The Influence of Light and Temperature on the Germination of *Brassica oleracea* Seed

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ABSTRACT

From February to May in 2010, I worked on the project of how light and temperature influence *Brassica oleracea* seed germination. All the work was performed at the seed & production physiology research department of Syngenta Seed B.V. in Enkhuizen, the Netherlands.

I investigated the influence of low temperatures and light conditions on germination, not only for the selection of seed batches by the company, but also to test the potential of this protocol as a tool for breeding for stress tolerance of germination. We used different varieties and seed batches, most of which were of the cauliflower variety, in order to determine if this tolerance is genetically determined. Paper, soil and agar were used as germination media at 10°C, 20°C and 30°C with and without light. Germination of the seeds was counted on a regular basis. After the experiments, the datasets were analyzed by excel and the germinator seed germination analysis program.

Clear differences were found between different varieties and seed batches of different production sites. However more research is needed to fully test our hypothesis.

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1. INTRODUCTION

I was very lucky to get the opportunity to do my thesis at Syngenta Seed B.V. in Enkhuizen, the Netherlands. The Syngenta seed businesses are part of Syngenta AG, a leader in the agribusiness industry of crop protection and seeds. I worked in the Seed & Production Physiology Research Department of Syngenta Seed B.V. with the project, "the effect of environmental conditions on the germination of *Brassica oleracea* seed". My supervisor was Dr. Frank Lanfermeijer, one of the scientists in the Seed & Production Physiology Research Department. All the work was performed in the laboratory of that department in Enkhuizen. I studied the effect of low temperatures and the presence or absence of light on the germination of cauliflower, an agronomic variety of *Brassica oleracea*. The best germination temperature of cauliflower is 21°C (Cross, 2008). However, cauliflower will germinate at temperatures as low as 10°C (Naeve, 1997). During this study the idea developed that germination at low temperatures could be used to test the stress-tolerance of germination of different seed-batches, or, in other words, the ability of seed batches to germinate under less than ideal conditions.

Objectives:

1. To offer a scientific basis for seed selection for the company.
2. To find out whether the cold and dark conditions during seed germination can be used as a tool for breeding for stress tolerance.

Research question:

What is the influence of temperature and light on germination of *Brassica* seeds?

Sub questions:

1. What are the differences between different varieties?
2. Are there any differences within a variety, for instance between different seed

batches?

3. What is the mechanism?
4. Can it be used for breeding purposes, for instance as tool to select for stress tolerance of germination?

Outline Report:

In chapter 2, the project is introduced and the current state of the knowledge on germination and the impact of temperature and light conditions are described. In chapter 3, the material and methods that were used during the study are described. The experimental results, the analysis and discussion of the results are given in chapter 4. In chapter 5, the research question and sub questions are discussed and some conclusions and recommendations are made.

2. LITERATURE

2.1 *Brassica*

Brassica is a genus of the mustard family (*Brassicaceae*). Within this genus we find species that are annual, biennials and perennial herbaceous plants. However, most of the commercial species are annual or biennial, although in the agriculture they forced to behave as annuals. Members of the genus are amongst others mustard, cabbage, collards, bok choy, kale, cauliflower, broccoli, and turnip. Crops from this genus are sometimes called *cole crops,* which is derived from the Latin *caulis,* meaning *stem or cabbage.* The wild ancestor of the crops is native in the Western Europe and Mediterranean and temperate zone of Asia with about 40 Old World species, Syngenta Seed B.V. focuses on *B. rapa*, *B. napus* and *B. oleracea. B. oleracea* which was the subject in this study is already cultivated for thousands of years. It can be classified by the edible part: Leaf type (kales), flower and thick scape type (cauliflower) and stem type (cabbage). These crops grow well in a cold environment. Warm temperatures will restrain their growth and decrease the quality. They are very healthy for people because of their low calorie content and high Vitamin C content.

2.2 Seed Germination and Dormancy

Germination of a seed starts with the uptake of water, restoration of respiration, initiation of protein synthesis and the restart of other metabolic activities. After enough water has been taken up the embryo emerges from seed, generally radical first. (Bewley *et al.,* 1983). This moment is usually considered to be the moment of germination. However, when the seed is buried in the soil, the seedling also has to penetrate the soil before it emerges and can start photosynthesis. Besides the basic requirement for water, oxygen and appropriate temperature, the seed may also be sensitive to light and nitrate (William et al., 2006).

Many species of seed nevertheless fail to germinate even when these requirements are satisfied. This phenomenon is called dormancy. Dormancy is a block to the completion of germination of an intact viable seed under favorable conditions under which it otherwise would germinate (William et al., 2006).

There are two types of dormancy: Seed coat-imposed dormancy and embryo dormancy. Seed coat-imposed dormancy results from the seed coat which prevents the embryo from growing. The seed coat hampers water uptake and oxygen exchange and it limits growth of embryo due to mechanical constraint. The seed coat can also retain germination inhibitors or it can be responsible for the production of those germination inhibitors (such as abscisic acid (ABA)). In the case of seed coat imposed dormancy, the seed will only germinate when the seed coat and other surrounding tissues (such as testa, pericarp) either are removed or damaged. Embryo dormancy is characterized by the fact that removal of the seed coat does not result in germination.

Usually, the balance between the two antagonistic plant hormones, ABA and gibberellic acid (GA) controls germination. When ABA (germination inhibitor) has the upper hand the seed will not germinate, while when GA (germination promoter) has the upper hand the seed will germinate (Taiz et al.. 2006). *In vitro* the status quo can be manipulated by various treatments: for instance by adding the hormones or Fluridone (an ABA synthesis inhibitor) which stimulates germination, or paclobutrazol (a GA synthesis inhibitor) which inhibits germination (Hedden et al., 1985, Yoshioka et al., 1998).

There is another way to differentiate seed dormancy. It can be separated in primary dormancy and secondary dormancy. One speaks of primary dormancy when the seed becomes dormant as part of the developmental process leading to mature seed. It can be broken by after-ripening (The common method which is used to release dormancy is by reducing the moisture content of seed to a certain level by drying). Secondary

dormancy is dormancy that is induced in mature seeds as a result of unfavorable germination or storage conditions (Taiz et al., 2006). Secondary dormancy can be lost and re-introduced repeatedly as conditions change until the proper germination conditions become available (William et al., 2006)

2.3 Temperature and light effects on germination

2.3.1 Temperature

Temperature affects both the capacity for germination and the rate of germination (Bewley *et al.*, 1986). Different species have different temperature ranges within which they germinate. At very low temperatures and very high temperatures the germination of all seeds is prevented. A rise in temperature does not necessarily cause an increase in either the rate of germination or in its percentage (Mayer, 1963). A certain species generally has an optimum germination temperature at which percentage of germination is the highest. Below and above the optimal temperature germination will decrease (Fig. 1). For cauliflower, optimal germination take place around 20°C. However, at less than optimal temperatures some seeds will germinate.

