WAGENINGEN UR

The influence of climatic factors on resistance of barley to different rust species

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PLANT BREEDING WAGENINGEN UR

Abstract

In this study, a hypothesis that climatic factors might be effective on resistance of barley to rust fungi was proposed. Two experiments were set up to prove this hypothesis.

In the first experiment, barley accessions were collected from Evolution Canyon (EC). There are two opposite slopes with different climate conditions in EC: African slope (AS) and European slope (ES). AS stands for hot and dry environments, and ES stands for wet and cool environments. It was assumed that accessions from ES might be more resistant to rusts because of natural selection. Accessions were inoculated with eight rust species including five non-host resistance rusts (*P. persistens*, *P. hordei-murini*, *P. hordei-secalini*, *P. graminis*. f. sp. *lolii* and *P. triticina* Swiss) and three partial resistance rusts (*P. hordei* isolate, *P. striiformis* f. sp. *hordei* and *P. graminis* f. sp. *tritici*). The results showed that accessions from ES were more resistant than from AS to four rusts (. *hordei-secalini*, *P. triticina* Swiss, *P. hordei-murini* and *P. striiformis* f.sp. *hordei*). The preliminary results that obtained in this study could be used as a first indication and as reference for repeat experiment. The results indicated that the hypothesis might be true.

In the second experiment, barley accessions were collected by the Focused Identification of Germplasm Strategy (FIGS). The plants were inoculated with *P. persistens*. In a previous study, the same plant materials were tested with *P. triticina*. It showed that barley accessions from cool and wet environments were more resistant than accessions from hot and dry environments to *P. triticina*. In this study, the results indicated that distributions of barley accessions susceptibility to *P. triticina* and to *P. persistens* were associated. Therefore, the eco-geographic distribution of both *P. triticina* and *P. persistens* resistance in barley was not random but was linked to climatic factors. It could be assumed that barley accessions from cool and wet environments would be more resistant than accessions from hot and dry environments to *P. persistens*.

Key words: Barley, *Hordeum vulgare*, Nonhost resistance, Plant resistance, *Puccinia*, Rusts, Evolution canyon, Climatic factor, Environment factor

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1 Introduction

The aim of this thesis is to study the contribution of climate and environmental factors on barley resistance level to different heterologous rusts. In this report, firstly basic principles of plant defence and definition of nonhost resistance will be described; secondly the research background will be given.

1.1 Basic concepts of plant defence

Plants are continuously suffering from countless stress factors. Plant pathogens are one of the main stress factors causing disease and subsequent symptoms in plants. *Puccinia* rust (Basidiomycota, Uredinales, Pucciniaceae) is an obligate fungus that can form haustoria inside plant cells. Rust fungi can infect plants successfully by negating all potential defence mechanisms in the plants and absorbing nutrients of the plants (Figure 1).

Plants can protect themselves through a multi-layered defence (da Cunha, McFall et al. 2006), containing physical (wax layers, cell wall structures etc.), chemical preformed barriers (phenols, alkaloids etc.) (Nuernberger and Lipka 2005) and induced defence (apposition, papilla etc.) (Wolter, Hollricher et al. 1993). The induced defence is activated by pathogen-associated molecular patterns (PAMPs) perception, the recognition of essential compounds of the pathogen. PAMP-triggered immunity (PTI) is induced upon the recognition of PAMP (Chisholm, Coaker et al. 2006).

PAMPs involved in host and related non-host pathogens are similar or even the same. Therefore, PTI in host plants can also be triggered by host pathogens, but are suppressed within hours (Caldo, Nettleton et al. 2006, Truman, Zabala et al. 2006). The host pathogens are able to suppress PTI by excreting conserved proteins, named effectors. Effector-Triggered Susceptibility (ETS) is induced upon the suppression of PTI by the effectors. However, Effector-Triggered Immunity (ETI) is able to be activated by the recognition of avirulence (*Avr*) genes of the pathogens by R-genes in the plant. The ETI therefore becomes an elicitor of resistance, initiating a hypersensitive response (HR) in the plant (Figure 2).

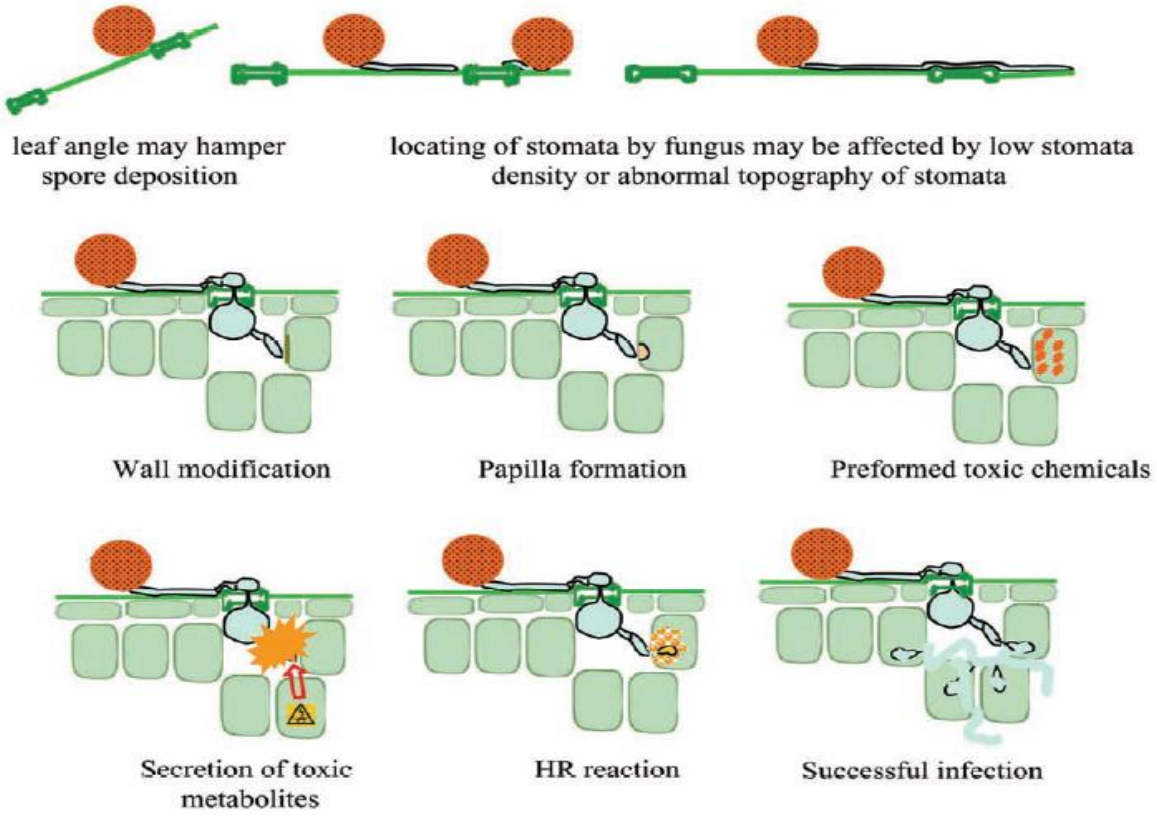

Figure 1. Scheme illustrating the summary of performed and inducible defence responses in plants that are potentially involved in non-host resistance. Plants may have one, two or more defensive features against non-host pathogens. Successful infection occurs when a pathogen can negate all potential defense mechanisms. Adapted from Jafary (2006).

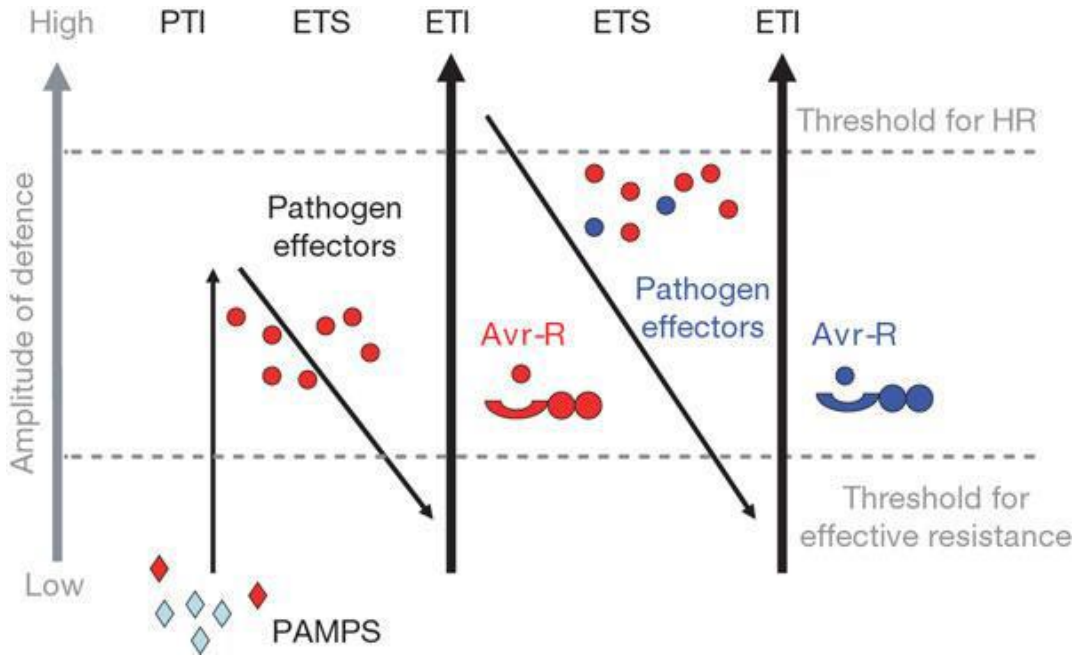

Figure 2. Schematic "zig-zag" overview of plant defence. The plant is able to recognize the pathogen, leading to immunity. However, the pathogen is able to evade or suppress the defence response by secreting effectors. (Jones and Dangl 2006).

