Effect of cold temperature on coleoptile length and emergence of F_9 recombinant inbred lines of naked spring barley.	143	RILs & Temperature	"Cold" room	7th March 2011

3.3 Material and Methods

The Tibet-37 accession was sourced from the John Innes Centre, Norwich, UK, and comprised a hull-less landrace originally collected from the Tibetan plateau at 4,500 meter altitude. This was used as the pollen donor and was crossed with Taiga. Flowers of the Taiga mother plants were emasculated before anthesis, and the Tibet-37 spikes at anthesis were inverted and suspended above them, and allowed to shed pollen freely. Foreign pollen was prevented from entering by a protective sleeve covering the mother and pollen-donor spikes (Fig 3.1). Using a rapid micro-plant growing technique, it was possible to advance to the F_6 generation (145 RILs) within two years. In this technique uniculm plants are raised in cells in trays of dimensions 36 x 22 x 5 cm (L*W*D) 40 cells per tray (Fig 3.3) and these achieved maturity in 75 d instead of 110–120 d.

Twenty two seeds were obtained from the initial cross and all had white testas. F_1 seeds were grown in 1 L pots and from which 17 plants were black seeded and 5 were white seeded. Seeds of black and white plants were bulked separately. The 30 universal white phenotypes of all descendants of the 5 white F_2 generations led me to believe that these may not all be the result of a genuine hybridization cross. In addition there mature plants had phenotypic traits similar to the mother plant ‘Taiga’. Therefore, erring on the side of caution, white seeded plants were excluded from subsequent analyses. In F_2 500 black seeds and 300 white seeds were taken randomly and sown in micro plant trays containing John Ines potting soil No 3. 340 plants were harvested from black F_2 population and 68 plants from

white population. Out of 340 plants 200 were black and 40 were grey. However, white population remained white. In F_3 a single seed was taken from each ear and planted in micro plant trays shown in Fig 3.2 (Acquaah, 2007, 2012). Among the 200 black plants 142 plants were harvested with black colour and 35 plants with grey and 40 grey plants remained grey. From the 68 white plants, 63 plants successfully matured from which seeds were harvested. In the F_4 these plants were planted as single seeds from each ear. At maturity 122 black and 9 grey plants were harvested from black F_4, from grey plants 41 plants were harvested. From the white F_4 population 48 plants were harvested. Similarly these harvested F_5 plants were also planted as single seed method in micro plant trays. The plants harvested as F_6 seed were as under: from 122 black plants 68 black, from grey 47 plants. From the white population 30 plants were harvested. Details are given in Fig 3.2.

During the early generations, (F_2 to $F_{4)}$ seedlings were tested for coleoptile length and shoot length. The method of seedling germination and measuring CL was similar to that used in previous experiments in chapter two. Heritability was calculated in F_2 to F_4 generations for CL. Heritability in the broad sense was calculated according to Acquaah (2007, 2012).

H^2 (B.S.) = $\{VF_2\text{-}1/2(VP_1+VP_2)/F2\}$

Where, VF_2 = variance of F_2, VP_1 = variance of parent 1, VP_2 = variance of parent 2, H^2 (B.S) = broad sense heritability. Observed data were analyzed using SPSS, ver.16.

The degree of genetic advance was calculated for each character in F_2, F_3 and F_4 generations according to Breese (1972).

Genetic Advance (G A) = $K.\sqrt{SD}. H^2$

Where,

SD = standard deviation of phenotypic variance
H^2 = broad sense heritability in fraction.
K = selection intensity.
(The value for K = 1.755 in this study at 10% selection pressure)

From the F_8 generation 104 spring naked barley RILs along with their parents were sown at the Henfaes Research Centre, because not all of the 113 RILs in the F_7 generation produced enough viable seed. Three sowings were done in the field on 20th January, 10th February and 4th March 2011 in a randomized complete block design with three replications. Ten seeds of each line were planted by hand at 50 mm depth. Two I-buttons per replication were placed at 50 mm depth in the soil to record temperature every 3 hours until harvesting.

Once germination was completed, (22-30 days after sowing for SD1, 20-28 d for SD2 and 18-25 d for SD3), plants were carefully excavated from the field per genotype per replication for recording coleoptile length, shoot length and final emergence percentage. The plants were washed with tap water to separate soil from seedlings and coleoptile length was measured with ruler from point of emergence of the coleoptile to its tip (Fig 2.3).

In previous experiments only early maturing 104 F_8 RILs were used. At the time of this experiment from 145 F_8 RILs, 143 were matured and used. Ten seeds of 143 F_8 spring naked barley RILs and two parental varieties were sown in seedling trays (36 cm x 23 cm x 9 cm) size containing, John Innes No 3 potting soil with three replications in cold room at Thoday Building Bangor University. The seed were sown at 50 mm depth. The germination was completed between 20-30 days after sowing. Same traits were recorded as in field experiment. The average cold room temperature was 4^0C. Data recorded were analyzed analysis of variance and descriptive statics with SPSS ver.16.

Fig 3.1 Flowers of Taiga genotypes were emasculated three days prior to pollination. Ears were covered with paper bags to prevent foreign pollens before and after pollination.

Fig 3.2 Recombinant inbred lines (RILs) from a cross of Taiga x Tibet-37 naked barley were generated to F_6 using single seed descent. This flow diagram indicates how many seeds were used in each generation. The descent from the 17 black F_2 generation resulted in 115 F_7 RILS of varying phenotype. (B=Black, G= Grey and W= White).

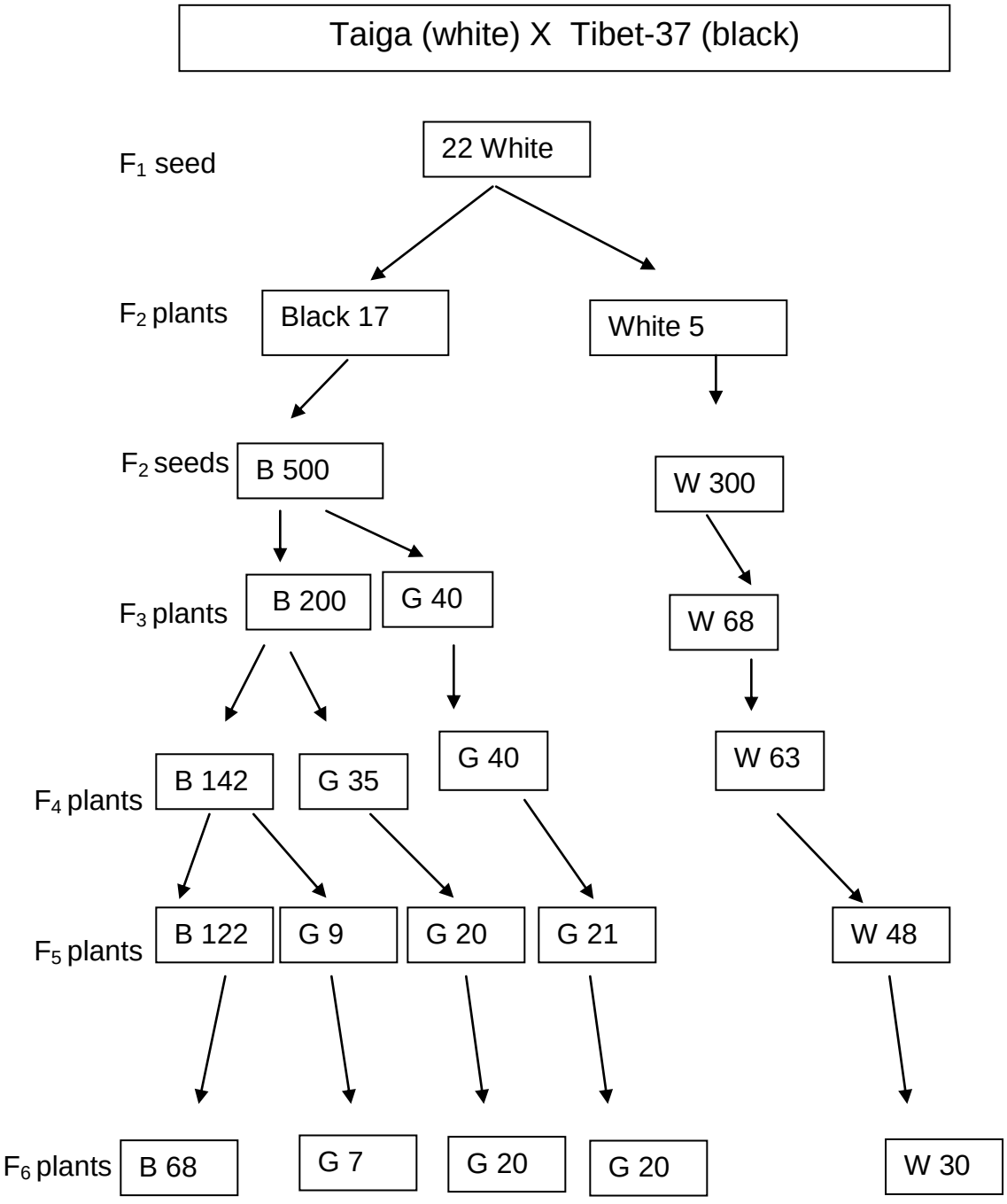
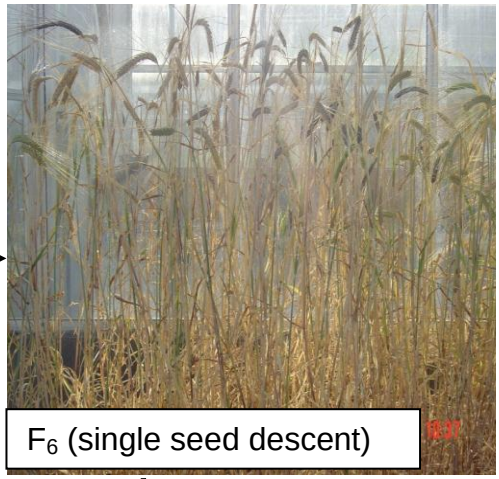
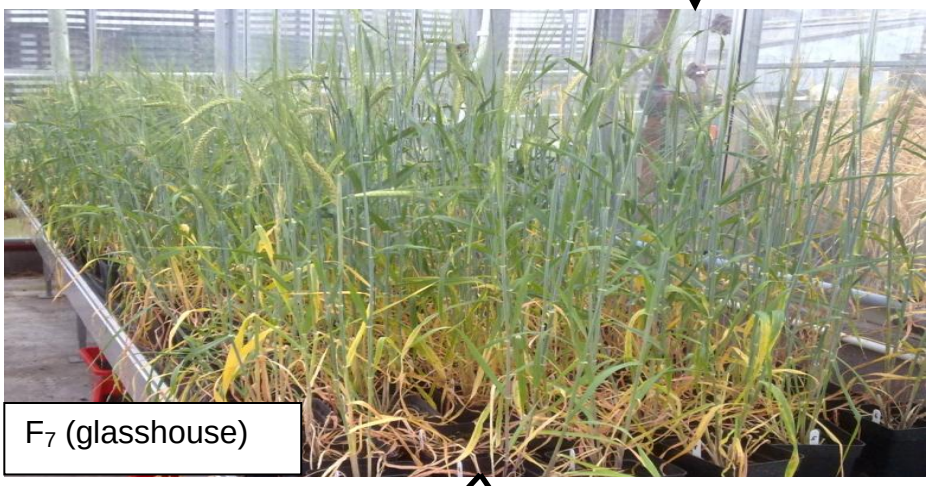