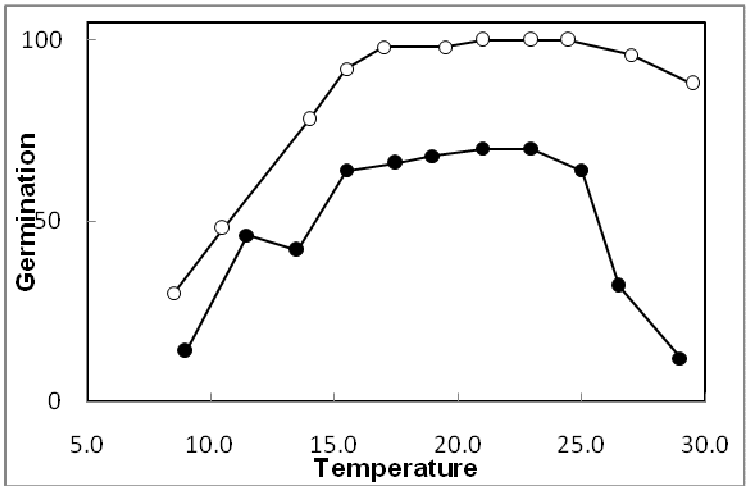


Figure.1 The relationship between temperature, light, and germination. Seeds of cauliflower var. Bruce were germinated over temperature range of 8-30°C. The open circles indicate germination in the presence of light, the filled circles indicate germination in the absence of light. Germination (%) was recorded after 8 days.

2.3.2 Light

In many plant species germination is inhibited by continuous white light. The seeds of these species are dark-germinating. However, in some seeds dormancy is broken by light, however, their germination can also be inhibited by prolonged exposure (Bewley et al., 1986). The importance of light as a factor in germination has long been recognized. Already at the end of the nineteenth and the beginning of the twentieth century, a number of papers by Cieslar (1883), Gassner (1915) and by Lehman (1913) analyzed this phenomenon (Mayer *et al.*, 1963). It is possible that the light sensitivity of seeds has some relation to their germination in their natural habitat although this view is contested by others (Niethammer, 1922). Under natural conditions, seeds may be shed so as to fall on the soil, enter the soil or be covered by leaf litter, thus creating different conditions of light during germination. Among the species which have been investigated for their light response during germination at least half showed a light requirement. Kinzel (1926) lists hundreds of plant species which he divides into several categories. He found that about 270 species germinate above 20°C in the light and about 114 species germinate at the same temperature in the dark and about 190 species germinate in the light and about 81 species germinate in the dark after severe frost. After mild frost, about 52 species germinate in the light and 32 species germinate in the dark. In 1963, Mayer found 33 species are indifferent to light or dark.

Light which breaks seed dormancy is perceived by the phytochromes in the seeds. Phytochromes are the pigment which participates in the breaking of dormancy. A phytochrome can exist in two states: the Pr form (absorbs red light) and the Pfr form (absorbs far- red light). If Pfr is left in the seed at the end of the irradiation sequence, dormancy is terminated, but if Pr is left, dormancy is retained. When Pr is activated by red light (peak at 660 nm), it is converted to Pfr. When Pfr absorbs far-red light (peak at 730 nm), it is converted to Pr (Bewley et al., 1986). In more than 200 species it has been shown that germination is controlled by the Pr↔Pfr reaction. In about half of them germination can be triggered, just by a short period red light given. A quarter

of them need the repeating light to obtain a proper level of Pfr. However, a quarter of them are inhibited by the long period exposure (Chinese Biology Education, 2003).

2.3.3 The interaction between light and temperature

The effects of light conditions and temperature on germination show an interaction. The light requirement frequently depends on the temperature. For example in absence of light, Grand Rapids lettuce seed is dormant above 23°C (Bewley et al., 1986).

It is well established that synthesis and perception of the phytohormone gibberellin (GA) is essential for light and cold responses leading to seed germination. GA is required for seed germination. GA biosynthesis is related by both light and temperature at the level of transcription in the imbibed seed. The final step in active GA biosynthesis is catalyzed by the enzyme gibberellic acid 3-oxidase (GA3ox), and importantly, the expression of both seed-expressed GA3ox isoforms is promoted by both light and low temperatures. Light and cold have also been shown to increase the levels of bioactive gibberellins in the seed. SPATULA (SPT) is a basic helix-loop-helix transcription factor. It is a repressor of GA3ox expression in dormant seeds. It will repress germination to a small but significant extent in the absence of light. PIL5 is the related transcription factor in germination control. It will repress the seed germination in the dark (Penfield *et al*., 2005).

2.4 Seed Quality

Seed quality can encompass many parameters. For a commercial seed company, seed quality means high germination speed, uniformity of seedlings, high percentage of germination, uniformity of genetics and free of pests and diseases. However, in nature plants want their offspring to have variability in order to establish at least some offspring under certain conditions. Therefore, seed germination will show variation.

Seeds germinate at different times. They will differ in growth. They require slight differences in germination conditions. The size of the seedlings differs and some seedlings show deviations from normal plants. These phenomena can result from the genetic background, from the growth conditions during seed maturation or from less optimal conditions during germination. This variability is an undesired trait for the high seed quality that is pursued by commercial seed companies. They want good storability, high germination percentages and a high uniformity, both in shape and in genetics. Several studies have focused on this aspect of seed physiology (Wobus and Weber, 1999; Finch-Savage and Leubner-Metzger, 2006; Gutierrez *et al.*, 2007; Holdsworth *et al.*, 2008a; Holdsworth *et al.*, 2008b; Franklin and Quail, 2009). One method used to estimate seed quality is the standard germination test which is conducted under ideal and standardized conditions in the laboratory, according to the protocols of the International Seed Testing Association (ISTA) or Association of Official Seed Analyst (AOSA). The Seed and Production Physiology Department in Enkhuizen wants to elucidate factors involved in seed quality. They study the genetics behind seed quality, the impact of germination conditions and the involvement of maternal conditions during seed production. My research is part of this effort.

3. MATERIAL AND METHODS

3.1 Seeds

The *Brassica* seeds used are obtained from the different Syngenta production sites. Several different batches are used as indicated in Table 1. All seed batches were untreated and unprocessed. All of the varieties belong to cauliflower segment, except: batches 2009-B106, 2010-B0001, 2010-B0002, 2010-B0003, and 2010-B0004, which belong to the Chinese kale segment.

Table 1. The characteristics of seeds material used.