1.2 Nonhost and near-nonhost resistance

Nonhost resistance is the most common, complete and durable type of immunity of plants to potential pathogens (Heath 2000). The majority of plant pathogens can only infect a few or even one plant species or genera (Jafary 2006). If all individuals within one plant species are resistant to a certain parasite or pathogen species, this plant species is a nonhost to this specific parasite or pathogen species (Heath 1981). Heterologous (= inappropriate or nonhost) pathogens refer to the pathogen species that are unadapted to a particular plant species (Atienza, Jafary et al. 2004).

However, it has been shown that some heterologous pathogens can somewhat infect some genotypes of nonhost plant species under special conditions (Niks 1987). For example, some barley accessions are susceptible in the seedling stage to wheat leaf rust fungus (*Puccinia triticina*) and the wall barley leaf rust (*Puccinia hordei-murini*) (Niks 1987, Zhang, De la Rosa et al. 1994, Hoogkamp, Chen et al. 1998). Therefore, near nonhost (=intermediate host) status has been proposed, when only few accessions show only moderate susceptibility to a normally heterologous pathogen (Niks 1987).

Nonhost resistance is proposed to be a multi-component phenomenon (Heath 2001). The resistance of most plants to most potential pathogens results from a continuum of layered defences (Heath 2003, da Cunha, McFall et al. 2006), including physical and chemical preformed or constitutive factors. The potential pathogens should deal effectively with the defence that plant species count against unadapted microbial intruders (Niks and Marcel 2009). Nonhost resistance to haustorium-forming specialized pathogens is based on poor haustorium formation on nonhost status, which is termed pre-haustorial resistance (Heath 2002, Collins, Niks et al. 2007, Hardham, Jones et al. 2007). After the would-be pathogens negating physical and chemical barriers, they may be recognized at the plasma membrane of plant cells (Jafary 2006). The mechanism of nonhost resistance is that callose deposits are formed (Perumalla 1989) and phenolic compounds or silica are deposited on the cell wall (Heath and Stumpf 1986) when the plant is infected by heterologous pathogens. The plant species are full host species to the pathogen when all or a majority of individuals within the plant species are susceptible to the pathogen.

1.3 Barley-*Puccinia* model system

The study of genetics of nonhost resistance normally requires interspecific crosses. However, the progeny that is obtained from crosses between host and nonhost species suffers often from sterility and abnormal segregation. It hinders to identify individual genetic factors. Barley (*Hordeum vulgare*) is an ideal model species for nonhost resistance studies. Comparing with other cereals such as wheat, barley is a diploid crop with a quite simple. Meanwhile, barley is nearly nonhost to some heterologous rust species, and rare individuals are susceptible to some heterologous rusts (Figure 3) (Atienza, Jafary et al. 2004). Therefore, "Barley-*Puccinia*" model is a good model for inheritance studies of nonhost resistance. This model system was developed to study the inheritance of nonhost resistance, and the specificity and diversity of genes involved in nonhost resistance.

In previous studies, it was reported that the climatic factors would have an effect on natural selection for some crop traits (Spieth 1979, Epperson 1990). For example, because of eco-climatic factors, accessions originated from sites with high pressure of powdery mildew are more resistant to powdery mildew (Paillard, Goldringer et al. 2000). Thus, it can be assumed that environmental factors influence resistance of barley to rust fungi. In this Msc thesis, two experiments are set up to prove this hypothesis; the details of the projects will be described below.

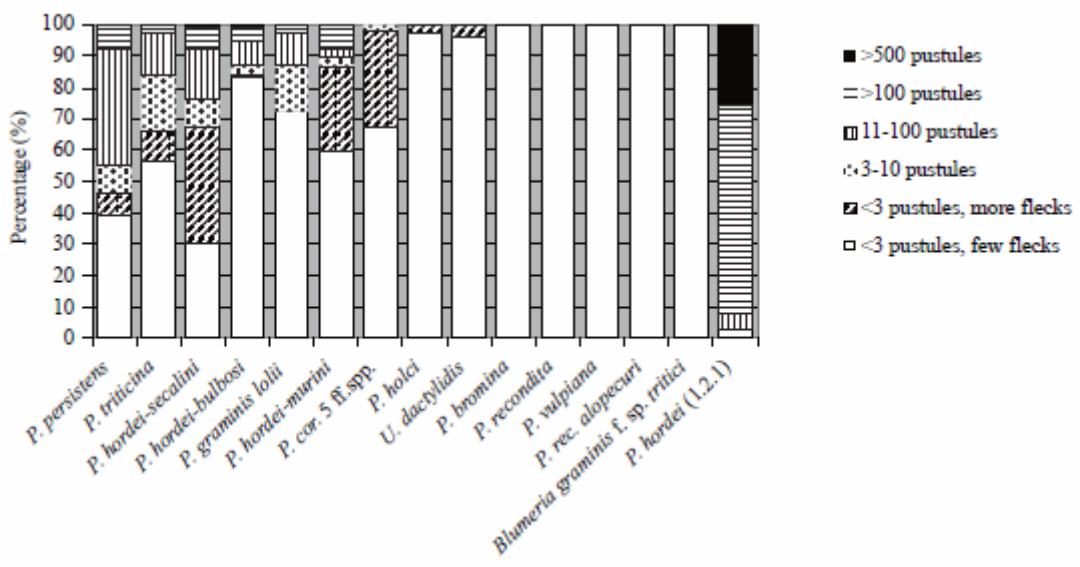

Figure 3. Percentage of barley accessions (n= 110) per susceptibility class for 13 heterologous rust fungi, the wheat powdery mildew and the barley leaf rust (*P. hordei*), determined at the seedling stage (Atienza, Jafary et al. 2004).

1.4 Near nonhost resistance of barley accessions in Evolution Canyon

Evolution Canyon (EC) model is used as a natural laboratory for studying local and global macrocosmic ecological theatres across life (Nevo 1995, Nevo 1997, Nevo 2001). In Evolution Canyon, diverse prokaryotes and eukaryotes share the same rock, soil and macroclimatic background, but the micro-ecology is sharply subdivided (EC). There are two opposite slopes with different climate conditions in EC: African slope (AS) and European slope (ES) (Figure 4). AS stands for the south-facing slope, and ES stands for the north-facing slope in the canyon. The solar radiation in the AS at EC in Mount Carmel is 200%-800%

higher than that of the ES (Pavlícek, Sharon et al. 2003). The higher solar radiation leads to higher temperature and drought stress in AS. On the contrary, the environment in ES is wet, cool and temperate (Pavlícek, Sharon et al. 2003). Therefore, biodiversity divergence across 200 meters between AS and ES displays global divergent patterns (Nevo, Fu et al. 2012)

The sessile, predominantly inbreeding plant wild barley, *H. spontaneum*, in seven stations at EC I (Figure 4), was tested genotypically and phenotypically. It was found that there were significant inter- and intraslope diversity differences, with a striking genetic distance between the mid-slope stations on opposite slopes (*D*=0.481). The distance of these barley populations across 200 metres, and the genetic distance is the same as the distance between the *H. spontaneum* populations which are separated by 100km. The results also suggested that local, regional and global adaptive patterns could be generated by ecological stress (Nevo 2006).

Figure 4. Wild barley (*Hordeum spontaneum*) in "Evolution Canyon". The Nei genetic distance, *D* = 0.481, is based on SSRs from nuclear DNA. Source: Nevo et al., 2005.

In previous researches, behavioural traits and genetically adaptive complex strategies to cope with the mesic ES and those to cope with xeric AS in wild barley were compared (Lavie, Stow et al. 1993, Gutterman and Nevo 1994). There are large genetic distance between interslope plants in wild barley (Figure 5) and extremely interslope physiological divergence is existed in patterns of germination (Gutterman and Nevo 1994).

Thus, micro climatic selection of wild barley drives both interslope adaptive radiation and incipient sympatric speciation in EC I. It was also proven by Nevo that microclimatic selection is more important than migration and genetic drift on genotypes and phenotypes of organisms. Generation of appropriate evolutionary genotypes and phenotypes can be forced by ecological stress in microclimatic and macroclimatic environment. (Nevo 2006).

Meanwhile, as rust pathogens prefer wet, temperate, cool-mesic ES ecology compared with dry, high solar radiation, warm-xeric AS ecology, so rust disease can be more serious on ES than on AS in EC I. Therefore, accessions in ES are bearing more pathogen invasion. It may lead to accessions form ES having stronger immune system, so these accessions are more resistant to heterologous rusts than accessions from AS. It can be assumed that plants on ES are more resistant to rust pathogens than those on AS in EC I because of selection of pathogen pressure.