Fig 3.2 Illustrations of various stages in the generation of F_8 RILs from the Taiga X Tibet-37 cross. (See text section 3.3 for details).

F_3 (uniculm microplants)



F_6 (single seed descent)



F_7 (glasshouse)

F_8 (germination. nets to exclude binds)

F_8 (mature plants in field)

3.4 Results

Effect of sowing depth on coleoptile and shoot length in early generations of two naked spring barley Taiga and Tibet-37 cross (Experiment 3.1).

There was a highly significant difference between generations for CL and SL (Table 3.1.1). It was further revealed that means of CL in F_2- F_4 with black testa were higher or nearest to means of male parent Tibet-37. However, the means of the white testa population were more or less similar to their female parent Taiga (Table 3.1.2).

High heritability along with high genetic advance has been observed for black testa generation for coleoptile length (Table 3.1.3).

Table 3.1.1 The dependence of coleoptile length (CL) and shoot length (SL) on seeding depth and (breeding) generation. ANOVA (mean squares) of F_2, F_3 and F_4 generations of naked spring barley along with their parents for sowing at 25 or 50 mm depth in the glasshouse. ** Significant at 0.01 level

Source	DF	CL	SL
Depth	1	41616.42**	38856.14**
Generation	7	2544.827**	54205.13**
Depth * Generation	7	214.386**	21378.7**
Error	1880	23.078	1024.019

Table 3.1.2 The dependence of coleoptile length (CL) and shoot length (SL) on seeding depth and (breeding) generation. Mean performance of F_2, F_3 and F_4 generations spring barley along with their parents for coleoptile length (CL) and shoot length (SL) in two sowing depths (25 and 50 mm) at glasshouse. The results for black and white phenotypes are expressed separately.

	CL (mm)			SL (mm)		
Genotypes	**25mm**	**50mm**	**Mean**	**25mm**	**50mm**	**Mean**
Taiga	**23**	**34**	28	**209**	**151**	**180**
Tibet-37	**30**	**42**	36	**214**	**176**	**195**
F_2B	28	42	35	159	168	163
F_2W	25	34	29	201	204	203
F_3B	30	44	37	169	164	166
F_3W	28	37	32	172	153	162
F_4B	29	42	36	161	171	166
F_4W	22	35	28	141	149	145

Table 3.1.3 Heritability (BS) and genetic advance (GA) for Coleoptile length in F_2, F_3 and F_4 generations. (All data pooled).

Genotype	Taiga	Tibet-37	F_2B	F_2W	F_3B	F_3W	F_4B	F_4W
Variance	43	69	67	65	70	35	60	52
H^2 (BS) %			66	64	69	33	59	51
GA			41	40	42	24	37	34

Effects of different temperature on coleoptile length and seedling emergence of RILs of naked spring barley genotypes in field and cold room conditions (Exp 3.2 and 3.3).

There was highly significant difference between RILs for coleoptile length and emergence percentage ($P<0.01$) (Table 3.2.1). The results showed that there was highly significant variation for CL and EP among Temperature and RILs * temperature (Table 3.2.1).

Heritability estimates were high for coleoptile length (87%) and 56% for emergence percentage (Table 3.2.1). Heritability estimates must accompany a high genetic advance to be reliable. The results indicated that maximum genetic advance was observed in emergence percentage (61%) followed by coleoptile length (42%). High heritability and genetic advance for coleoptile length and emergence percentage indicate the presence of additive gene effects.

The minimum and maximum coleoptile length was 35 and 56 mm in field sowing date 1 and 2 and 38 and 56 mm in sowing date 3 (Fig 3.2.1). For further details see appendix 3.1. The genotype Tibet-37 had the longest CL (56 mm) in all 3 sowing dates, RIL-92 was at lowest position with CL value 35 mm in sowing 1 and 2 and variety Taiga was at the lowest end with CL value (38 mm) in sowing date 3 (Fig 3.2.1). The mean emergence percentage was highest in Tibet-37 (93%, 100% and 96% in SD1, SD2 and SD3 respectively). RIL-78, RIL-98 and RIL-49 were at the lowest end in SD1, SD2 and SD3 respectively) (Fig 3.2.2).

In the case of cold room experiment the minimum and maximum CL was 45 and 65 mm (Fig 3.2.2). Highest CL was observed in Tibet-37 followed by RILs-23 and RIL-49. Lowest CL was Taiga followed by RIL-91(Fig. 3.2.3). In the case of emergence percentage it was revealed that EP ranged between 63-96% (Fig 3.2.3). The highest average EP was observed in Tibet-37 and lowest was in RIL-75 followed by Taiga.

Genotype Tibet-37 showed longest CL (58 mm) in combined four sowing dates and Taiga displayed shortest CL (39 mm) (Fig 3.2.4). Similarly for the trait EP genotype Tibet-37 was on highest EP (96%) and Taiga was on lowest end (58%) (Fig 3.2.4). Highest CL (57 mm) was observed in cold room temperature followed by 3rd field sowing (49 mm) and lowest (44 mm) was in 1st field sowing (Fig 3.2.5). In the case of EP highest (86%) was in cold room ad lowest (53%) in 1st field sowing (Fig 2.2.6).

There was strong correlations between CL and EP (R^2=0.671) in combined three sowing dates in field (Fig 3.2.7). The positive correlation was also observed between CL and EP (R^2=0.320) in cold room (Fig 3.2.8). Significant correlation was observed for CL (R^2=0.397) (Fig 3.2.9) and EP (R^2=0.013) in combined field and cold room experiment (Fig 3.2.10).

Analysis of variance was also performed using seed colour as a factor. It was found from the data that highly significant variation was observed between the

seed colour for all three traits studied (Table 3.2.2). Heritability was highest in CL (99%) and EP and SL (97%). Highest CL was displayed by black seeded RILs (58 mm) followed by grey (55 mm) (Table 2.2.3). EP was highest in black seeded RILs (86%) followed by grey (84%) and lowest in white seeded RILs (67%). Grey seeded RILs showed highest SL (186 mm) followed by black seeded RILs (182 mm).

It was revealed from the data presented in (Table 3.2.4) regarding correlation that, there was highly significant positive correlation between CL and EP (r=0.653), CL with SL (r=0.620) and significant negative association with seed colour (r=-0.849). Highly significant positive correlation was also observed between EP and SL (r=0.611). Significant negative association was also observed between EP and SL with seed colour (r=-0.527 and -0.455 respectively).

Table 3.2.1 The dependence of coleoptile length (CL) and emergence percentage (EP) on different experiments (temperature) of recombinant inbred lines (RILs) grown at 50 mm depth, 3 sowing dates in field (Experiment 3.2) and one sowing date in cold room (Experiment 3.3). (Data from all experiments combined.) ANOVA (mean squares) and heritability values for Coleoptile length (CL) and emergence percentage (EP). ** Significant at p<0.01 level.

Source	DF	CL (mm)	EP (%)
RILs	105	144.91**	678.06**
Experiments (Temperature)	3	9294.65**	66318.78**
RILs*experiments	315	20.62**	353.03**
Error	848	7.66	236.79
V_G		22.06	259.51
V_E		7.66	236.79
V_P		58.51	541.74
H^2 (BS)		87	56
GA		42	61

Table 3.2.2 The dependence of coleoptile length (CL) and emergence percentage (EP) on seed colour of recombinant inbred lines (RILs) grown at 50 mm depth, 3 sowing dates in field (Experiment 3.2) and one sowing date in cold room (Experiment 3.3). (Data from all experiments combined.) ANOVA (mean squares) and heritability values for Coleoptile length (CL) and emergence percentage (EP). ** Significant at p<0.01 level.