Number	Name	Batch#	Year	Production area	Remarks
2009-B106	A12		2009	South Africa	rapid cycling Chinese kale; First harvest; First sample
2010-B0003	A12[1]		2009	South Africa	rapid cycling Chinese kale; First harvest; Second sample
2010-B0004	A12[2]		2009	South Africa	Chinese kale; Second harvest
2009-B043	Aldrin	2233770	2008	South Africa	
2009-B036	Americo	2642760	2008	South Africa	
2009-B044	Baker	2532789	2009	South Africa	
2009-B045	Baker	1509277	2004	South Africa	
2009-B046	Baker	1890065	2005	South Africa	
2009-B140	Baker	2681787	2004	South Africa	
2009-B141	Baker	2533384	2009	South Africa	
2010-B060	Bruce	136742	2009	South Africa	
2008-B001	Cadillac				
2009-B001	Cadillac				
2010-B005	Cadillac				

2010-B059	Cartier	136731	2009	South Africa	
2009-B037	Cartion	2534360	2009	South Africa	
2009-B002	Clapton	2281074			
2009-B030	Clapton	2315925	2008	Chile	
2009-B031	Clapton	2583885	2009	Chile	
2009-B032	Clapton	2217871	2007	Chile	
2009-B038	Cleobis	2558464	2009	South Africa	
2010-B061	Cleobis	2558465	2010		Predecessor of 2010-B062; not-coated; not dormant
2010-B062	Cleobis	2572176	2010		Coated (dormant)
2010-B130	Cleobis	2816344			
2009-B029	Karnabit	2530006	2009		Similar to 2010-B136
2009-B034	Kornalu	2633128	2009	South Africa	
2008-B003	Monaco		2008	South Africa	Broccoli; Production experiment PvdW
2009-B107	SL101		2009	South Africa	rapid cycling Chinese kale (A12DHd) with a calabrese (GDDH33) QTL ， First harvest; First sample
2010-B001	SL101[1]		2009	South Africa	rapid cycling Chinese kale (A12DHd) with a calabrese (GDDH33) QTL ， First harvest; Second sample
2010-B0002	SL101[2]		2009	South Africa	A12 with a broccoli QTL; Second harvest
2008-B004	Solis		2008		benchmark 2008
2010-B137	Solis	2846404	2010	South Africa	B137, B138 and B139 are from one production. Size fraction: 2.00-2.25
2010-B138	Solis	2846402	2009	South Africa	B137, B138 and B139 are from one production. Size fraction: 1.75-2.00
2010-B139	Solis	2846406	2009	South Africa	B137, B138 and B139 are from one production. Size fraction: 2.25-2.50
2009-B041	Spacestar	2260214	2008	South Africa	

2009-B042	Spacestar	2340971	2008	Chile	
2010-B024	Spacestar	2705043	2010	South Africa	Fertilizer trial SAF
2009-B056	Tetris	1299242	2005	South Africa	

3.2 Germination

Germination can be tested on different substrates. Common practice at the Seedtech laboratory is to study germination on paper or on soil. Both methods are used. Also an additional method of germination on agar medium is used.

3.2.1 Paper germination

Paper germination was studied in the presence or absence of light. For experiments in the light transparent trays were used. For experiments in the dark gray trays were used. The dimension of the tray was 44cm x 29 cm. (Appendix 1, photo 1, 2). Two trays are used, one tray on the top as a lit, one at the bottom in which one layer filter paper and one layer light blue paper were positioned. Four hundred and twenty ml demineralized water was added to create a humid condition. A number of small pre-wetted germination beds (dark blue filter paper; dimensions 7.9 cm x 7.9 cm) were placed on the paper and 50 seeds were distributed on these beds. A maximum of 12 beds per tray were used. The trays were closed and put into transparent or opaque plastic bags depending on the desired light condition. Subsequently the seeds were placed in germination cabinets of which the temperature and light was set. Germination of seeds was counted on a regular basis. In the case of germination in the dark counting was performed in a room with safe light (green). To test the role of abscisic acid in cold germination, water was replaced by a fluridone solution (12mg/l). (Appendix 1, photo6) As a control in the fluridone experiment the tray were humidified with 420 ml water in which 1ml aceton per 1l was dissolved.

An alternative method to study the effect of fluridone was used to determine the effects of the fluridone incubation. More than 200 seeds were put in a container with 200ml of either a fluridone (12mg/l) or control solution (1ml aceton/l) and incubated for two hours at room temperature and in the light on a shaking table. The seeds were collected seeds with a mesh and rinsed with water. Then 50 seeds were placed in the germination trays; 2x 50 for the incubation in the light, 2x 50 for the incubation in the dark and germination was monitored.

A second alternative method to treat the seeds with fluridone was used to compare our methods with a standard protocol. Two hundred seeds were put in one bag and several bags were put in a bottle with the fluridone or control solution. In this setup the incubation was mixed every 15 minutes by a spoon (Appendix 1, photo 7). Then 50 seeds were placed in the germination trays; 2x 50 for the incubation in the light, 2x 50 for the incubation in the dark. Subsequently, germination was monitored.

3.2.2 Soil germination

Soil germination was studied in the presence or absence of light. About 40 ml soil was put in a small plastic box (8cmx12cm) and approximately 40 ml demineralized water was added. Then 50 seeds placed in a box. A maximum of 8 small plastic boxes were placed in either transparent or dark gray trays, depending on the light condition wanted. The trays were closed and put them in the cabinet of which the temperature and light condition was set. Subsequently, germination was monitored.

3.2.3 Agar germination

Agar germination was also studied in the presence or absence of light. Water-Agar plates were prepared as follows. Ten g Micro-Agar was transferred to a Medium

bottle and 1 l of demineralized water was added. The bottle was placed at 120 °C for 1 hour in an autoclave. When bottle was cooled down and the 25 ml of the solution was poured into the petri dishes. Plates were allowed to cool down and solidify in the flow cabinet and were stored until used in the cold room. Fifty seeds were distributed on each agar petri dish. Subsequently the seeds were placed in germination cabinets of which the temperature and light was set. Subsequently, germination was monitored.

3.2.4 Software

Data was analyzed with excel. Germination curves were analyzed with the Germinator Program (Joosen *et al.*, 2010). This program fits the germination during time to a sigmoidal germination curve. Curve-fitting program is a module written for Microsoft Excel. It is used in combination with the least sum of squares method to find the right parameters to fit the curve to a four parameter Hill function: $y=yo + (G_{max}*t^b)/(T_{50}{}^b+t^b)$.. In this equation "y" is the cumulative germination percentage at time t, "y0" is the intercept on the y-axis at t=0 (usually 0), "G_{max}" is the maximum cumulative germination percentage (≤100), "b" is controlling the shape and steepness of the curve and "T_{50}" is the time required for 50% of viable seeds to germinate (Joosen *et al.*, 2010).

4. RESULTS AND DISCUSSION

In order to test of Arabidopsis can be used as a model system for *Brassica*, experiments to compare germination of Arabidopsis with *Brassica* were performed. Germination of Arabidopsis is known for its dependence on a stratification treatment, hence, several Cauliflower varieties (Bruce, Karnabit and Cartier) and a single Arabidopsis ecotype (Columbia) were germinated with and without a stratification treatment. The stratification treatment consisted of 3 days at 12°C in the dark (cold room). As expected, germination of Arabidopsis was stimulated by the stratification treatment, both speed and total germination was increased (Fig. 2). Surprisingly, all cauliflower varieties already germinated during the stratification treatment and no major differences in this behavior were observed between the three varieties. Hence only Cauliflower var. Bruce is shown in Fig. 2. Because this germination of *Brassica* varieties in the dark could be of interest for seed physiology and production, this phenomenon was studied in more detail.