In this case, nonhost resistance to different rust species in wild barley on AS and ES should be compared to prove this hypothesis. There are five stations based on the sites in ECI: #1 and #2 stations on the AS, #4-5 on the valley bottom, and #6-7 and #7 on the ES (Figure 6). Thus, the

hypothesis can be substantiated with the result that resistance of barley accessions from ES is significantly higher than resistance of accessions from AS. So this experiment was set up to prove this hypothesis.

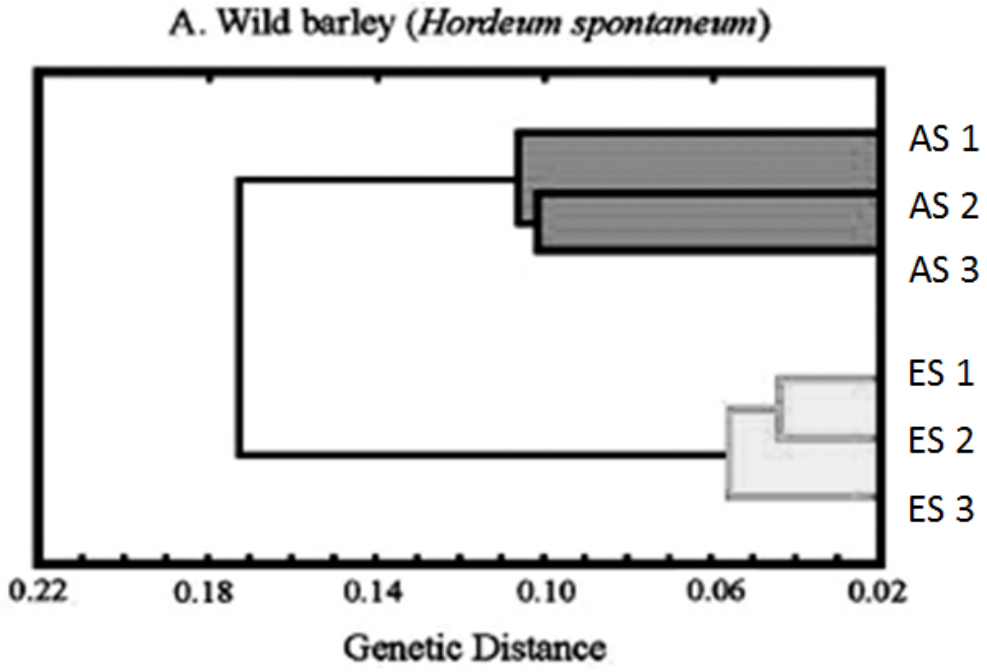

Figure 5. Dendrograms derived from Nei's genetic distances (*D*) between the subpopulations of wild barley in "Evolution Canyon". Source: Nevo et al., 2006. AS=African slope; ES= European slope.

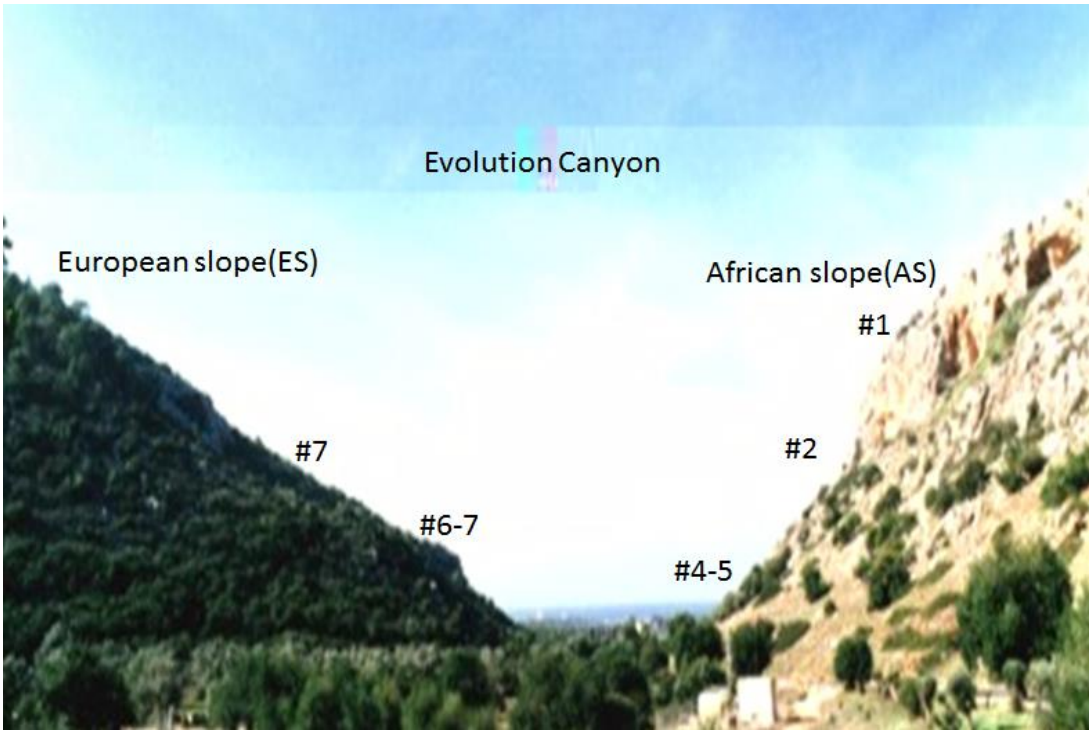

Figure 6. "Evolution Canyon", lower Nahal Oren, Mount Carmel, Israel. Wild barley (*Hordeum spontaneum*) grows on the opposite slopes. The green, wet, cool-mesic "European slope" (ES) extremely contrasts with the drought stressful, high solar radiation, warm-xeric "African slope" (AS). There are five stations: two on the AS (#1 and #2), one at the valley bottom (#4-5), and two on the ES (#6-7 and #7). (Nevo, Beharav et al. 2005)

1.5 Near non-host resistance of barley accessions from ICARDA to *P. persistens*

Study of resistance genes is one of the key subjects of plant resistance breeding. The novel disease resistance genes that are valuable genetic resources could be searched in *ex situ* genebanks or germplasm collections (Leonard and Szabo 2005, Fehser, Beike et al. 2010, Vurro, Bonciani et al. 2010). The traditional methods of germplasm collection such as present in core collections are not efficient when looking for novel genes, because the number of genebank accessions (crop landraces and wild relatives) is extremely big around the world (Pessoa-Filho, Rangel et al. 2010, Xu 2010). Therefore, the Focused Identification of Germplasm Strategy (FIGS) is introduced as a new approach in selecting genetic variation for only one trait at a time by constructing small subsets of accessions (Endresen, Terje et al. 2011).

It is based on traits and driven by users to improve crop traits by selecting potentially useful germplasm. This approach selects accessions that are most likely bearing a selection pressure from collection sites for the sought after trait. It maximizes the likelihood that accessions with specific adaptive traits could be included in subsets (Mackay and Street 2004).

Natural selection poses a main impact on disease resistance traits, it therefore leads to a restricted distribution of disease resistance traits (Qualset 1975). The crop traits are not randomly distributed and the distribution could be geographically structured (Hakes and Cronin 2011). The association between geographic, eco-climate and morphological traits in barley was reported by using the FIGS strategy (Endresen 2010).

It was asserted that the distribution of wheat landraces with resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) was not random but associated with geographical or climate-zone characteristics (Bari, Street et al. 2012). In addition, a large panel of barley landraces composed of 1774 geo-referenced accessions obtained from the ICARDA collection were screened to determine the percentage of accessions with susceptibility to *P. triticina*. The result showed that resistance to *P. triticina* in barley accessions is not randomly distributed but may tend to be associated with agro-eco-climatic factors (Jafary, unpublished) (Figure 7). In this study, a similar experiment was set up to detect whether the distribution of accessions with resistance to *P. persistens* is linked to environmental factors.