Source	DF	CL (mm)	EP (%)
Seed Colour	2	6489.89**	14165.74**
Error	432	6.98	128.61
V_G		2161.01	4679.04
V_E		6.98	128.61
V_P		2167.86	4807.65
H^2 (B.S)%		100	97

Table 3.2.3 A comparison of coleoptile length (CL) and emergence percentage (EP) on seed colour of recombinant inbred lines (RILs) grown at 50 mm depth in cold room (Experiment 3.3). Minimum, maximum and mean values for Coleoptile length (CL) and emergence percentage (EP).

Seed Colour		CL (mm)	EP (%)
Black (n=67)	Mean	58	86
	SEM	0.19	0.81
	Minimum	51	56
	Maximum	68	100
Grey (n=47)	Mean	55	83
	SEM	0.21	1.04
	Minimum	48	56
	Maximum	65	100
White (n=31)	Mean	44	66
	SEM	0.25	0.94
	Minimum	40	56
	Maximum	49	89

Fig 3.2.1 Means value of coleoptile length in 104 F_8 RILs along with their parents in field. 1 graph per sowing date.

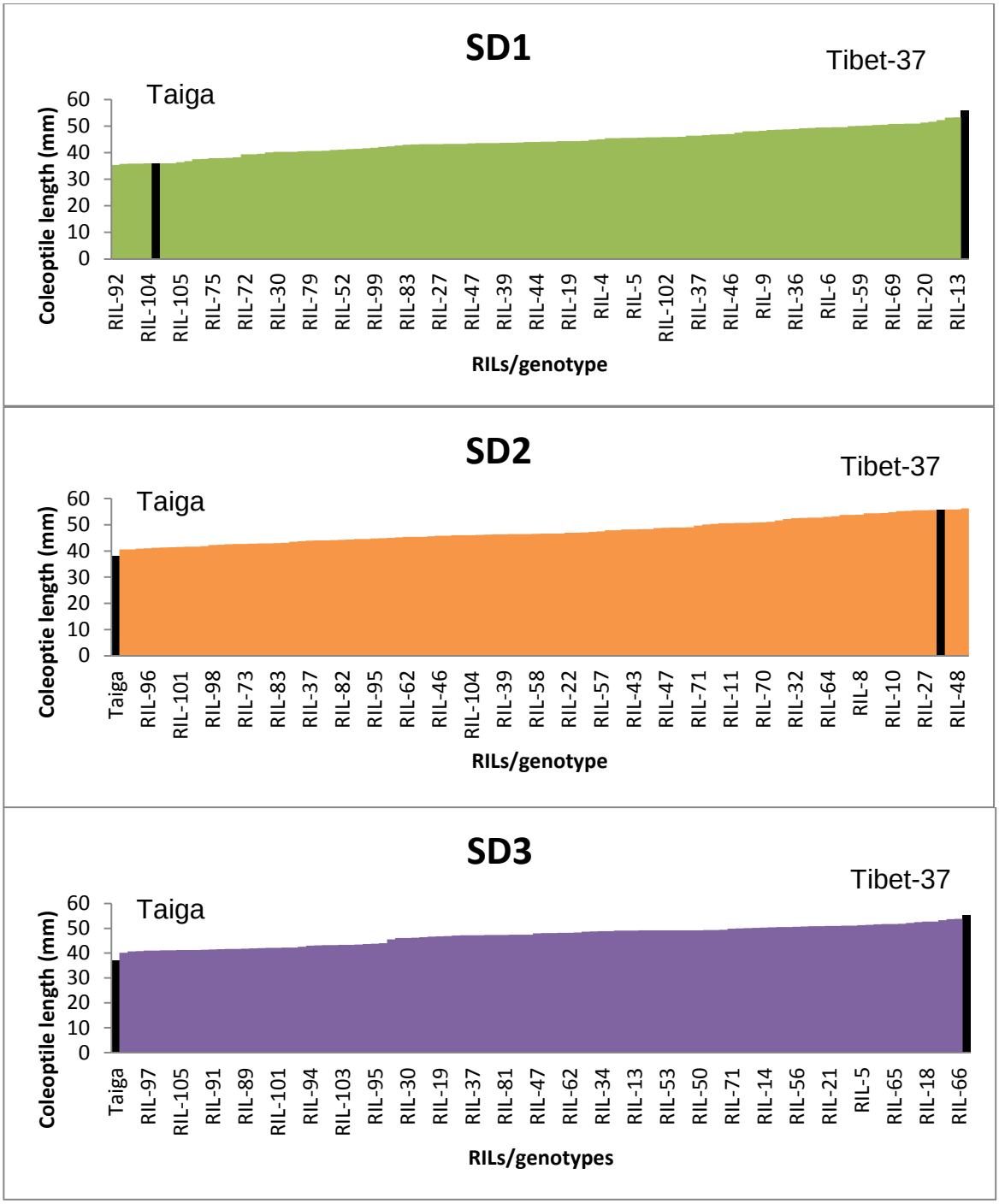


Fig 3.2.2 Means value of emergence percentage in 104 F_8 RILs along with their parents in field 1 graph per sowing date.

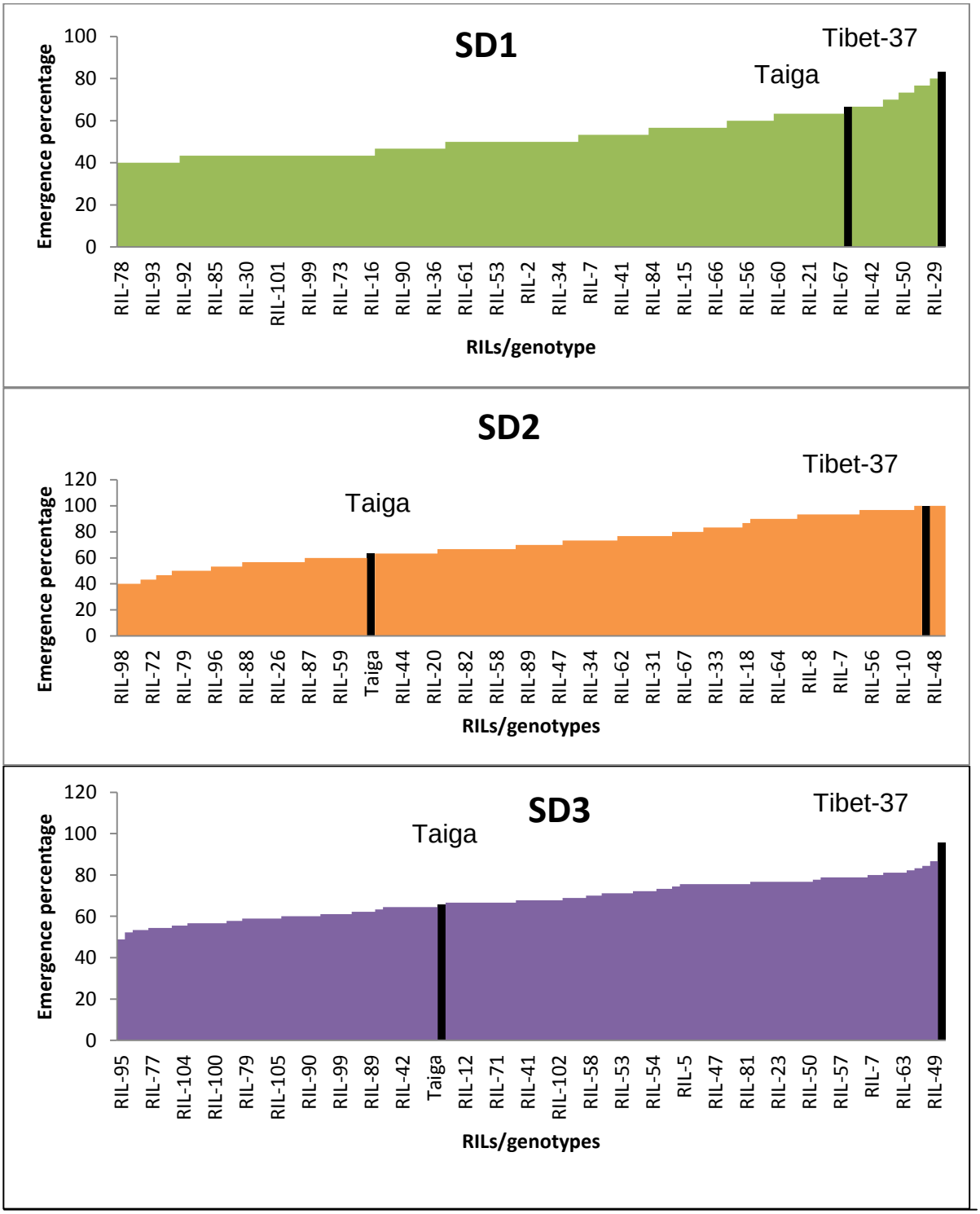


Fig 3.2.3 Mean value of coleoptile length, emergence percentage and shoot length in F_8 RILs along with their parents in cold temperature room.