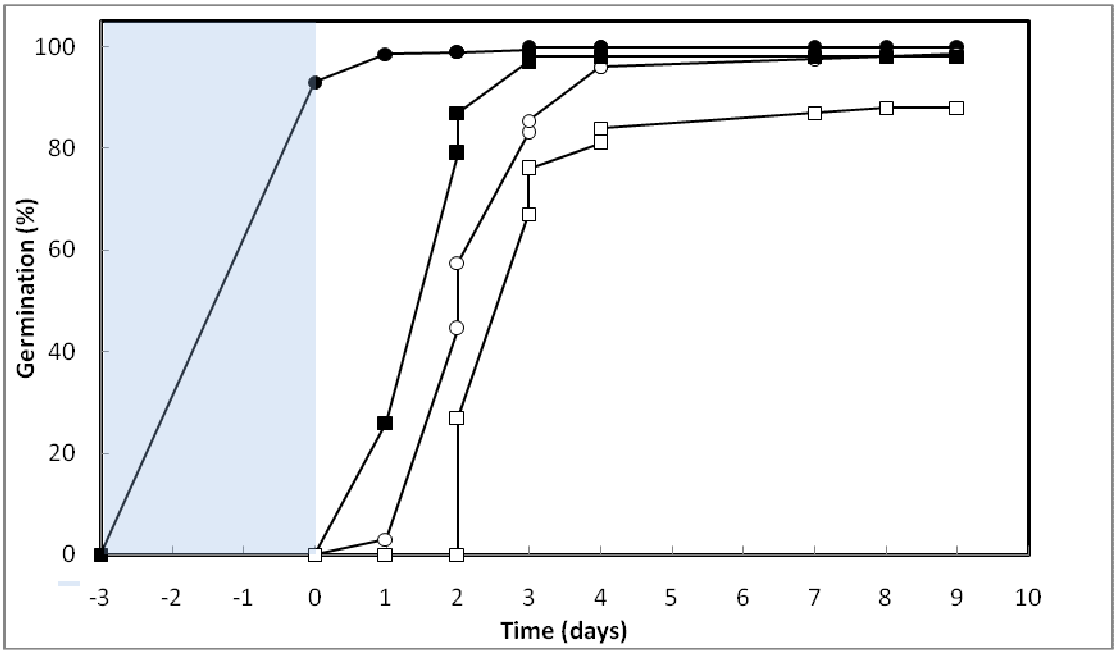


Fig. 2 Germination of Cauliflower var. Bruce (circles) and Arabidopsis eco. Columbia (squares) with and without stratification treatment. Germination was studied using paper. The light blue area indicates the stratification period at 12°C and in the dark. After the cold period trays were transferred to illuminated germination cabinets at 25°C.

For 10 *Brassica* varieties germination in the absence and presence of light at 10°C

was characterized. In Fig. 3 germination of seeds of cauliflower var. Bruce and Spacestar is shown as an example of the datasets that were obtained. These dataset were analyzed using the Germinator Germination Curve Fit Procedure (it has to be stated that for the curve fit procedure only the data points were used, which were obtained before the seeds were transferred to 20°C). In Fig. 3 the parameters and curves which were obtained by the curve fit procedure are graphically explained and shown. It becomes clear that the two parameters, G_{max} and T_{50}, characterize the germination characteristics of the varieties, hence, these parameters are used to describe germination of the ten varieties. G_{max} represents the germination percentage and the T_{50} describes the germination rate. It can also be observed that the fit procedure results in good fits even when the datasets are less than perfect (e.g. in the case of cauliflower var. spacestar).

But before the effects of light and darkness are discussed it should be noticed that transfer of the seeds to 20°C after a period of 10°C did not result in germination of the, until then, not-germinated seeds. However, it is known that these seed batches germinate with a high G_{max} up to 100% (data not shown) when placed immediately at 20°C. This suggests that at 10°C some seeds will germinate will others enter a condition of secondary dormancy.

In Fig.4A and B the obtained germination parameters of ten *Brassica* varieties are shown. Moreover, of two varieties also germination characteristics of two different productions (harvests) were shown. First of all it can be observed that variation of the light conditions does not affect the germination rate (Fig.4B) within one variety. Only in the case of Cauliflower var. Solis an effect of light conditions on the T_{50} can be observed in Fig 4B. However, this is not due to an effect of light on germination rate. Because total germination of this variety was so low (Fig.4A) the fit procedure on this data set failed to estimate a reliable T_{50} although a G_{max} could be obtained. However, there is a difference in rate between varieties. Most varieties have a T_{50} around 100 h. A few have a T_{50} around 140h. Interestingly this difference can also be observed

between the A12 and SL101 varieties. These two varieties are closely related to each other. The SL101 variety is the A12 variety in which a seed vigor quantitative trait locus (QTL) has been introduce by breeding (Table 1). Apparently this QTL has a positive effect on the germination rate. Another deviating variety is the Moncao variety which has a T_{50} of approximately 85 h. However, this variety belongs to the broccoli segment (Table 1). Therefore the results with these varieties suggest that the germination rate at 10°C is mainly determined by the genetic background and is not affected by light conditions.

Larger differences were observed between the G_{max} values, not only between varieties but also between light treatments. Some varieties germinated up to 100% in both conditions, whereas others did not perform that well. However, there appeared to be a relation between sensitivity towards light conditions and maximum germination at 10°C in the dark: the higher the germination in the dark is the less the impact of light on germination is. In the context of the seed vigor QTL it could be observed that the QTL improved both dark and light germination. SL1011 and SL1012 are harvested together A121 and A122, respectively. When one compares the simultaneously harvest batches an improved G_{max} due to the QTL can be observed and also when G_{max} is improved the inhibition by light is reduced.

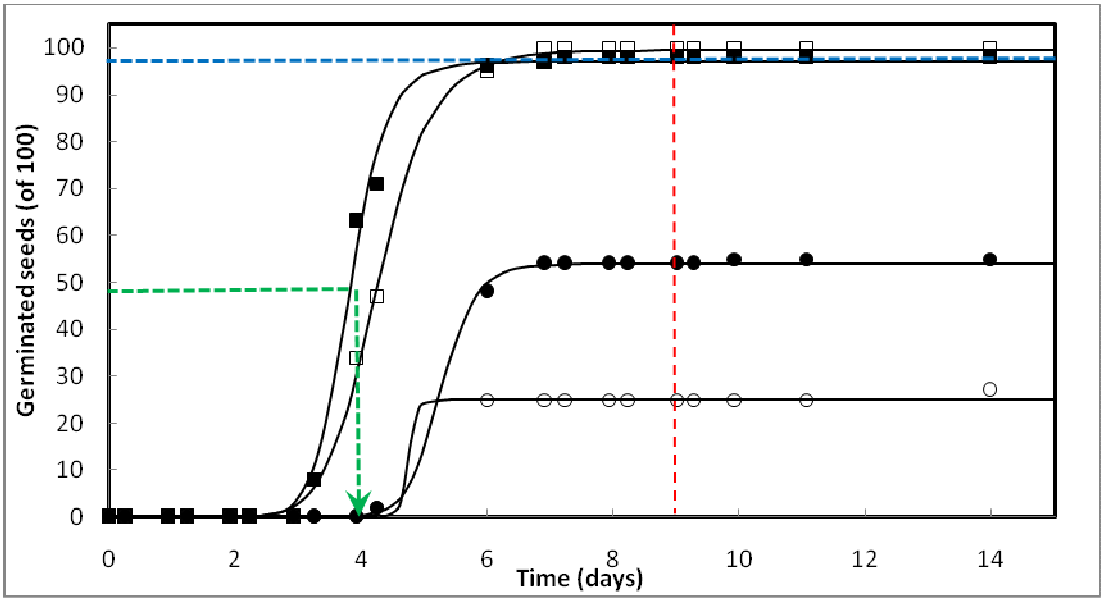


Figure 3. Germination of Cauliflower var. Bruce and Spacestar in the absence or presence of light