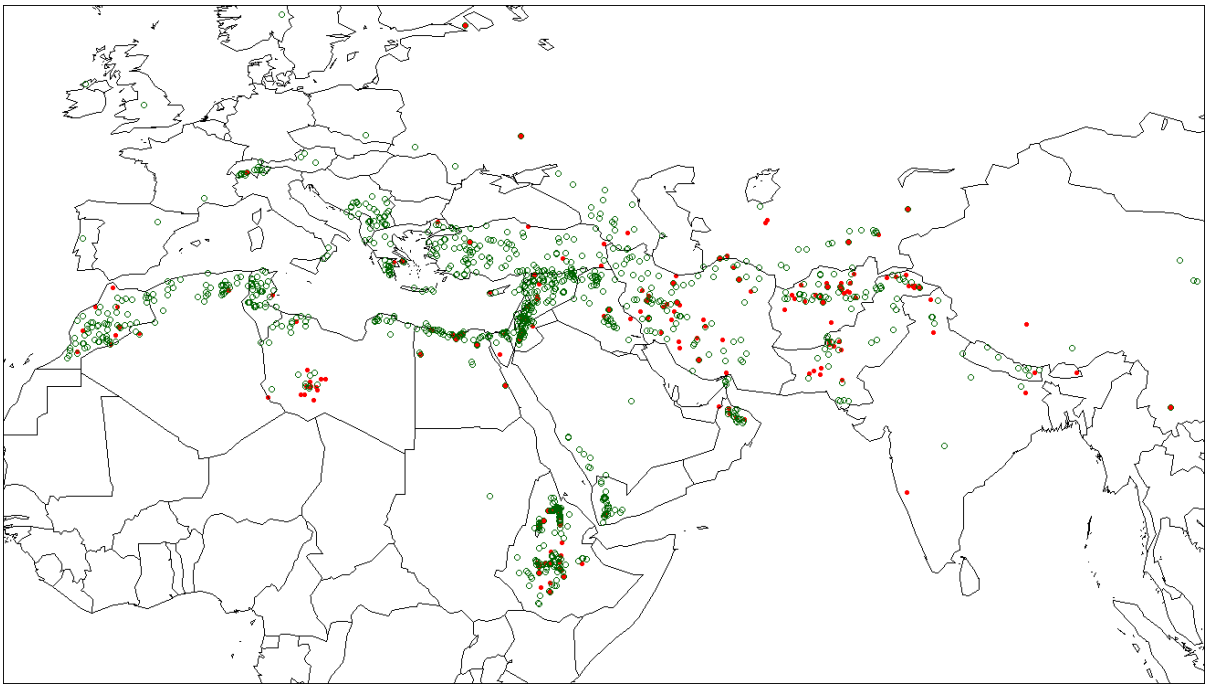

Figure 7. Distribution dot: distribution map of barley accessions subset. Explanation: Susceptible accessions in red stand for susceptible accessions to *P. triticina* (at least 1 pustule), in green stand for resistant accessions to *P. triticina* (0 pustule) (Jafary, unpublished).

1.6 Research objectives of this thesis

Two projects were proposed for this thesis. In the first project, two objectives were included: one was to detect the influence of climate factors on resistance of barley to heterologous rusts; the second was to find relationships between different rust species.

In the second project, one of the objectives was to detect whether there is a link between climate factors and resistance of barley to *P. persistens*. In a previous study, the distribution map of barley accessions to *P. triticina* was already developed (Figure 7). Therefore, the other objective was to check whether the distribution of resistance of barley to *P. persistens* and *P. triticina* are associated.

2 Materials and methods

2.1 Materials

2.1.1 Plant materials

A collection of wild barley (*H. vulgare* ssp. *spontaneum*) accessions from opposite slopes in Evolution Canyon I was subjected to the infection experiments. These accessions were from five stations (#1, #2, #4-5, #6-7, #7) in ECI, and ten single spike progenies were collected from each station. There were around ten seeds in each single spike progeny. In this experiment, seeds were limited, so the first five progenies from each station (#1-1, #1-2, #1-3, #1-4, #1-5, #2-1…) were used for *P. persistens* (*P.p*), *P. hordei-secalini* (*Phs*), *P. hordei-murini* (*Phm*), *P. triticina* Swiss (*Pts*), *P. graminis*. f. sp. *lolii*, and *P. striiformis* f. sp. *hordei*. After all seeds of the five progenies were finished, the next five progenies (#1-6, #1-7, #1-8, #1-9, #1-10, #2-6…) were tested for *P. graminis* f. sp. *tritici*, *P. graminis*. f. sp. *lolii*, and *P. hordei*. Three lines; line SusPtrit, line L94 and line Wheat 8860 were used as references. SusPtrit, which is an experimental barley line with exceptional susceptibility to rust fungi, was used as the susceptible control. L94 is less susceptible than SusPtrit to most heterologous rusts. Wheat is nonhost resistant to most heterologous rusts and susceptible to *P. triticina* (Niks and Marcel 2009), so Wheat 8860 is immune to most heterologous rusts except *P. triticina* Swiss.

A collection of geo-referenced barley landraces (n=1313) were provided by ICARDA, and these accessions were collected by considering different climatic factors: climate data, longitude and latitude, altitude, and soil. They were numbered and divided into 21 trays (84 seedlings per tray) for growing (Figure 8). In this experiment, three reference lines were used: line SusPtrit, line L94 and line Vada. Vada is immune to most heterologous rusts except *P. hordei*, so it was used to check whether there was contamination of *P. hordei* in each tray.

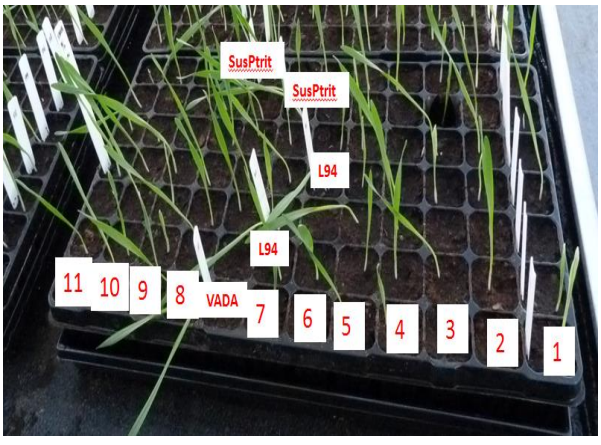

Figure 8. Barley accessions of ICARDA material were grown in these trays: each tray contains 84 seedlings.

2.1.2 Pathogen materials

Five heterologous rusts, *P. persistens* (*P.p*), *P. hordei-murini* (*Phm*), *P. hordei-secalini* (*Phs*), *P. graminis*. f. sp. *lolii* (*Pgl*) and *P. triticina* Swiss (*Pts*); three host pathogens, *P. hordei* isolate 1.2.1 (*P. hordei*), *P. striiformis* f. sp. *hordei* (*P. striif*) and *P. graminis* f. sp. *tritici* (*Pgt*) were used. The spores of different rusts were multiplied on their respective host plant (Table 1).

Table 1. Pathogen material used in this study.

Pathogen/Isolate or forma specialis	Host plant	Place of collection
P. hordei-secalini	*Hordeum secalinum* Schreb.	Mesquer (France)
P. hordei-murini Rhenen	*Hordeum murinum* L.	Rhenen (The Netherlands)
P. triticina. Swiss	*Triticum aestivum*	Wageningen
P. graminis. f. sp. *lolii*	*L. perenne*	Wageningen
P. persistens	*E. repens*	Wageningen
P. graminis f. sp. *tritici*	*Triticum aestivum*	Hungary
P. striiformis f. sp. *hordei*	*Hordeum vulgare*	Wageningen
P. hordei isolate 1.2.1	*Hordeum vulgare*	Wageningen

2.2 Methods

2.2.1 Near non-host resistance of barley accessions in Evolution Canyon

2.2.1.1 Inoculation

The inoculation method was developed by Dr. R.E. Niks (Wageningen UR Plant Breeding): Seedlings were grown in boxes (37 cm wide, 39 cm long) (Figure 9). Inoculations were performed in a settling tower (Figure 10). For each rust fungus, five progeny per station were inoculated. Each progeny was represented by one seedling, and each box contained 25 wild barley progeny and line L94, line SusPtrit and line Wheat 8860 as references. The inoculations were carried out with freshly collected spores, i.e. about 5 mg spores per plant box, resulting in a deposition of about 300 urediospores per cm^2. An inoculation with the barley pathogen *P. hordei* was carried out using about 1.5 mg spores per plant box. Twelve days after sowing, completely unfolded primary leaves were fixed in a horizontal position, the adaxial surface facing up. The inoculum was applied in the setting tower. The plants were incubated overnight in a dew chamber for 9.5 h (17-18°C) at 100% relative humidity. In the morning, the boxes were transferred to a greenhouse compartment at about 22/18 °C (day/night). Inoculation was performed with only one pathogen species per day. To prevent cross contamination the setting tower and other tools were cleaned with 96% ethanol. Furthermore, atypically large and compatible pustules were considered as contamination by *P. hordei*. In case of *P. graminis*, colour and spore morphology were also used to discriminate possible contamination by other rust species (Atienza, Jafary et al. 2004).

Table 2 Pathogens and the amounts of pathogen used in this experiment.

Pathogen/Isolate or forma specialis	Amount (mg)
P. hordei-secalini	*5*
P. hordei-murini Rhenen	*5*
P. triticina Swiss	*5*
P. graminis. f. sp. *lolii*	*5*
P. persistens	*5*
P. graminis f. sp. *tritici*	*3*
P. striiformis f. sp. *hordei*	*2*
P. hordei isolate 1.2.1	*1.5*

Figure 9. Barley seedlings were grown in 37*39 boxes, in two rows.

Figure 10. Photo of settling tower. The spores could be sprayed over the plants in a uniform density by using the settling tower. Boxes with plants are put in at the bottom of the tower.

2.2.1.2 Evaluation

At least 12 days after inoculation the level of infection was quantified for each seedling. The number of pustules per leaf was counted on the adaxial leaf surface (Figure 11). For heterologous rusts, infection frequency (IF) which is the number of pustules per square centimetre was calculated to quantify the level of infection (Jafary, Szabo et al. 2006). The adjacent pustules on the leaf often merged if the accession was more susceptible to *P. graminis* f.sp. *triticina*; and two or more pustules would be produced from the same infection site if the accession was more resistance to *P. striiformis f. sp. hordei*. Therefore, IF was not accurate to measure the resistance level to *P. striiformis f. sp. hordei*. In this case, sporulating area (%) (SA) is the percentage of leave area with pustules per square centimetre, and it was used to quantify resistance of barley accessions to *P. striiformis* f. sp. *hordei*. The latency period (LP) of each plant was evaluated by estimating the period (h) at which 50% of the ultimate number of pustules became visible for *P. hordei* (Jafary, Albertazzi et al. 2008).