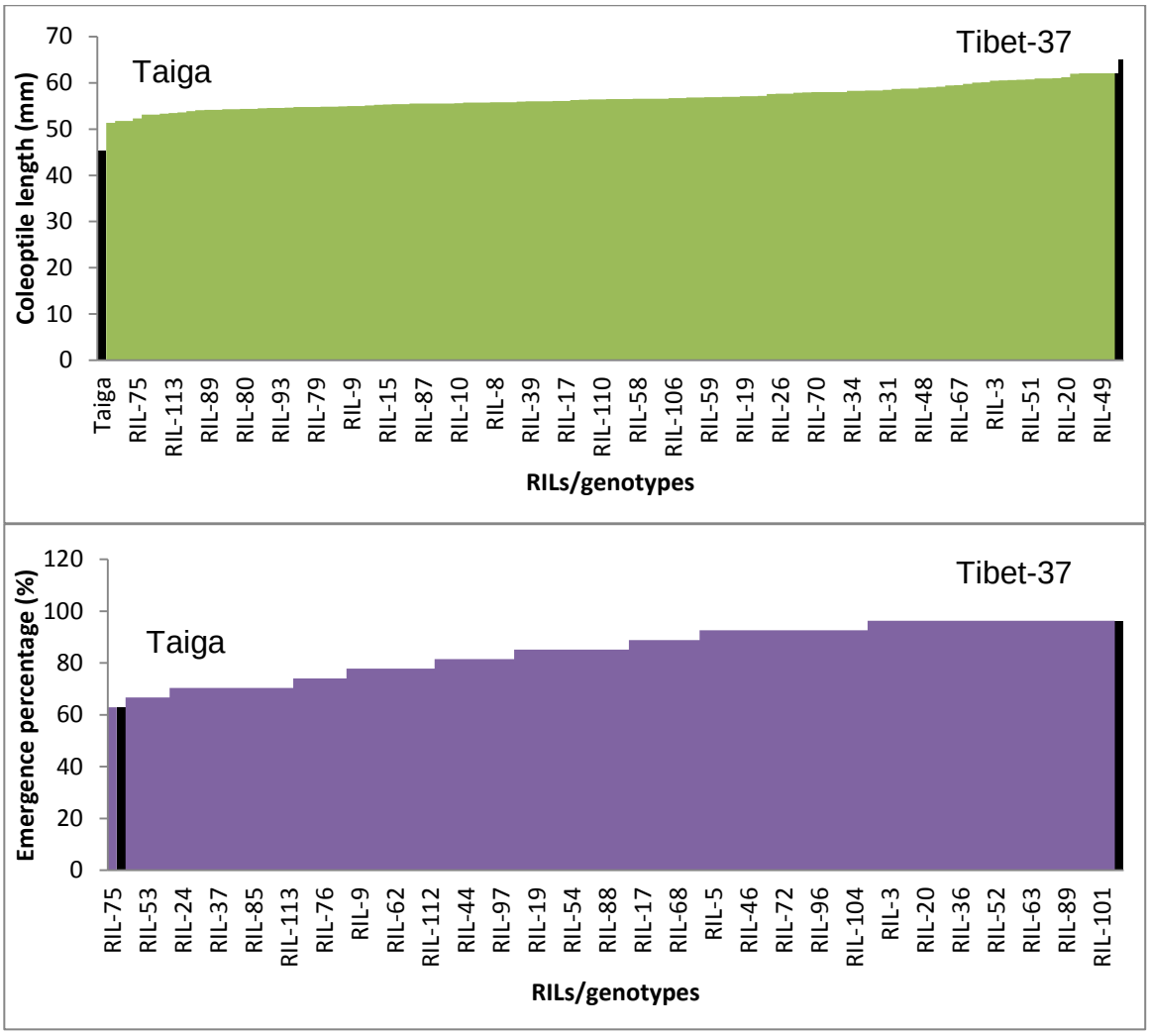


Fig 3.2.4 Mean value of coleoptile length and emergence percentage in F_8 RILs along with their parents in combined (3 field sowing and one cold temperature room).

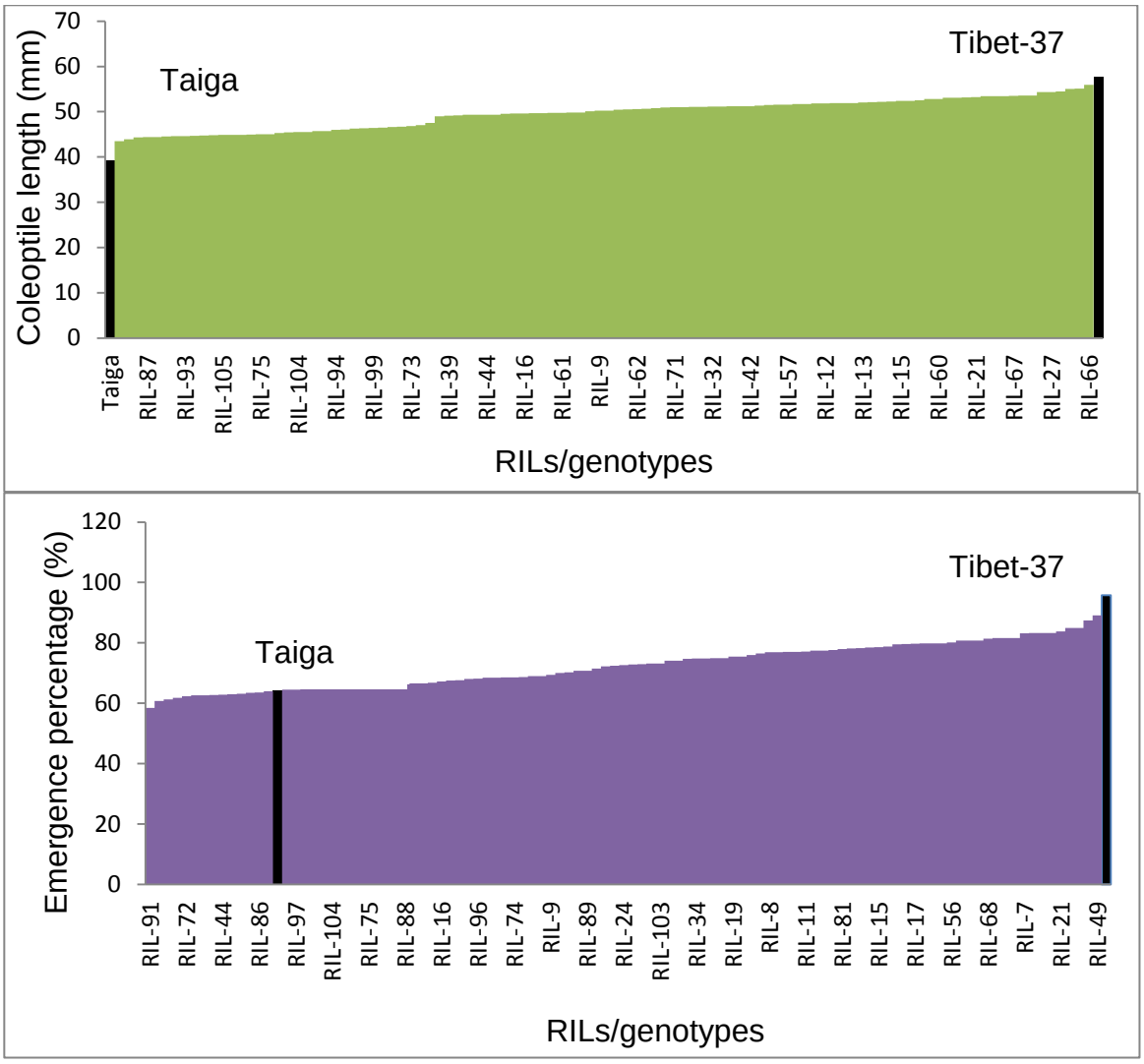


Fig 3.2.5 The effect of temperature (and light) on coleoptile length recombinant inbred lines (RILs) of spring barley. Data pooled from two experiments as described in Table 3.3.1. Mean ± se (n = 108; where error bars are not visible, they are smaller than the symbols). In these experiments, temperature was only one of several environmental variables – see details for experiments 2.2 and 3.2.3. In summary, blue diamond's represent seedlings in field (-2.2 to 6.9°C) (with a day/night rhythm) while red squares represent data from dark "cold" room (4.4°C). Most of these data are means of considerable environmental variation (Fig 3.2. 11). These data show not only a major light dependence of CL, but also, possibly, a temperature dependence (that may be light dependent).

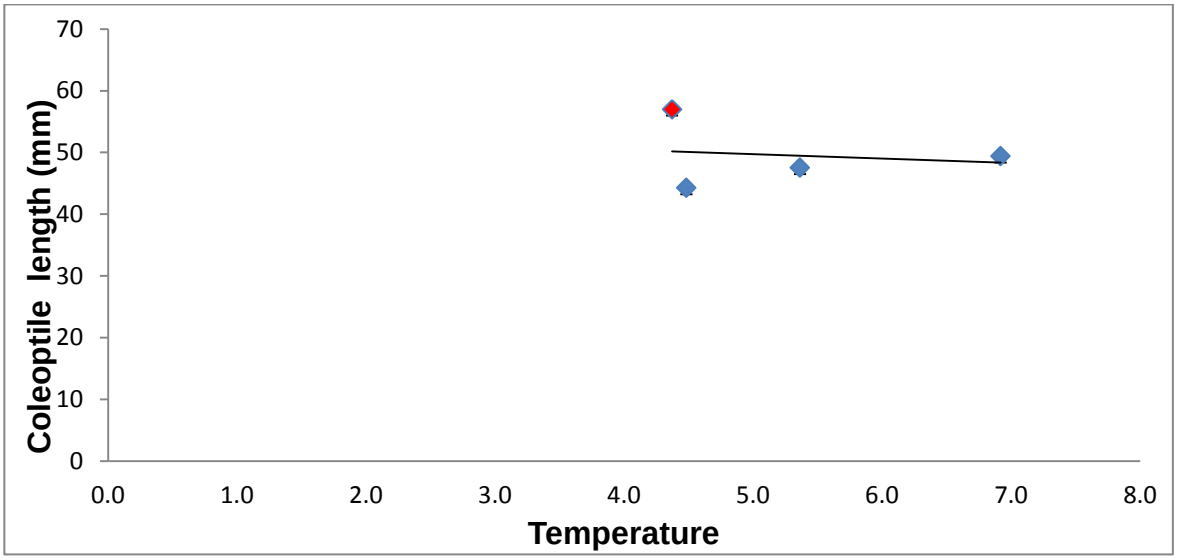


Fig 3.2.6 The effect of temperature (and light) on emergence percentage (EP)on recombinant inbred lines (RILs) of spring barley. Data pooled from two experiments as described in Table 3.3.1. Mean ± se (n = 108; where error bars are not visible, they are smaller than the symbols). In these experiments, temperature was only one of several environmental variables – see details for experiments 3.2 and 3. 3. In summary, blue diamonds represent seedlings in field (-2.2 to 6.9°C) (with a day/night rhythm) while red squares represent data from dark "cold" room (4.4°C). Most of these data are means of considerable environmental variation (Fig 3.2.11). These data show not only a major light dependence of CL, but also, possibly, a temperature dependence (that may be light dependent).