at 10°C. Bruce (□, light; ■, dark). Spacestar (SAF, 2010) (○, light; ●, dark). The black smooth line indicates fitted curve obtained by Germinator. Indicated are the different parameters, which are obtained by the curve fit procedure for Cauliflower var. Bruce in the dark. Blue dash: Maximum germination (G_{max}); green dashed arrow points at the time at which half of G_{max} is reached (T_{50}). These parameters were calculated for all varieties used. The red line indicates the moment when the seeds were transferred to 20°C. For curve fitting only the data points before the transfer to 20°C were used.

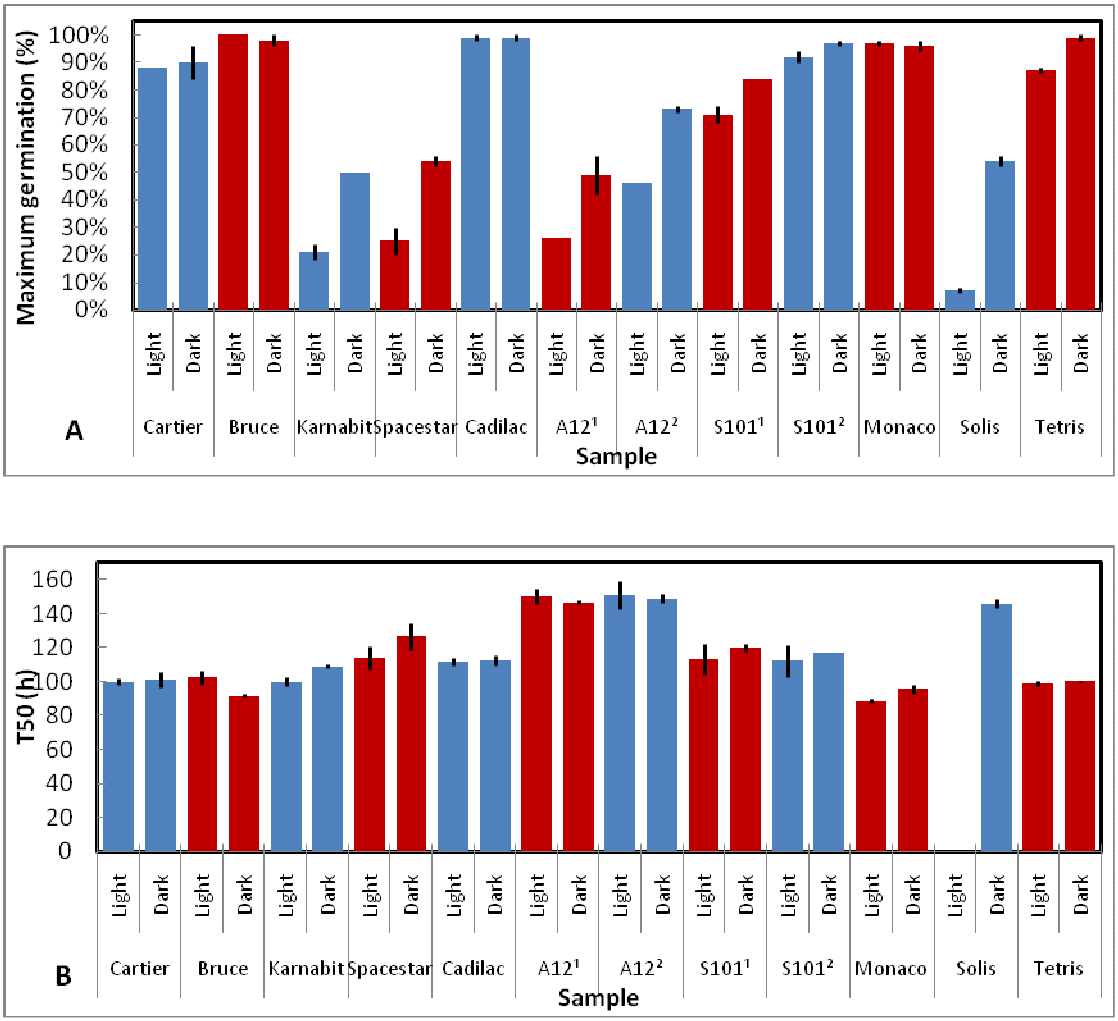


Figure 4. Maximum germination (A) and T_{50} (B) of 12 different seed batches of 10 different varieties of *Brassica* in the absence and presence of light at 10°C. Of the varieties A12 and SL101 two different batches, which were harvested at different moments, are tested. Error bars indicate the standard deviation.