Figure 11. Pustules on the leaf.

2.2.2 Near non-host resistance of barley accessions from ICARDA to *P. persistens*

P. persistens was used as the pathogen material in this experiment. Seedlings were grown in trays. Each accession was represented by one seedling, each tray containing 84 seedlings, including line SusPtrit, line L94 and line Vada. Seedlings were inoculated with 15 mg of spores of *P. persistens* on first leaf per accession. Twelve days after sowing, the spores of *P. persistens* were sprayed directly on seedlings. The inoculation method was described in 2.2.1.1. Around 12 days after inoculation, pustules per seedling leaf was counted.

2.2.3 Data analysis

Two sample t-test was carried out for all rust species to check if there is significant difference between resistance of accessions from African slope and European slope to different rust fungi.

The Spearman's coefficient of rank correlation was carried out to quantify the association between different rust species.

The Chi-square test was applied to check the null hypothesis assuming independent distribution of barley accessions with resistance to *P. triticina* and *P. persistens*.

3 Results

3.1 Near nonhost resistance in Evolution Canyon

3.1.1 Disease tests with different rust species.

In this study, five heterologous rusts (*P. hordei-secalini, P. persistens, P. graminis* f.sp. *triticina, P. graminis*. f.sp. *lolii, P. triticina* Swiss, , *P. hordei-murini*) and three host pathogens (*P. hordei, P. striiformis* f. sp. *hordei* and *P. graminis* f. sp. *tritici*) were inoculated on plants collected in Evolution Canyon (station #1, #2, #4-5, #6-7, #7) and references (L94, Susptrit, Wheat 8860). The data presented in this report are the average of infection parameters (IF, SA, LP) over the experiments of each rust fungus.

In this experiment, barley accessions from the same station were regarded as one subset, because they grew in a similar environment. To compare resistance of barley from different stations to different rust species, the percentages of resistance progeny in each station were calculated. An example of resistance progeny percentage calculation is shown in Table 3. Susptrit was used as susceptible reference and 20% of IF (Susptrit) was considered as the critical value to distinguish resistant and susceptible progeny; the progeny would be marked as "R" when its IF was lower than IF(Susptrit). The percentage of resistance progeny (R progeny) of each station could be calculated. If resistance progeny percentage was "0", it indicated that barley from this station were susceptible to the rust; and if resistance progeny percentage was "1", it indicated that barley from this station were resistant to the rust.

Table 3. An example of calculation of resistant progeny percentage. "0" stands for plants from this station which were susceptible to the rust, and "1" stands for plants from this station which were resistant to the rust.

P. persistens			
Station	Progeny	IF	Resistant level
#1	1	2.9	
	2	0.3	R
	3	1.8	
	4	0.8	
	5	0.0	R
Susptrit		1.5	
20% of Susptrit		0.3	
Resistance percentage			2/5*100%=40%

Table 4. Percentage (%) of resistant progeny to different rusts in each station

Percentage (%) of resistant progeny							
Station	*P. hordei-secalini*	*P. graminis* f. sp. *lolii*	*P. hordei-murini*	*P. triticina* Swiss	*P. persistens*	*P. striiformis* f.sp. *hordei*	*P.graminis* f. sp. *tritici*
#1	25	0	60	60	40	20	50
#2	25	75	20	100	0	20	0
#4-5	100	0	100	80	0	25	0
#6-7	20	0	100	100	0	80	0
#7	100	0	100	100	0	100	0

The percentages of resistance progeny in each station are shown in Table 4. Accessions from station #7 were resistant to *P. hordei-secalini, P. hordei-murini,* and *P. striiformis* f. sp. *hordei*, while accessions from station #4-5, #6-7 and #7 were fully susceptible to the other rust species (*P. graminis* f. sp. *lolii, P. persistens, P.graminis f. sp. tritici*). It indicates that accessions from the same station were resistant to some rusts but were susceptible to the other rusts.

In Figure 12, Infection frequency of heterologous rusts (IF), sporulating area (%) (SA) of *P. striiformis* f. sp. *hordei* and latency period (LP) of *P. hordei* were shown through column chart. The infection phenotypes for *P. persistens* and *P. striiformis* f. sp. *hordei* were immune to L94; and SusPtrit was the most susceptible accession to all rust species, while line Wheat 8860 was immune to most rust fungi except *P. triticina* Swiss (Figure 12).

IFs or SAs of accessions from station #6/ #7 to five rusts (*P. hordei-secalini, P. hordei-murini, P. triticina* Swiss, *P. graminis* f.sp. *tritici* and *P. striiformis* f. sp. *hordei*) were significantly lower than accessions from station #1 and station #2 (Figure 12, A, B,C, F, G). However, there was no significant difference among IFs or LPs of accessions from all stations to *P. persistens* and *P. hordei* (Figure 12, E, H).

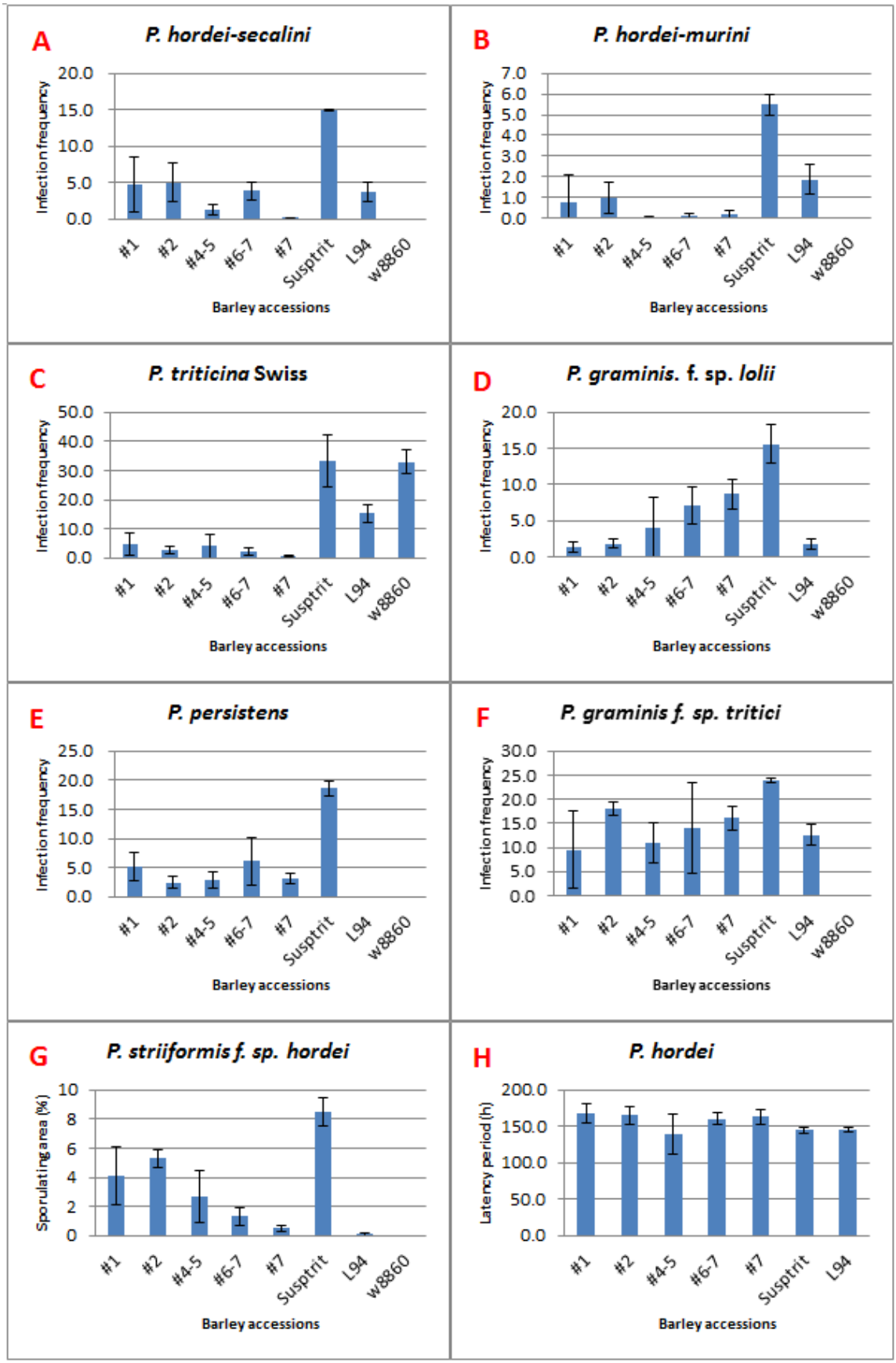

Figure 12. A-E, infection frequency of accessions from Evolution Canyon (#1, #2, #4-5, #6-7, #7)and reference lines to five heterologous rusts; H, latency period of *P. hordei* in lines from five station in EC I and reference lines.

Table 5. Independent T-test for equality of means of infection parameters expressing level of resistance of plants from AS and ES. Explanation: AS includes station #1 and #2 and ES includes station #6-7 and #7.