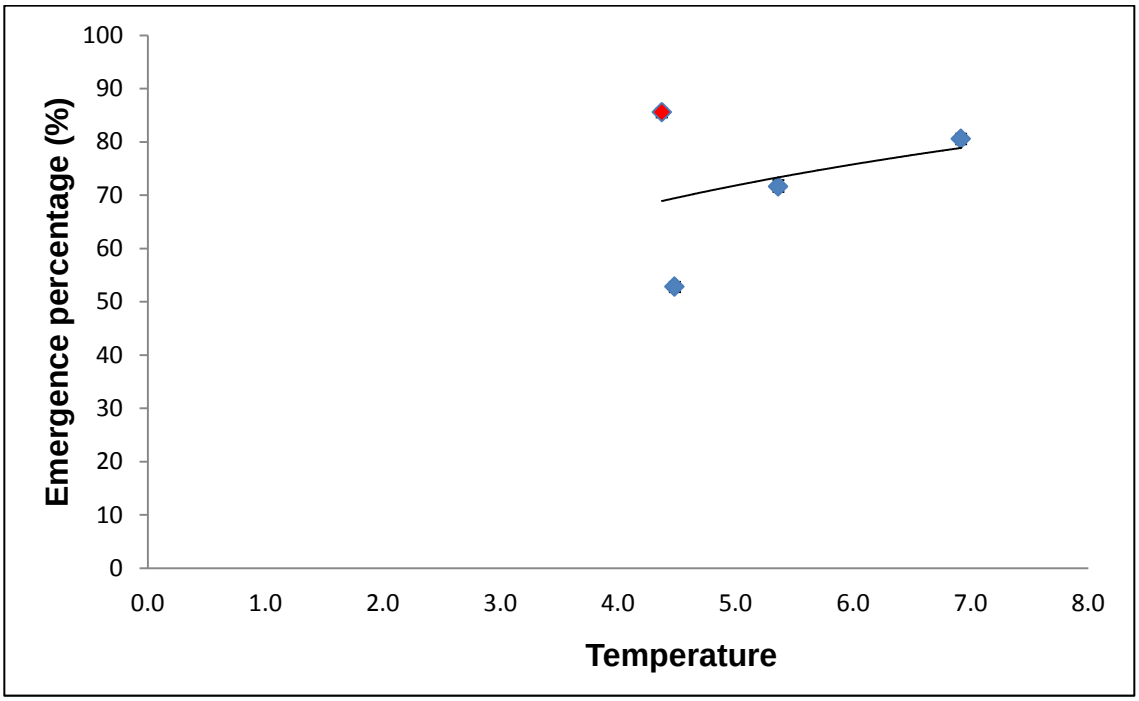


Fig 3.2.7 Correlation of CL and EP between naked barley RILs combined for all sowing dates in field (mean of each genotype (n=3)).

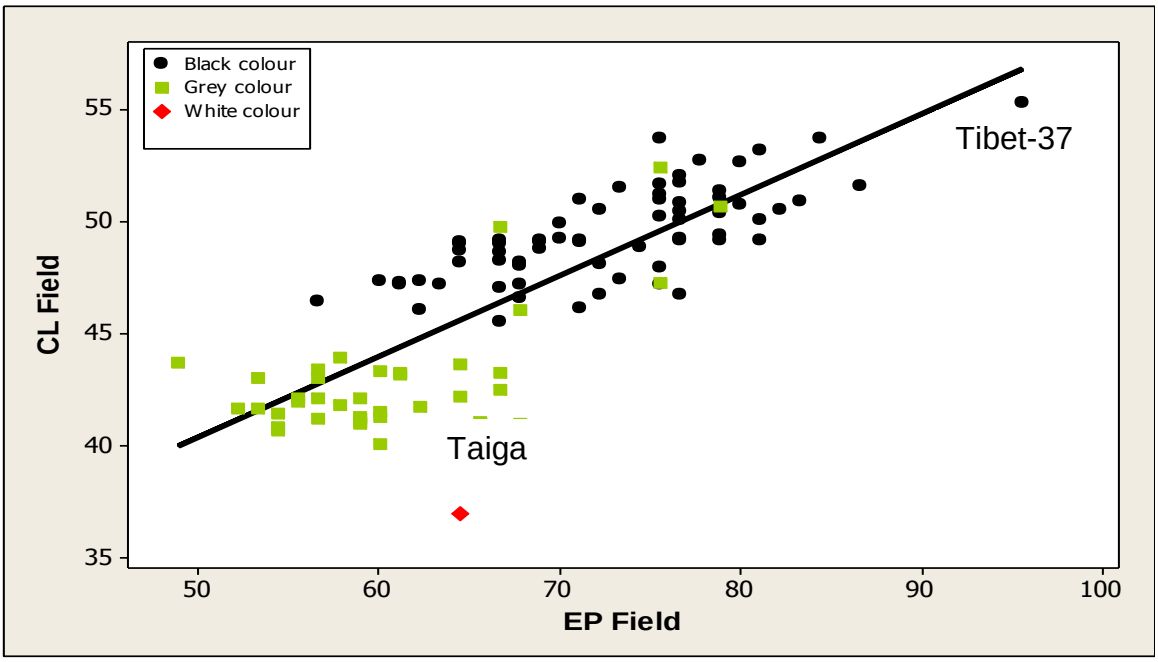


Fig 3.2.8. Correlation of CL and EP between naked barley RILs in cold room. (mean of each genotype).

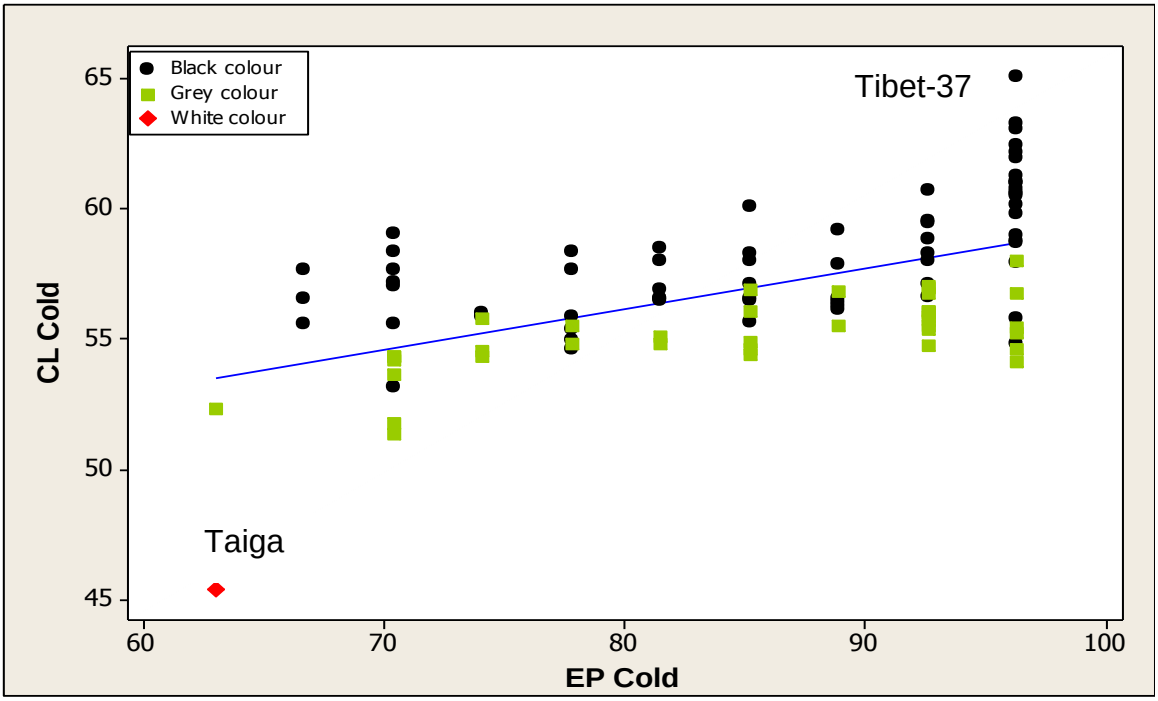


Fig 3.2.9 Correlation of CL between naked barley RILs in field sowing and cold room (mean of each genotype (n=4)).