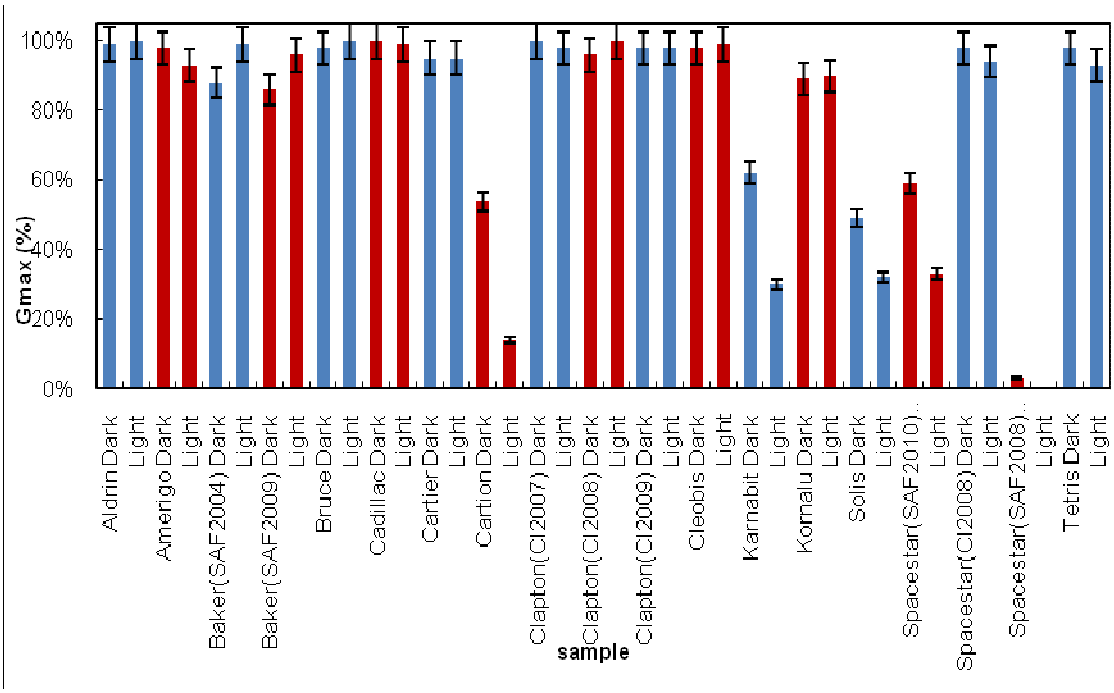


Figure 5. Maximum germination of 19 different seed batches of 13 different varieties of *Brassica* in the absence and presence of light at 10°C. Error bars represent the standard deviation of 2 replications.

In order to prove the observation above further and characterize more varieties, more *Brassica* varieties, 19 varieties, were used to do germination in the absence and presence of light at 10°C. Some of the varieties where already tested in the previous experiment. Also germination of one variety but from different production sites or different harvest years was characterized.

In Fig. 5 the G_{max} of 13 *Brassica* varieties are shown.

Most of the batches show a germination up to 100% in the dark. Only Cartion, Karnabit, Solis, Spacestar (SAF2010 and SAF2008) showed germination of less than 60% in the dark. The observation that the higher germination in the dark is the less the impact of light on germination can also be observed again (Fig. 5). The results with cauliflower var. spacestar show that one genotype can result in seed batches with different sensitivities towards light and temperature. While the batch from Chile 2008 (CL2008) shows a high tolerance against low temperature and light conditions the other two batches are sensitive to different degrees (both from South-Africa but

harvest in 2008 or 2010; SAF2008, SAF2010). However, different productions of cauliflower var. Clapton and Baker showed a consistently a high germination at low temperatures in the dark. These observations might indicate that the sensitivity of germination of different seed batches for low temperatures is influenced by the production circumstances. However, a variety needs to be sensitive to these conditions. Apparently the cauliflower varieties Clapton and Baker are not sensitive to these conditions and therefore will always perform good at low temperatures. The cauliflower var. Spacestar on the other hand can perform good but when during production harmful conditions are met germination will become sensitive to low temperatures.

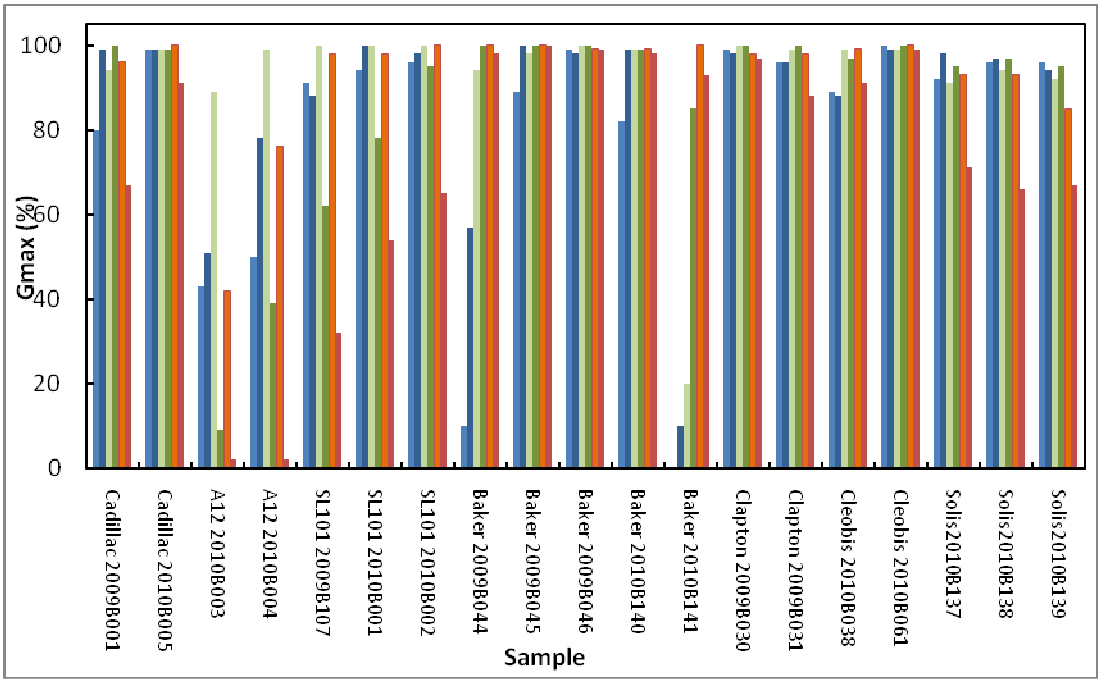


Figure 6. Maximum germination of 19 different seed batches of 7 different varieties of *Brassica* in the absence and presence of light at 10°C, 20°C and 30°C. The blue (light, dark) bar indicates germination at 10°C, green (light, dark) bar indicates germination at 20°C and orange (light, dark) bar indicates germination at 30°C. The intensity of the color indicates terminated in the dark (dark color) or in the light (light color).

In order to get information on the relation between germination at low temperatures and the temperature dependence of germination, germination of 19 seed batches was characterized in absence and presence of light at 10°C, 20°C and 30°C, respectively.

These seed batches belong to 7 different varieties (Fig. 6). Within varieties variation between different production sites, harvest years or different post-harvest processes was sought.

It again becomes clear that some varieties can become sensitive to low temperatures (Fig. 6). For the cauliflower var. Baker for which in the previous experiment no sensitive batches were observed now some seed batches were obtained which were sensitive. Apparently, the change of becoming sensitive can be low for some varieties and high for others, but more observations are needed to confirm this.

In general, it can be observed that the optimal temperature for the germination of cauliflower is around 20°C. Differences between varieties were that germination of some varieties was more affected by high temperatures than by lower temperatures while for others it was *vice versa*. Some of them germinated well at all the conditions.

As mentioned above, the seed vigor QTL improved G_{max} and T_{50} of SL101 at 10°C. The similar phenomenon was showed at 20°C and 30°C. A12 had smaller G_{max} at 20°C and 30°C in the dark and light as SL101. This suggests that the QTL affects germination in general.