Criteria measured	Position	N	Mean	Std. Deviation	Std. Error Mean	Sig. (1-tailed)
IF	AS	9	4.8	3	1	
(*P. hordei-secalini*)	ES	9	2.2	2.2	0.7	0.024*
IF	AS	10	0.9	1	0.3	
(*P. hordei-murini*)	ES	8	0.2	0.1	0.1	0.029*
IF	AS	10	3.6	2.8	0.9	
(*P. triticina* Swiss)	ES	9	1.3	1.2	0.4	0.016*
IF	AS	5	0.6	0.5	0.2	
(*P. graminis* f. sp. *lolii*)	ES	10	4.9	2.6	0.8	0.000*
IF	AS	10	1.1	0.9	0.3	
(*P. persisten*)	ES	9	1.2	0.9	0.3	0.374
IF	AS	10	4.7	1	0.3	
(*P.striiformis* f.sp. *hordei*)	ES	9	0.9	0.5	0.2	0.002*
IF	AS	9	5.9	5	1.7	
(*P. graminis* f. sp. *tritici*)	ES	9	9.4	3.5	1.2	0.052
LP	AS	8	166.2	11.8	4.2	
(*P. hordei*)	ES	6	161.3	7.7	3.1	0.197

* indicates a significant difference at the 0.05 level (1-tailed).

The data analyses of mean values of infection parameters expressing level of resistance of lines from African slope (AS) and European slope (ES) are presented in Table 5. It was shown that for IF to *P. hordei-secalini*, *P. hordei-murini*, *P. triticina* Swiss, *P. striiformis* f. sp. *hordei*, lines from ES were significantly lower than from AS, indicating that lines from ES were significantly more resistant to these rust fungi. IF to *P. graminis*. f.sp. *lolii* from ES was significantly higher than that from AS, indicating that lines from ES were significantly more susceptible to *P. graminis*. f.sp. *lolii*. For IFs to other heterologous rusts, lines from ES were not significantly higher than these from AS and for LP to *P. hordei*, lines from ES were not significantly lower than these from AS (Table 5).

3.1.2 Correlations between different rusts species.

We quantified the association in nonhost resistance to six rust species by the Spearman's coefficient of rank correlation. Correlation coefficients (*r*) among the infection parameter frequency of resistance to six rusts are shown in Table 6. The highest positive correlation was between SA to *P. striiformis* f. sp. *hordei* and IF to *P. hordei-murini* ($r = 0.7$), correlation between SA to *P. striiformis* f. sp. *hordei* and IF to *P. triticina* Swiss was slightly lower ($r = 0.6$), and both of the correlations were significant. Correlations among five rust species (*P. persistens, P. hordei-secalini, P. striiformis* f. sp. *hordei, P. hordei-murini, P. triticina* Swiss) were intermediately positive.

Table 6. Correlations among quantified traits for different rusts.

Criteria measured	IF (*P. persisten*)	IF (*P. hordei-secalini*)	SA (*P.striiformis* f.sp. *hordei*)	IF (*P. hordei-murini*)	IF (*P. triticina* Swiss)
IF (*P. persisten*)	...	...	...	...	...
IF (*P. hordei-secalini*)	0.2	...	...	...	...
SA (*P.striiformis* f.sp. *hordei*)	0.3	0.6	...	...	...
IF (*P. hordei-murini*)	0.4	0.3	0.7*	...	...
IF (*P. triticina* Swiss)	0.3	0.5	0.6*	0.2	...

*. Correlation is significant at the 0.05 level (2-tailed).

3.1.3 Correlation between LP of *P. hordei* and nonhost resistance to *P. graminis* f. sp. *lolii* and *P. graminis* f. sp. *tritici*.

We also quantified the association in nonhost resistance against *P. graminis* f. sp. *lolii* with the level of partial resistance against *P. hordei* and *P. graminis f. sp. tritici* by the Spearman's coefficient of rank correlation. We used the measurements of the LP of *P. hordei* on test lines as a parameter for the partial resistance to *P. hordei*.

The correlation between LP of *P. hordei* and IF of *P. graminis* f. sp. *lolii* was negative and significant ($r = -0.5$), which was similar to the correlation between LP of *P. hordei* and IF of *P. graminis* f.sp. *triticina* ($r = -0.4$). If LP of *P. hordei* was higher, it indicated that this accession was more resistant to *P. hordei*. The results indicated that the correlation between both *P. hordei* and *P. graminis* f. sp. *lolii*, and *P. hordei* and *P. graminis* f.sp. *triticina* was positive on resistance level. The correlation between *P. graminis* f.sp. *triticina* and *P. graminis* f. sp. *lolii* was also positive but not highly correlated ($r = 0.3$) (Table 7).

Table 7. Correlations among quantified traits for different rusts.

Criteria measured	IF (*P. graminis* f. sp. *tritici*)	IF (*P.striiformis* f.sp. *hordei*)	LP (*P. hordei*)
IF (*P. graminis* f. sp. *tritici*)	...		
IF (*P. graminis* f. sp. *lolii*)	0.3	...	...
LP (*P. hordei*)	-0.3	-0.5*	...

*. Correlation is significant at the 0.05 level (2-tailed).

3.2 Near non-host resistance of barley accessions from ICARDA to *P. persistens*

3.2.1 Disease test in each tray

The number of pustules in line SusPtrit, L94, and Vada in each tray are presented in Table 8. It is shown that line L94 was immune to *P. persistens* with HR (0 pustules). In most cases, line Vada was resistant to *P. persistens* (0 pustules), but there were several pustules in some Vada seedlings. The percentages of susceptible accessions in each tray were calculated and presented in Table 8: in most trays the percentage was around 50%, the highest was 74% in tray 15 and the lowest was 38% in tray 2.

3.2.2 Proportion of susceptible barley accessions

In a previous study, the experiment of *P. triticina* test was finished by Jafary: 88% (1567 out of 1774) of the barley accessions were resistant (0 pustules) to *P. triticina* (Figure 13). In this experiment, 43% accessions (569 out of 1313) were resistant (0 pustules) to *P. persistens* (Figure 14). The number of accessions with susceptibility (at least 1 pustule) to *P. persistens* was higher than that of *P. triticina.* It indicates that barley was easier to infect by *P. persistens* than *P. triticina.*

3.2.3 Chi-square test of numbers of barley accessions classified according to susceptibility to *P. triticina* and to *P. persistens*

The number of barley accessions was classified according to susceptibility to *P. persistens* and *P. triticina* in Table 9. 527 accessions were resistant to both *P. persistens* and *P. triticina*; 637 accessions were susceptible to *P. persistens* but resistant to *P. triticina* ; 42 accessions were susceptible to *P. triticina* but resistant to *P. persistens*; and 97 accessions were susceptible to both rust fungi. Chi-square test was used to detect the association of distribution of barley accessions with resistance to *P. persistens* and *P. triticina*. The null hypothesis assumed that resistance to *P. persistens* and *P. triticina* in barley accessions was distributed independently. The null hypothesis was rejected with a high probability $P< 0.001$, suggesting that there is an association between the distribution of the accessions with resistance to *P. persistens* and *P. triticina*. It could be also concluded that when more accessions susceptible to *P. persistens* exist in one place, there would be more accessions susceptible to *P. triticina* as well.

Table 8. Susceptible accessions analyses

DATE		Number of susceptible accessions (pustule>0)	% susceptible accessions	Average IF of susceptible accessions (pustule>0)	IF SusPtrit	IF Vada	IF L94
D1	tray 1	35	54	13	32	0	0
	tray 2	23	38	23	15	0	0
	tray 3	34	49	21	35	0	0
D2	tray 4	36	54	33	23	0	0
	tray 5	38	55	19	40	0	0
	tray 6	44	65	29	120	8	0
D3	tray 7	45	69	31	83	2	0
	tray 8	32	53	43	63	1	0
	tray 9	30	53	27	57	0	0
	tray 10	30	50	25	45	0	0
D4	tray 11	31	53	28	33	0	0
	tray 12	39	60	13	33	0	0
	tray 13	41	62	21	38	0	0
	tray 14	35	66	20	50	0	0
D5	tray 15	45	74	23	38	0	0
	tray 16	31	53	23	57	2	0
	tray 17	41	58	19	73	2	0
	tray 18	34	53	17	45	0	0
D6	tray 19	43	69	30	65	2	0
	tray 20	32	52	26	63	0	0
	tray 21	31	49	18	50	0	0

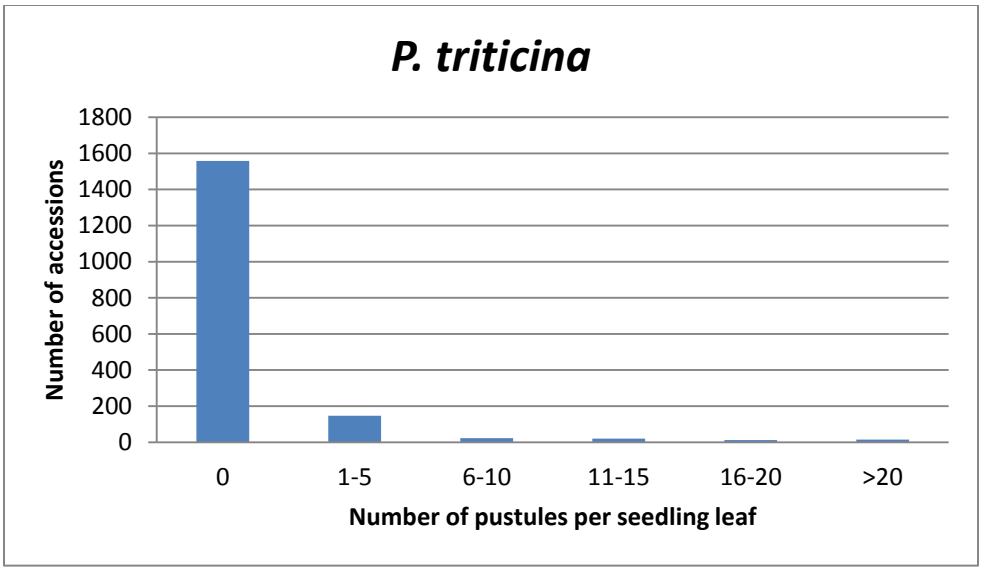

Figure 13. Numbers of barley accessions, tested in the seedling stage, per level of susceptibility to *P. triticina* isolate Flamingo. Source: Jafary, unpublished.