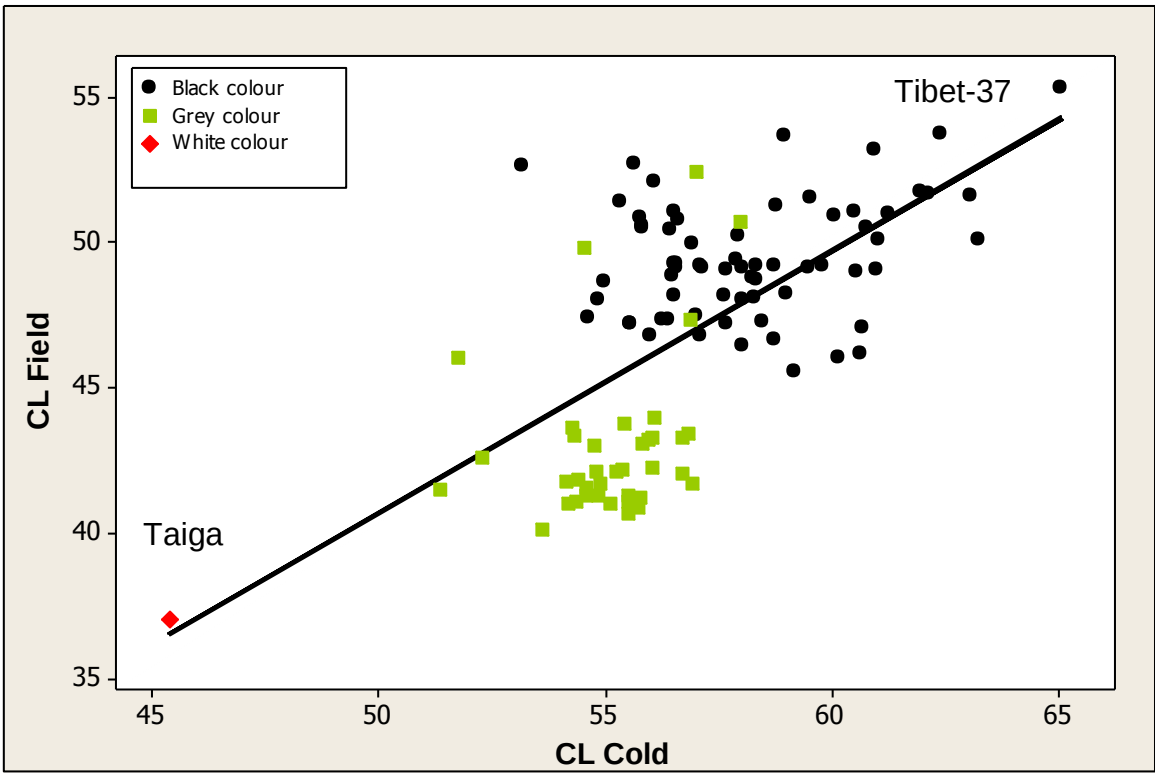


Fig 3.2.10 Correlation of EP between naked barley RILs in field sowing and cold room (mean of each genotype (n=4)).

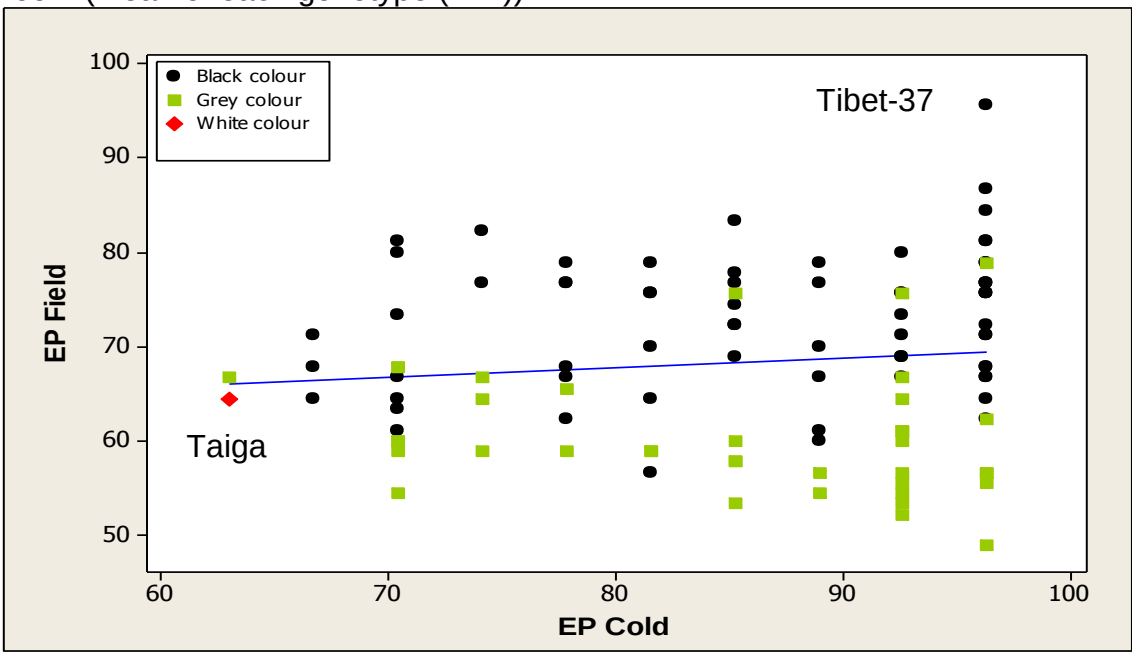
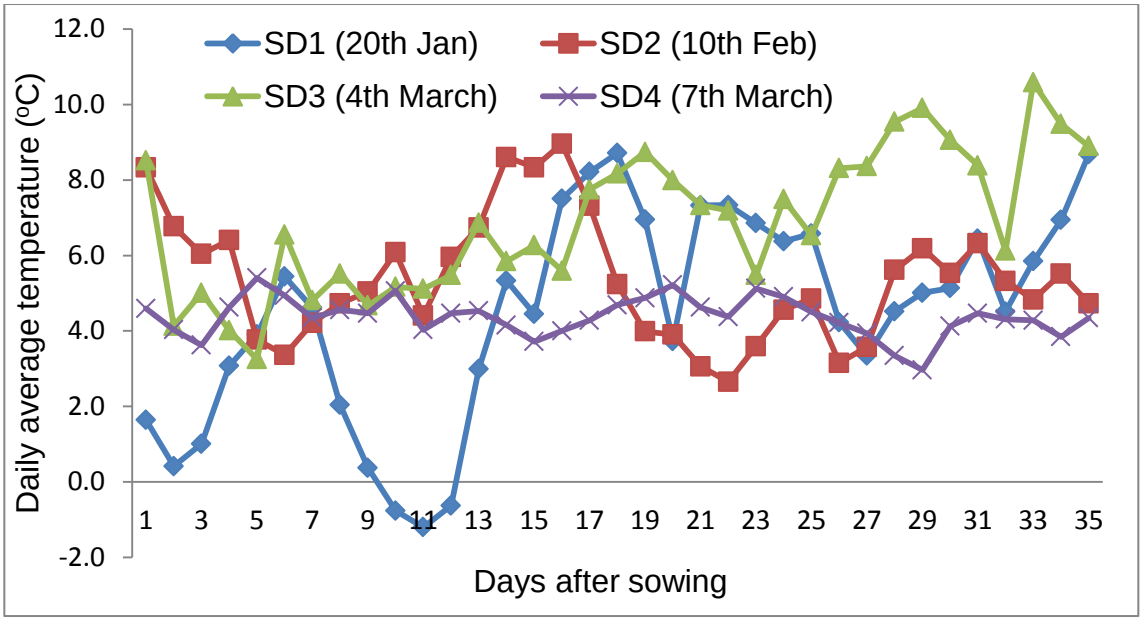


Fig 3.2.11 Average daily temperature for three sowing dates at Henfaes field and one sowing in cold room at Thoday Building, Bangor University (January- April 2011).



3.5 Discussion

There was a wide variation in CL and EP between Taiga and Tibet-37 and their RILs. EP and CL are correlated and heritable and both were associated with seed colour. It was also observed that the RILs were moderately stable in between the sowing dates for these traits. This was because of weather conditions in early sowing date (SD1) soil was frosted.

Coleoptile length showed significant variation between the RILs. The RILs with longer CL showed highest emergence percentage and the most rapid speed of emergence. These findings are agreement with results presented by earlier researchers in barley (Grando and Ceccarelli 1995, Portmann *et al*., 1996, Box *et al*., 1999, Platz *et al*., 1999, Takahashi and Takeda 1999, Takeda and Takahashi 1999 and Takahashi *et al.*, 2001, 2008 and Gulnaz *et al*., 2011). In wheat, varieties with longer coleoptiles generally have faster, more even emergence and improved early vigour (Rebetzke *et al*., 2005).

In the RILs, there was significant correlation between CL, EP and SL. In contrast poor correlation of coleoptile length with plant height was reported by earlier (Awan *et al*., 2007). We can predict that the traits with high heritability and high predicted genetic advance selection for the traits will be effective to develop superior lines for a particular trait.

Significant variation such as in seed colour in Turkey (Akinci and Yildirim, 2009) and Oman barley landraces (Jaradat *et al*., 2004) was observed in earlier research. In the present study, there was a significant variation observed for seed colour in barley RILs associated with CL, EP and SE. There was also significant negative correlation of these traits with seed colour. No evidence had been found in earlier reports of the relationship between seed colour and CL or other seedling traits. This finding is one of the key outcomes of this study.

The white testa trait was pure breeding from the F_2 stage onwards, supporting the view that these were produced by self-fertilization of the white seeded mother variety, Taiga (Figure 3.1). If seed coat colour is determined by the female parent, the effect of segregation does not appear until the F_2 generation. The female parent of this cross was white and the F_1 were all white, implying maternal cytoplasmic inheritance of this trait. The F_2 showed segregation for seed colour of 17 black : 5 white plants. This is not significantly different to the expected 3:1 ratio (Chi squared = 0.06; $P > 0.5$) and is consistent with the hypothesis that black seed colour is dominant over white and the trait is controlled by a single gene. Seeds with a grey colour were observed at the F_3 generation, and this may be due to the effect of another gene. Reciprocal crosses could be carried out to

check segregation ratios in this cross. Thus, in the case of Tibet-37, the black seed coat is a useful genetic marker to indicate whether hybridization has occurred where Tibet-37 is the pollen donor.