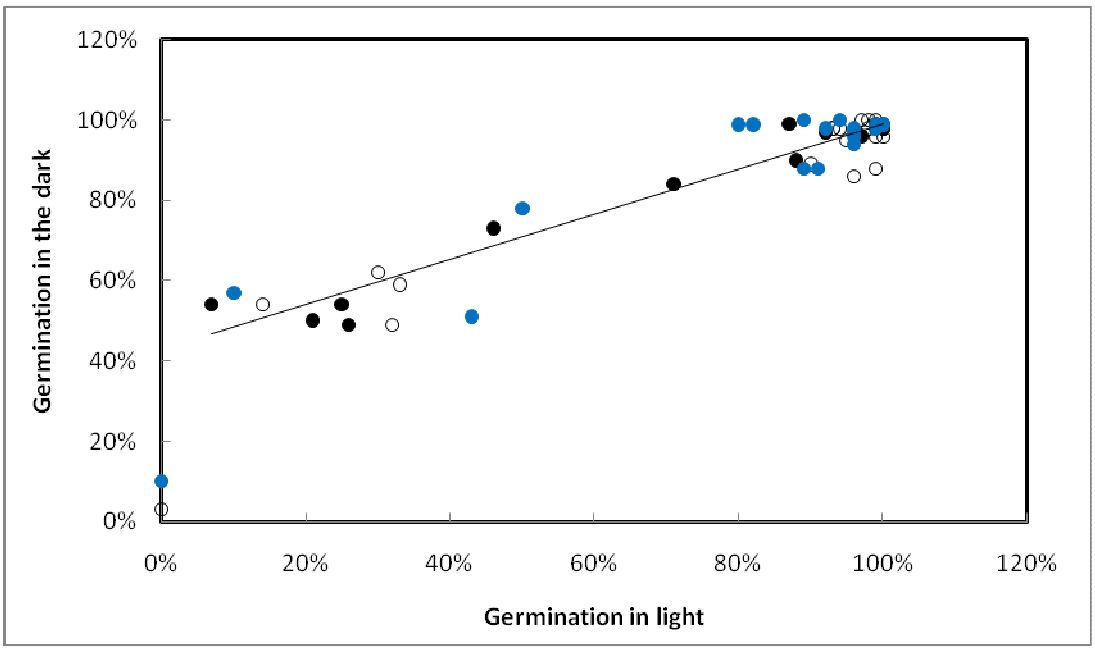


Figure 7. The relation between maximum germination in the light and maximum germination in the dark at 10°C. Graph shows the accumulated data of Figs. 4A (open circles), 5 (black closed circles) and 6 (blue closed circles). The regression line is calculated using the data of all experiments, excluding the two data points close the origin. The line is described by the equation: $y = 0.56x + 0.43$ ($R^2 = 0.9035$).

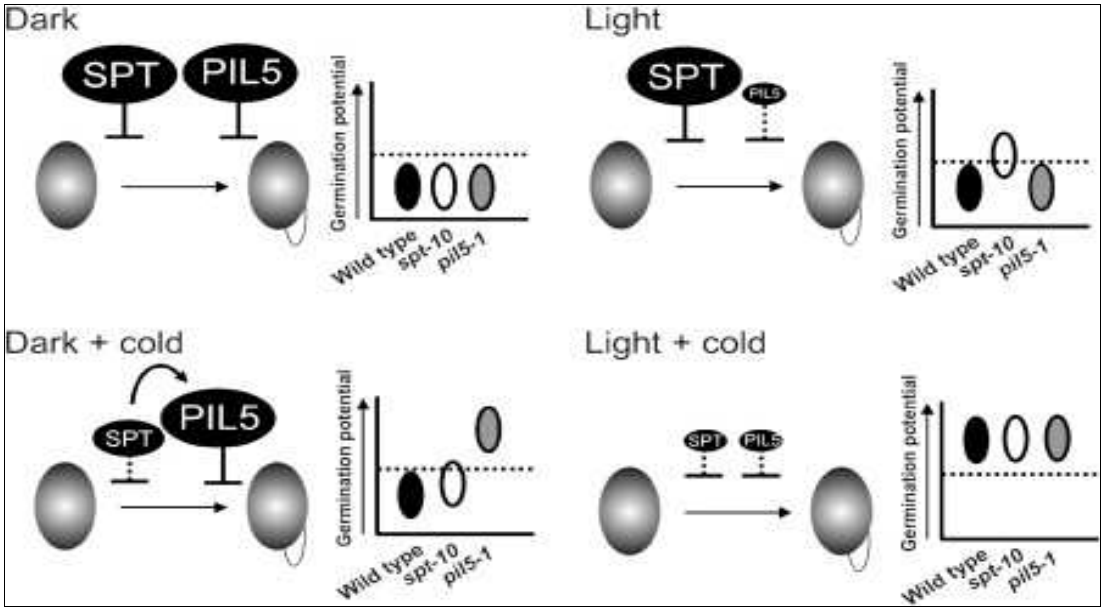


Figure 8. A Model Representing the Control of Seed Germination by SPT and PIL5 after Light and Cold Treatment in Arabidopsis. The relative repressive activity of SPT and PIL5 under each condition is represented by changes in the size of either protein symbol. A given population of seeds exhibits a range of germination potentials, depicted by the ovals (right), and if this exceeds a critical threshold (shown by the dashed line) in an individual seed, then germination will occur. In dark stratified seeds SPT activity appears to be dependent on PIL5 (denoted by arrow). Adapted from Penfield *et al.* 2005

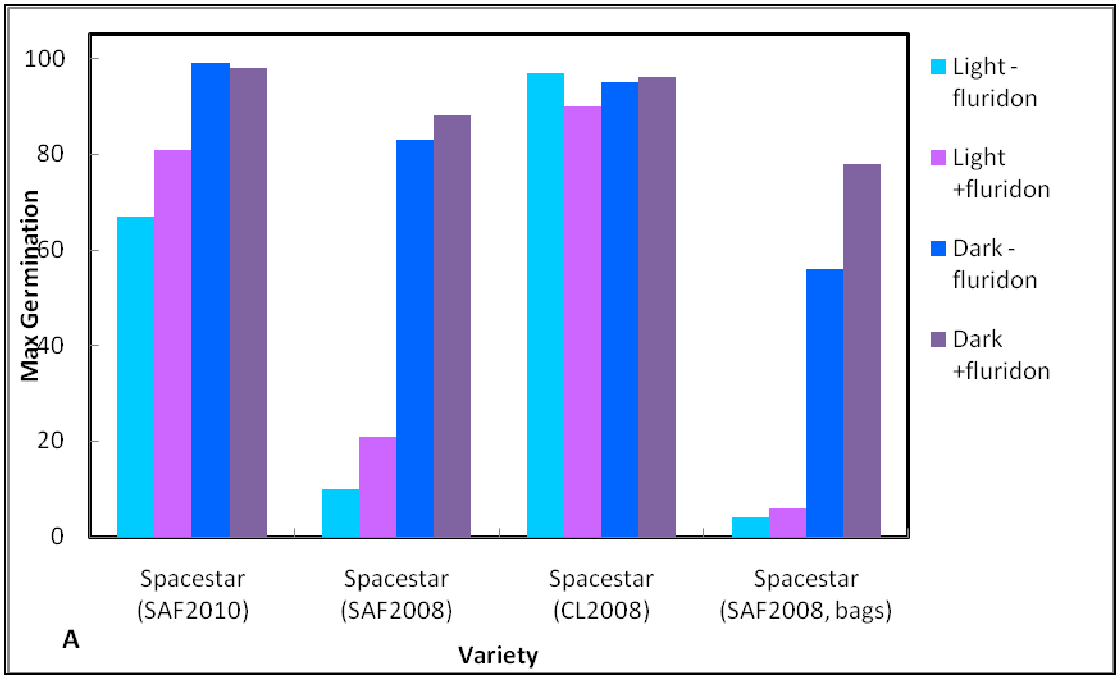
A relation between a decreased G_{max} in the dark and an increased inhibition by light was already observed in Figs.4A, 5 and 6. However, in Fig. 7 this relation is better visualized. This relation suggests that when a seed is in prime condition it can cope with a reasonable amount of stress. However, if a seeds is not at an optimal condition stress (low temperature) will reduce its germination adding more stress in the form of light will then reduce germination even further. And the more a seed is stressed by the first condition the more impact the second stress has. The reason why germination in the dark stays reasonable until it really collapses (the two points at the origin) while in the light there is a more gradual drop till almost nil germination needs further investigation.