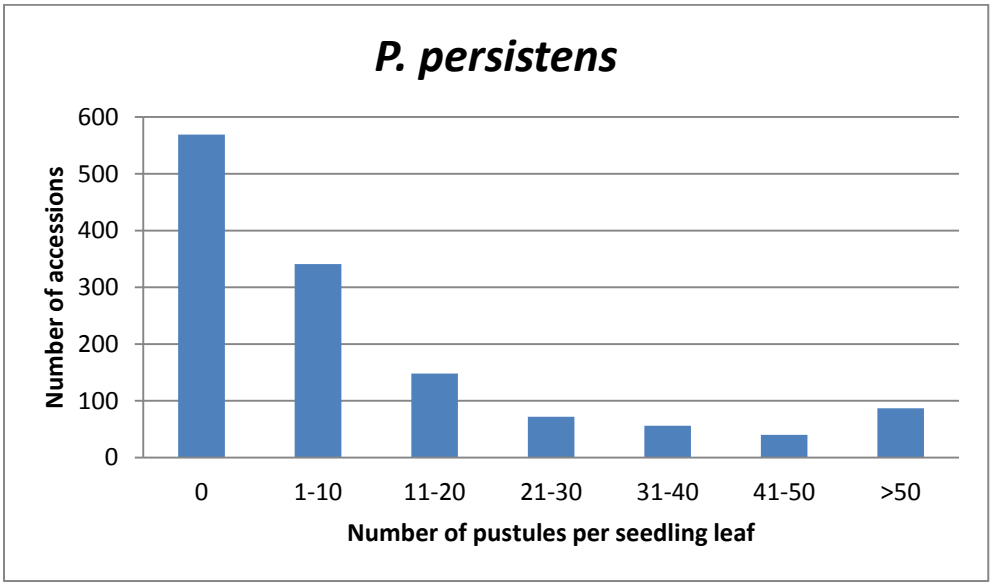

Figure 14. Numbers of barley accessions, tested in the seedling stage, per level of susceptibility to *P. persistens*.

Table 9. Numbers of barley accessions classified according to susceptibility to *P. triticina* and to *P. persistens*. The figures in blue are the numbers expected in case of absence of association between rust species and probability of infection.

Pustules of *P. persistens*			
Pustules		0	At least 1
of *P. triticina*	0	**527** (508)	637 (656)
	At least 1	42 (61)	**97** (78)

4 Discussion

4.1 The influence of climate factors to nonhost resistance of barley to heterologous rusts in Evolutionary Canyon.

4.1.1 Resistance of barley accessions to different rust fungi in ES and AS.

In this study, the first aim was to prove the hypothesis that barley accessions from ES were more resistant than accessions from AS to heterologous rusts. As the environment of ES is wet and cool, which is suitable for most pathogens, more pathogens are able to live well in ES. Therefore, accessions in ES are bearing more pathogen invasion. Biotrophic pathogens may co-evolve with the immunity of their host plant species (Thordal-Christensen 2003). It may lead to accessions from ES having stronger immune system, so these accessions are more resistant to heterologous rusts than accessions from AS (Nevo 2006). In the previous study, it was also indicated that there is a tendency that European accessions were more resistant than African accessions to most heterologous rust fungi (Table 10).

To prove this hypothesis, barley accessions from both ES and AS were infected by seven heterologous rusts and *P. hordei*. IF (infection frequency) to different rusts were calculated to compare resistance levels of different rusts. Barley is more resistant to rusts when IF is lower. So it was assumed that IF of accessions from ES would be lower than that from AS, and it would be written as IF (ES) and IF (AS) for short below.

It was shown that for four rusts including *P. hordei-secalini, P. triticina* Swiss, *P. hordei-murini* and *P. striiformis* f.sp. *hordei*, their IF (ES) were significantly lower than IF (AS). On the other side, there was no significant difference between IF (AS) and IF (ES) to *P. persistens*, *P. graminis* f. sp. *tritici*, and *P. hordei*.

In total, 3 out of 5 near- nonhost rust species showed that IF (ES) were significantly lower than IF (AS). It indicated that barley accessions from ES were more resistant than accessions from AS to the three rust species (*P. hordei-secalini, P. triticina* Swiss and *P. hordei-murini*). Meanwhile, there is a tendency that accessions from wet and cool environment are more resistant than accessions from hot and dry places in terms of the three near-nonhost rusts.

It was shown in the results that accessions from some stations such as station #7 were resistant to some rusts but were fully susceptible to the other rusts. A previous study suggested that barley contains both genes for general defence to several heterologous rusts and genes for rust species-specific resistance to heterologous rusts (Hoogkamp, Chen et al. 1998, Atienza, Jafary et al. 2004). In addition, the near-nonhost resistance of barley to heterologous rusts is controlled by QTLs with overlapping rust specificity. The QTLs have influence on only one or two rust species, and only few effect more than four rusts (Jafary 2006, Jafary, Albertazzi et al. 2008). Therefore, it indicates that nonhost resistance of barley to rusts is controlled by a combination of non-specificity and specificity of the genes (Niks 2014). In this case, it may suppose that accessions from some stations such as #7 contain only QTLs of resistance to specific rust fungi (*P. hordei-secalini, P. hordei-murini,* and *P. striiformis* f. sp. *hordei*).

Table 10. Percentage of barley germplasm (*n*= 109) susceptible to seven heterologous rust fungi.

	No. of accessions	*P. hordei-murini* Cordoba	*P. hordei-murini* Rhenen	*P. agro-pyrina*	*P. triticina* Flamingo	*P. hor-secalini*	*P. graminis lolii*	*P. coro-nata*[a]
Level of agronomic application								
Wild species (*H. spontaneum*)	6	40	17	50	83	50	83	17
Line from land race and research lines	30	80	33	50	57	60	45	3
Cultivar released before 1945	17	70	0	70	0	18	18	0
Cultivar released in 1945 or later	44	45	7	55	25	16	16	2
Unknown	12							
Origin[b]								
Europe	56	48	5	59	13	30	16	2
North America	7	85	0	33	50	33	33	0
South America	13	46	0	50	33	25	17	0
Africa	10	77	70	20	80	50	30	0
Asia	12	91	23	85	70	92	54	0
Unknown	5							
Morphological traits[b]								
6-row	35	71	17	54	40	45	34	3
2-row	68	56	12	54	26	24	20	1
Naked seeded	10	90	60	50	80	80	70	0
Covered seeded	93	59	9	55	26	26	19	2
Black seeded	7	85	71	0	71	57	43	14
White seeded	96	46	9	58	25	29	23	1
All accessions	109	58	14	54	36	32	29	3

4.1.2 Correlations between different rusts species.

The highest positive correlation was between SA *P. striiformis* f.sp. *hordei* and IF to *P. hordei-murini*, it indicated that the distribution of barley accessions with resistance to *P. striiformis* f.sp. *hordei* and to *P. hordei-murini* was highly associated in EC. The distribution of barley accessions with resistance to *P. striiformis* f.sp. *hordei* and to *P. triticina* Swiss was also highly associated in EC. The distributions of accessions with resistance to other five heterologous rusts (*P. persistens, P. hordei-secalini, P. striiformis* f.sp. *hordei, P. hordei-murini, P. triticina* Swiss) tended to be positively and moderately correlated. The distributions to *P. hordei, P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *lolli* were also positively associated. It suggested that environmental factors may be effective to resistance of barley to these rusts. It may indicate that environment factors can affect general defence of barley to different rust species.

Eight rust species used in this study belong to three different groups of rust fungi of grasses: *P. hordei, P. persistens*/ *P. triticina*, and *P. graminis*. Due to coevolution of biotrophic pathogens with their host plant species (Thordal-Christensen 2003), it could be presumed that the resistance of barley to two related rust species is associated.

However, our evidence of such an evolutionary association is not conclusive. The effect of environmental factors on barley resistance to two less-related rust species (*P. striiformis* f.sp. *hordei* and *P. hordei-murini*) was highly associated; and as for two closely related rust species (e.g., *P. hordei-secalini* and *P. persistens*), it was poorly associated (Figure 15). It was illustrated that evolutionary distance and host status to a certain potential pathogen were not associated (Jafary 2006). It seems that biological specialization in rust pathogens is not influenced by evolutionary distance but by other factors, such as geographical origin of pathogens (Wyand and Brown 2003) .