Other studies have identified segregation for colour traits in seed tissue other than barley. Two major QTL linked with kernel colour were identified on chromosomes 3(3H) and 5(1H) which explained 13% and 30% of the phenotypic variation, respectively (Gyenis, *et al*., 2007) (Table 3.5.1).

Table 3.5.1 Locations of QTLs for seed/kernel colour in barley RILs

Trait	Chromosome	R^2	Marker with greatest effect	Donor allele (parent)	Reference
Kernel colour	3(3H)	13.0	HvLTPPB	OUH602 alleles	Gyenis, *et al.,* 2007
Kernel colour	5(1H)	30.41	GBM1061	OUH602 alleles	Gyenis, *et al.,* 2007

Chapter 4

4.1 Final Discussion

Development of high-yielding cultivars requires a thorough knowledge of the existing genetic variation for yield and its components. Assessment of the extent of genetic variability within barley is fundamental for barley breeding programs and the conservation of genetic resources, and is particularly useful as a general guide in the choice of parents for breeding hybrids.

Poor establishment leading to lower yields can be a problem in covered (hulled) barley but it is a frequent and more serious problem in naked barley. There are many factors which are considered to be linked with establishment i.e. seed embryo damage during harvesting and seed cleaning, resulting in poor viability and germination. Naked barley has an uncovered embryo, and consequently some of the embryos may be damaged before the seed is planted with the result that germination percentage is affected (Thomson *et al.,* 2009). Besides damage to the grain, the length of coleoptiles, seed size and sowing depth also has a major effect on barley establishment. Cultivars having shorter coleoptile length do not have ability to emerge easily from deep sowing and may cause poor seedling emergence, which may affect crop yield later on.

Seedling vigour is a major breeding target in barley and other crops (Grando & Ceccarelli 1995, Trachsel *et al*., 2010) because it is closely associated with later crop growth and yield (Ellis, 1992). Simple traits such as seedling or plant height, seedling dry weight and kernel weight (Acevedo *et al*., 1991, Regan *et al*., 1992 and Trachsel *et al*., 2010), rapid shoot growth, shoot dry weight and shoot length have been identified as good indicators of a good seedling and early vigour in barley.

Earlier (Turk and Tawaha 2002) reported that barley varieties produced better germination in drought conditions from large seeds. It was further noticed from the previous results that seed size has no significant effect on seedling emergence however; depth of planting showed significant differences on seedling emergence (Tavakoli *et al*., 2008 and Molatudi and Mariga, 2009).

To overcome the causes of poor seedling establishment, a logical progression of this research was undertaken to determine the seedling traits that are most suitable for the regions where poor seedling establishment of barley is a major constraint. Initially nine spring naked land races and genotypes, along with one covered UK variety, were used to evaluate the seedling establishment in glasshouse as well as field. The barley genotypes used had distinct variation in the traits of interest. Then two lines with highly differing traits were crossed in order to study the segregation of these characteristics, and the likelihood of being able to use these in breeding programmes.

Preliminary investigations into the morphology of the coleoptile showed that hull-less barley may produce a shorter coleoptile than covered barleys. In addition, hull-less barley are more susceptible to growing an ‘abnormal’ coleoptile principally when the embryo is damaged. It is possible that a large proportion of variation for coleoptile length is under simple genetic control. Selection for improved coleoptile length in hull-less barley, hence, may perhaps improve establishment.

In this study it was found that cultivars with comparatively longer coleoptiles had higher emergence rates. Significant cultivar x depth and cultivar x temperature interactions indicated that cultivars sometimes responded differently to temperature and depth of planting. Significant genetic variations for coleoptile length existed in this study, suggesting that it will be possible to select for long coleoptile to improve seedling emergence under deep planting conditions.

Naked spring barley genotype Tibet-37 having long coleoptile length showed superior performance in all the traits in initial glasshouse as well as in field experiments in chapter 2 of this study. As we found significant achievements for seedling improvement with higher coleoptile length that was correlated with emergence percentage and speed of emergence.

The coleoptile length plays an important role in stand establishment, mainly in rain-fed Mediterranean environments where cereals are often sown when the soil profile is dry. Deep sowing is able to reduce the risk of germination and subsequent loss of the seedlings if drought immediately follows rainfall (Acevedo & Naji, 1987). In deep sowing CL plays a significant role in germination as well as uniform seedling emergence therefore, this trait may be given full attention while breeding varieties for better seedling establishment in cereals (Fick and Qualset, 1976, Whan, 1976, Rebetzke *et al*., 2007). However, deep sowing might have a negative effect on crop establishment if the coleoptile is shorter to ensure the emergence of the first leaf. Shoot length was significantly affected planting depth as also found by Tavakoli *et al*. (2008).

Coleoptile length showed significant variation between varieties and sowing depth and these results are in accordance with previous results reported by different scientists. Coleoptile length showed significant correlation with speed of emergence, generally varieties with longer coleoptile length showed better establishment compared to shorter coleoptile length. Similar results were also reported by (Paynter and Clarke 2010) who reported significant variation for coleoptile length in hull-less.

These results indicate that germination percentage was affected by sowing depth as well as genotypes in all the experiments. Results are partially in agreement with other studies that barley seedlings emerged more rapidly from large seeds

than from small seeds; however, there were not any differences in overall germination (Ching *et al*., 1977 and Turk and Tawaha 2002).

Regarding the early growth traits presented here, root dry weight and shoot dry weight were significantly affected by genotype and sowing depth. These results are in agreement with the results reported earlier that dry seedling weight was decreased by genotypes and deep sowing reduced the dry shoot weight (Molatudi and Mariga 2009).

After these results, a mapping population was developed by crossing genotypes with diverse origins and seedling traits, Taiga x Tibet-37, particularly for improvement of seedling emergence on the basis of coleoptile length. This was the first population that was specifically developed for establishment improvement in naked spring barley.

In earlier research, morphological markers were detected and well established in different members of Poaceae (grass family) family such as different growth, yield and disease resistance traits are related to markers such as purple hull, purple node, awned panicle and brown pericarp (Kinoshita, 1990).

The population was developed by the single seed descent method. In this, three categories of seed colour were observed (black, grey and white). Black and grey colour of seed turned out to be a useful morphological marker in this population because their colour was donated by male parent. On the other hand

performance of white seeded RILs was similar to the female parent and the conclusion was reached that these were selfs and not hybrids. Thus, testa colour was a useful distinguishing characteristic.

A significant achievement of this work was that the F_8 generation was produced within the time available for this thesis starting from fresh crosses, achieved using uni-culm plants grown in small cell seedling trays. Each generation was harvested about 75 days after sowing. This method has great promise once initial phenotypic selections have been made for rapidly cycling through generations to produce inbred pure lines, from which later field selections can be made.

This rapid cycling through generations meant that the F_2-F_4 generations could be evaluated for seedling traits, and coleoptile length was found to be significantly correlated with emergence percentage and speed of emergence with high broad sense heritability. Later on, F_7 was tested for agronomic traits in the glasshouse. Significant variation was observed for the traits studied data not presented here. F_8 was evaluated for coleoptile length and emergence percentage in three sowing dates under cold, damp conditions in UK field conditions at Henfaes Research Centre and one sowing date in a cold room. Coleoptile length was found to be highly variable and associated with seedling emergence. It was found that black seed RILs showed longer coleoptile length and significantly associated with emergence percentage.

To proficiently exploit genetic resources in plant breeding programs, it is first necessary to define whether useful genetic variation exist in the material and second, to develop the most economical process of introducing the possibly useful genes into commercially acceptable material (Kearsey, 1997). Barley landraces are genetically nearer to existing cultivars than to wild barley. Nevertheless, wide breeding is necessary to accumulate favourable alleles into appropriate linkage blocks (Thomas *et al.,* 1998). Landraces are present worldwide and indicate a source of useful alleles, as they frequently perform more stably above a range of environments than do modern cultivars (Ceccarelli, 1996).

Other work has shown that high genetic variation was reported for the morphological and grain yield traits, indicating barley landraces from Turkey that may be useful in selection and breeding programs (Akgun, *et al.,* 2012). Ethiopian barley landraces also showed significant variation for the traits of interest (Muhe and Assefa 2011). In the current study there was also a wide variability among landraces for most of seedling traits including CL, EP and SE. The availability of genetic diversity existing in these landraces for important seedling, agronomic as well as yield traits, may be transferred in backcrossing programs.

As here, wide crosses were made between land races and varieties. Our results indicated there was a wide variation between agronomic traits among the RILs of the mapping population. These finding should be useful for developing better seedling establishment, naked genotypes and higher yield in barley.

4.2 Conclusions and future work

Significant variations were found among cultivars for all the characters studied indicating possibilities of genetic improvement. The results of this study show that deep planting, in general, adversely affects seedling emergence. Coleoptiles that experience high temperatures during germination would be short, and might fail to reach the soil surface.

It appears possible to select for barley genotypes with long coleoptile length and short sub-crown inter-node. These combinations are desirable to improve emergence and stand establishment under moisture limiting conditions where deep planting is required. Significant cultivar x depth and cultivar x temperature interactions suggest that selection for desirable genotypes will be more successful when carried out under the target environments.

The new mechanism of SSD by uni-culm micro plant has proved significant in reducing the gap between the generations. Hence it is recommended that this might be play an important in development of conventional or double haploid populations which minimize the time of development of pure inbred lines in barley and other cereals.

Nowadays, single nucleotide polymorphism (SNP) markers have become prevalent because of numerous key advantages such as: markers are more

common in the genome and multiplexed SNP genotyping is more efficient and cost-effective than simple sequence repeats (SSR) genotyping (Shahin, *et al*., 2012). SNP chips have been shown to empower rapid probes of the rice genome at different levels of resolution for applications such as genetic diversity analysis, DNA fingerprinting, identification of QTL and linkage mapping and marker-assisted selection. Simultaneously, prosperity of genetic information can facilitate candidate gene location for imperative traits at fine-mapped gene and QTL regions.