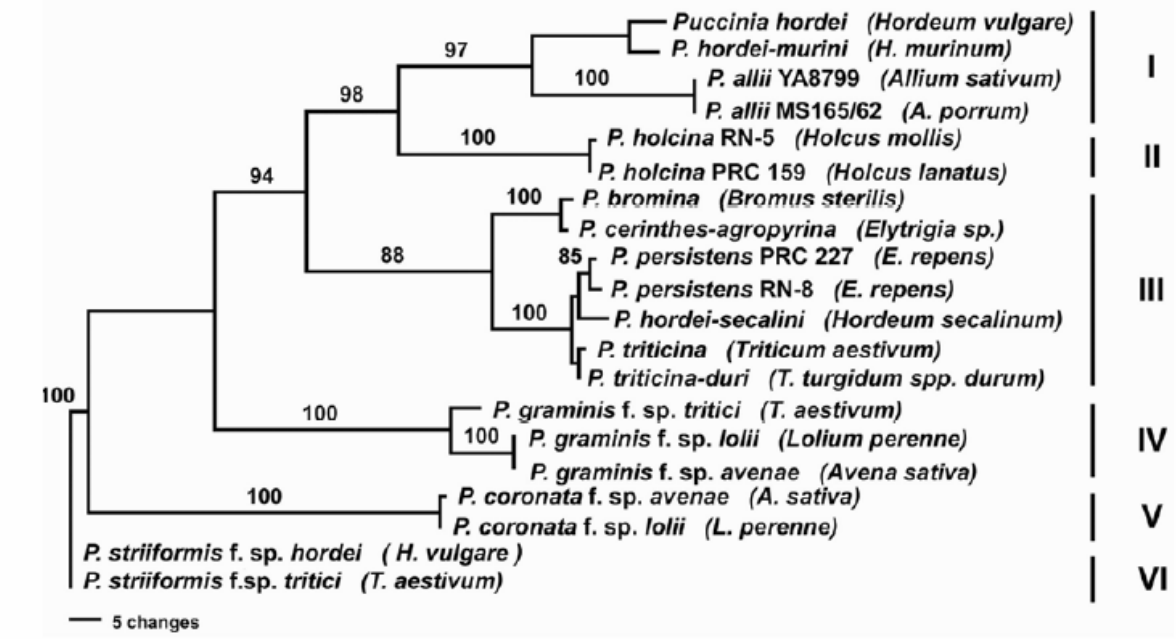

Figure 15. Parsimony tree from the analysis of nuclear ribosomal internal transcribed spacer (ITS) sequence data of selected cereal and grass rusts. Phylogenetic analysis resulted in four optimal trees, one of which is shown (tree length of 262 steps, Consistency index = 0.7634, homoplasy index = 0.2366, retention index = 0.8839, and rescaled consistency index = 0.6747). Numbers above branches indicate percentage of congruent clusters in 1,000 bootstrap trials and only values above 80% are shown. Rust hosts are indicated in parentheses. Clades are indicated along the right hand side. *Puccinia striiformis* DNA sequences were used as outgroup. (Jafary, Szabo et al. 2006)

4.1.3 Future work

In this experiment, germination rates of seeds from some stations was very low. For example, the germination rate of seeds from #7 was only 20% in some boxes. The number of seeds was also limited, which was not enough to do replication experiments for all rust species. Therefore, to study the influence of environmental factors on the resistance of barley to rust species, the here obtained results should be considered as a first indication and as reference for repeat experiments. More seeds from EC should be used to confirm or reject the hypothesis of this research. However, based on these preliminary results, it looks as if the hypothesis might be true.

4.2 Near non-host resistance of barley accessions from ICARDA to *P. persistens*

4.2.1 Reliability of data

In this study, line L94 was immune to *P. persistens*, and line Vada was resistant to *P. persistens* in most cases, except several Vada seedlings with few pustules. It corresponds with the previous study (Atienza, Jafary et al. 2004) that Vada and L94 are almost immune to *P. persistens* and Susptrit was extremely more susceptible than other accessions (Table 11). In addition, the number of susceptible accessions to *P. persistens* was around 50% in all trays, this result was also corresponding with the previous study that around 50% accessions were susceptible to *P. persistens* (Figure 3). It indicates that the reliability of data obtained in this experiment is high. Therefore, the data is useful and valuable for further study.

Table 11. Susceptibility (number of pustules per leaf), in seedling stage, of barley lines SusPtrit, Vada, L94 and other accessions tested barey germplasm to some heterologous rust fungi. (Atienza, Jafary et al. 2004)

Barley line	Heterologous rust									
	P. triticina Flamingo	*P. hordei-murini* Rhenen	*P. hordei-murini* Córdoba	*P. agro-pyrina*	*P. hordei-secalini*	*P. graminis lolii*	*P. coronata* f. sp. *holci*	*P. coronata* f. sp. *lolii*	*P. coronata* f. sp. *avenae*	*P. coronata* f. sp. *festucae*
Vada	0	0	2	8	0	0	0	0	0	0
Cebada Capa	0	0	0	1	0	0	0	0	0	0
Japan 1	32	125	0	3	6	0	1	1	1	1
Nigrinudum[a]	9	135	180	0	162	18	0	0	0	0
L92	87	360	12	2	163	0	1	1	1	1
Trigo Biasa[a]	134	245	200	192	432	158	1	0	0	1
Hassan	0	0	3	139	0	1	0	0	0	0
L94[a]	89	352	133	2	194	23	23	1	4	5
SusPtrit	708	575	225	438	657	166	10	0	5	5
SusPmur	19	700	517	37	475	142	6	56	5	1
Control = host[b]	618	138[c]	694	609	344	49[d]	300	396	500	32

4.2.2 Correlation between distribution of resistance of barley accessions to *P. triticina* and *P. persistens*

The aim of this experiment was to find association between distribution of barley accessions susceptibility to *P. triticina* and to *P. persistens*. The result shows that there is an association between them, which indicates that when there are more accessions susceptible to *P. triticina*, there would be more accessions susceptible to *P. triticina*.

It was illustrated that some QTLs were effective to both *P. triticina* and *P. persistens* (da Cunha, McFall et al. 2006). Hence, the effect of climate factors on barley resistance to the two closely related rust species may be highly associated.

This study shows that the eco-geographic distribution of both *P. triticina* and *P. persistens* resistance in barley is not random but is linked to climatic factors. In addition, barley accessions from cool and wet environment are more resistant than accessions from hot and dry environment to *P. triticina* (Jafary, unpublished). Thus, it could be assumed that barley accessions from cool and wet environments would be more resistant than accessions from hot and dry environments to *P. persistens*. It is likely that wet and cool climate has a positive influence on resistance of barley to heterologous rust species. It is not a surprising conclusion. Pathogens normally prefer to live in optimal climates, and this may lead to selection pressure on plants within the same environment. It was reported that eco-climatic factors would be

effective on natural selection and environmental differentiation for specific traits (Spieth 1979, Epperson 1990).

4.2.3 Future work

The results in this project are able to be connected with the geographic data on the origin of each accession, in order to determine the influence of climatic factors on barley resistance to *P. persistens*.

5 Conclusions

Both of the two projects were detecting whether environmental factors are associated with resistance of barley to rust fungi. In the first project, eight rust species including five heterologous rusts and three host rusts were tested. Barley accessions were collected from a valley which was called “Evolution Canyon”. Environmental conditions were sharply different on two slopes: African slope with hot and dry environment and European slope with cool and wet condition. The results showed that 3 (*P. hordei-secalini, P. triticina* Swiss, *P. hordei-murini*) out of 5 near-nonhost rust species and only one host rust (*P. striiformis* f.sp. *hordei*) showed that IF (ES) were significantly lower than IF (AS). Therefore, it may indicate that barley accessions originated from wet and cool environments are more resistant than accessions from hot and dry environments to these rust species (*P. hordei-secalini, P. triticina* Swiss, *P. hordei-murini, P. striiformis* f.sp. *hordei*).

The plant materials were tested for eight rust species in the same experiment. It therefore was able to find the correlation between different rusts. Correlations among five rust species (*P. persistens, P. hordei-secalini, P. striiformis* f. sp. *hordei, P. hordei-murini, P. triticina* Swiss) were intermediately positive. It may indicate that distributions of barley accessions with resistance to the five rusts were positively correlated. The distributions to *P. hordei, P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *lolli* were also positively associated. However, accessions from AS and ES originated in the same place, thus environment factors which influence resistance of barley to rust fungi are only solar radiation, temperature and humidity. More factors including altitude and soil may also be effective on resistance of barley to rusts. In addition, the number of seeds was limited. It thus may decrease the reliability of the conclusion.

On the contrast, more environmental factors were considered in the second project, as accessions were collected all over the world with geographical and spatial differentiation. The number of barley accessions was more than 1500, which was a much larger number than the number of accessions used in the first project. But the accessions were tested for only one rust fungus, *P. persistens*. In an earlier study, the same plant materials were used to check the association between climatic factors and barley accessions resistant to *P. triticina*. It illustrated that the eco-geographic distribution of *P. triticina* resistance in barley was not random but was linked to climatic factors. In this study, the results show that the eco-geographic distributions of barley accessions resistant to *P. persistens* and *P. triticina* are accociated. The distributions are not random but are linked to climatic factors. Compared with the first project, more environmental factors and barley accessions were included, but the accessions could be used to test only one rust species at a time. It also takes time to model the distribution map of barley accessions resistant to a specific rust.

6 References

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