During crossing, the linkage between traits may be broken, allowing genetic linkage map to be developed using molecular markers and marker assisted selection. A morphological marker for coleoptile length (black seed colour) has been observed in Taiga X Tibet-37 population. Quantitative trait loci (QTL) analysis must be done to develop genetic linkage map for coleoptile length, emergence percentage and other agro-morphological and yield traits. Genetic analysis could be done for seed colour in Taiga X Tibet-37 RILs either by using single marker analysis (Table 3.3) or by using new SNP technology 384-SNP OPA assay (Illumina BeadXpress platform) (Close, *et al,.* 2009).

Chapter 5

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Appendix

The means of coleoptile length (CL) and emergence percentage (EP) of RILs (F_8) generation in glasshouse and cold room temperature. (SD = sowing date. See Table 3.2 for dates.)

	CL						EP					
Genotype	SD1	SD2	SD3	SD1-3	COLD	SD1-4	SD1	SD2	SD3	SD1-3	COLD	SD1-4
RIL-1	51	51	51	51	56	52	57	80	93	77	96	82
RIL-2	46	48	50	48	55	50	50	60	93	68	96	75
RIL-3	47	52	54	51	60	53	50	93	93	79	96	83
RIL-4	45	47	50	47	61	50	50	57	93	67	93	73
RIL-5	46	56	52	51	59	53	53	93	80	76	93	80
RIL-6	49	52	52	51	57	52	67	80	80	76	81	77
RIL-7	44	55	53	51	57	52	53	93	93	80	93	83
RIL-8	44	54	53	51	56	52	50	93	87	77	78	77
RIL-9	48	45	53	49	55	50	57	50	93	67	78	69
RIL-10	50	55	54	53	56	53	57	97	80	78	85	80
RIL-11	44	51	52	49	56	51	60	73	90	74	85	77
RIL-12	45	50	52	49	60	52	43	73	83	67	96	74
RIL-13	53	45	49	49	61	52	60	57	77	64	96	72
RIL-14	43	56	52	50	58	52	53	93	80	76	96	81
RIL-15	46	56	52	51	55	52	57	100	80	79	78	79
RIL-16	46	49	48	47	56	50	43	50	87	60	89	67
RIL-17	51	54	51	52	56	53	50	93	87	77	89	80
RIL-18	53	53	52	53	53	53	73	87	80	80	70	78
RIL-19	44	46	50	47	57	49	47	77	93	72	85	75
RIL-20	51	49	53	51	61	54	70	63	80	71	96	77
RIL-21	46	54	53	51	60	53	63	97	90	83	85	84

	CL						EP					
Genotype	SD1	SD2	SD3	SD1-3	COLD	SD1-4	SD1	SD2	SD3	SD1-3	COLD	SD1-4
RIL-22	44	47	52	47	55	49	50	57	80	62	78	66
RIL-23	43	54	54	50	63	53	60	90	80	77	96	82
RIL-24	44	49	50	47	57	50	57	83	80	73	70	73
RIL-25	50	46	49	48	59	51	57	60	83	67	70	68
RIL-26	44	46	51	47	58	50	43	57	90	63	70	65
RIL-27	43	56	57	52	62	54	50	90	90	77	96	82
RIL-28	49	45	54	49	58	51	63	67	77	69	85	73
RIL-29	48	48	52	49	57	51	80	70	80	77	85	79
RIL-30	40	47	51	46	60	50	43	60	83	62	96	71
RIL-31	46	46	50	47	58	50	67	77	83	76	81	77
RIL-32	42	53	52	49	58	51	43	67	83	64	70	66
RIL-33	50	47	51	49	59	52	53	83	77	71	93	76
RIL-34	49	48	49	49	58	51	50	73	83	69	93	75
RIL-36	49	46	52	49	61	52	47	63	90	67	96	74
RIL-37	46	44	51	47	56	49	53	53	77	61	70	63
RIL-38	44	45	48	46	59	49	50	67	83	67	89	72
RIL-39	44	46	50	47	56	49	77	63	90	77	74	76
RIL-41	47	46	49	47	56	49	53	73	77	68	67	68
RIL-42	48	47	52	49	58	51	67	57	70	64	67	65
RIL-43	45	48	51	48	57	50	63	60	70	64	81	69
RIL-44	44	44	51	46	58	49	43	63	63	57	81	63

	CL						EP					
Genotype	SD1	SD2	SD3	SD1-3	COLD	SD1-4	SD1	SD2	SD3	SD1-3	COLD	SD1-4
RIL-45	46	46	50	47	56	50	50	63	70	61	89	68
RIL-46	47	46	55	49	57	51	63	53	90	69	93	75
RIL-47	44	49	52	48	58	51	67	70	90	76	93	80
RIL-48	49	56	56	54	59	55	63	100	90	84	96	87
RIL-49	45	55	54	52	63	55	77	93	90	87	96	89
RIL-50	52	46	50	49	58	52	73	67	90	77	78	77
RIL-51	47	51	54	51	61	53	50	90	77	72	96	78
RIL-52	41	48	51	47	59	50	43	70	90	68	96	75
RIL-53	44	51	53	49	57	51	50	73	90	71	67	70
RIL-54	42	49	53	48	58	51	43	83	90	72	85	75
RIL-55	43	53	52	49	57	51	63	90	90	81	70	78
RIL-56	49	51	53	51	56	52	60	97	90	82	74	80
RIL-57	44	47	56	49	59	52	50	97	90	79	96	83
RIL-58	49	47	53	49	57	51	53	67	90	70	89	75
RIL-59	50	47	53	50	57	52	60	60	90	70	81	73
RIL-60	43	53	54	50	61	53	63	97	83	81	96	85
RIL-61	43	45	51	46	61	50	50	83	80	71	96	77
RIL-62	47	45	52	48	58	51	47	77	80	68	78	70
RIL-63	50	54	55	53	61	55	53	100	90	81	96	85
RIL-64	43	53	55	50	56	52	57	90	90	79	81	80
RIL-65	46	54	55	52	62	54	53	93	80	76	96	81

	CL						EP					
Genotype	SD1	SD2	SD3	SD1-3	COLD	SD1-4	SD1	SD2	SD3	SD1-3	COLD	SD1-4
RIL-66	51	56	55	54	62	56	57	97	73	76	96	81
RIL-67	52	50	52	52	59	54	63	80	77	73	93	78
RIL-68	44	51	53	49	58	52	63	90	83	79	89	81
RIL-69	51	53	54	52	57	54	47	97	83	76	93	80
RIL-70	49	51	53	51	58	53	70	83	83	79	96	83
RIL-71	51	50	49	50	55	51	50	67	83	67	74	69
RIL-72	39	41	44	42	57	46	40	43	73	52	93	62
RIL-73	43	43	44	43	57	47	43	67	60	57	89	65
RIL-74	38	43	43	41	56	45	47	60	90	66	78	69
RIL-75	38	46	44	43	52	45	40	77	83	67	63	66
RIL-76	41	46	45	44	54	46	43	77	73	64	74	67
RIL-77	36	41	46	41	56	45	47	43	73	54	93	64
RIL-78	36	43	45	41	54	44	40	73	90	68	70	68
RIL-79	41	42	44	42	55	45	43	50	83	59	81	65
RIL-80	38	41	45	41	54	44	43	57	77	59	70	62
RIL-81	50	47	45	47	57	50	60	77	90	76	85	78
RIL-82	40	44	42	42	56	46	50	67	77	64	93	71
RIL-83	43	43	46	44	56	47	43	40	90	58	85	65
RIL-84	40	41	45	42	54	45	57	63	53	58	85	65
RIL-85	36	42	42	40	54	43	43	53	83	60	70	63
RIL-86	36	43	45	41	55	45	43	63	70	59	78	64

	CL						EP					
Genotype	SD1	SD2	SD3	SD1-3	COLD	SD1-4	SD1	SD2	SD3	SD1-3	COLD	SD1-4
RIL-87	36	42	45	41	56	44	43	60	60	54	89	63
RIL-88	41	41	43	42	55	45	47	57	77	60	85	66
RIL-89	38	43	45	42	54	45	43	70	73	62	96	71
RIL-90	41	44	45	43	54	46	47	60	73	60	70	63
RIL-91	37	43	45	41	51	44	47	63	53	54	70	58
RIL-92	35	44	46	42	55	45	43	57	60	53	85	61
RIL-93	38	41	44	41	55	45	40	47	83	57	96	67
RIL-94	41	43	45	43	55	46	43	40	77	53	93	63
RIL-95	44	45	43	44	55	47	40	47	60	49	96	61
RIL-96	41	41	42	41	56	45	50	53	77	60	93	68
RIL-97	38	42	43	41	55	45	40	70	67	59	81	65
RIL-98	39	42	44	42	57	46	40	40	87	56	96	66
RIL-99	42	44	44	43	56	46	43	67	73	61	93	69
RIL-100	42	43	44	43	56	46	43	50	77	57	93	66
RIL-101	41	42	44	42	55	45	43	50	77	57	96	67
RIL-102	46	48	44	46	52	47	57	70	77	68	70	68
RIL-103	40	44	46	43	56	46	43	67	90	67	93	73
RIL-104	36	46	45	42	55	45	43	73	50	56	93	65
RIL-105	36	45	42	41	56	45	40	80	57	59	74	63
RIL-106	40	45	44	43	57	47	43	77	63	61	93	69
Tibet-37	56	56	55	55	65	58	93	100	93	96	96	96
Taiga	36	38	37	37	45	40	67	63	63	64	63	64