In Arabidopsis a model has been developed which describes the influence of light conditions and temperature on germination (Fig. 8; Penfield *et al.* 2005). In this model it becomes clear that for Arabidopsis both light and cold are needed to remove either the blockade by darkness (PIL5) or the blockade by high temperatures (SPT). If one of them is not removed germination will not occur. In *Brassica*, however, we have a different situation at low temperatures light does not improve germination but inhibits germination. In order to adapt this model for *Brassica* the effect of light and darkness on the presence of PIL5 must be opposite to the one in Arabidopsis. Hence the level of PIL5 should be lower in darkness than in light. Although this model does not explain the *Brassica* situation completely, it gives us a clue for further study. The model by Penfield is very absolute. Our observations with *Brassica* might be explained by a more gradual effect of the levels of PIL5 and SPT on germination. However, the model of Penfield is a nice basis for further study.

Finally in order to characterization the role of ABA in the germination of *Brassica* at low temperatures and darkness the effect of fluridone on germination was studied. Fluridone was able to partly stimulated germination in the dark and light at 10°C. This means that the production of ABA plays a role in the reduced germination at low

temperatures. (Fig. 9A).

The methodological aspect of these experiments shows that incubating the seeds in the glass container without bags and on the shaking machine is better than incubating the seeds in the bags. Apparently the contact of the seeds with the solution is better when they are separate is in the first method. However, form Fig.9B it becomes also clear that the preincubation alone already removes some of the blockade imposed by light because dry seed, which means not pre-incubated with solution, germinate less in light then the mock-incubated seeds. This must be studied further because this shows that temperature sensitivity can be removed within a few hours by imbibitions at room temperature in the light.



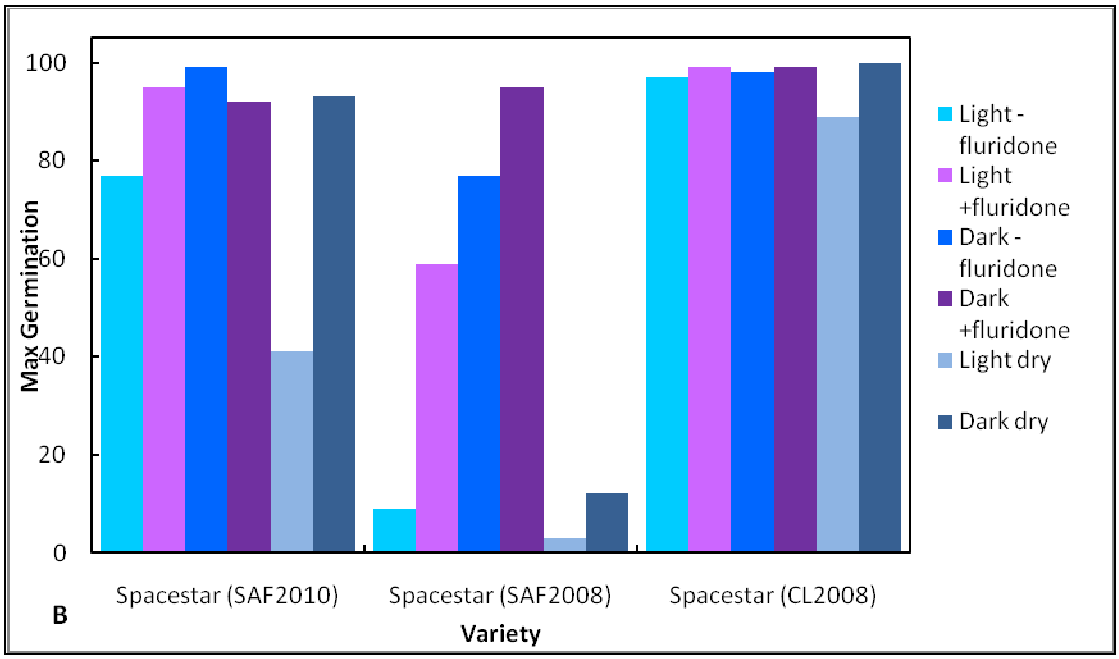


Figure 9. The effect of fluridone on the germination of Spacestar from different production sites in the presence and absence of light at 10°C. Because of doubt about the incubation methods two methods of fluridone treatment were compared. Seeds were either placed in a small mesh bag or lose in the fluridone solution (panel A). However, the preincubation in the solution was also doubted therefore a control was added in which the seeds were placed of the paper without pretreatment (panel B).

5. CONCLUSION AND RECOMMENDATIONS

The influence of light conditions on germination at low temperatures was studied. It could offer the basis of seed batch selection (a cold-germination test) with the consideration of stress tolerance based on temperature and light sensitive for the company. If they can germinate well at low temperatures, maybe they can resistant other stresses, as drought, as well. This might be good tool for breeders. However, the relation between being sensitive to low temperatures and being sensitive towards other stresses needs to be assessed.

However, it has become clear that not the sensitivity of germination for low temperatures is variety dependent but the ability to become temperature sensitive is. Apparently deleterious conditions during seed production trigger this sensitivity and it appears that different varieties have a different sensitive towards these deleterious production conditions. Which condition triggers the temperature sensitivity remains unclear.

After the experiments with these 40 seed batches of the different *Brassica* varieties, I think I might concluded that Bruce, Spacestar (CL2008), Cadillac, Monaco and Tetris are good seed batches. They are not very sensitive with light and can germinate well in a cold environment.

The model presented in Penfield *et al.* (2005) suggests some further experiments in which the role of SPT and PIL5 in the temperature sensitivity of germination of *Brassica* seeds might be determined.

REFERENCES

1. Cross Jacqueline, 2008. How to Grow Cauliflower.
2. Naeve L, 1997. Growing Cauliflower
3. A.M. MAYER and A. POLJAKOFF-MAYER, The Germination of Seeds, 1963, Botany Department Hebrew University Jerusalem,
4. William E. Finch-Savage and Gerhard Leubner-Metzger, Seed Dormancy and the Control of Germination, 2006
5. http://en.wikipedia.org/wiki/Brassica
6. http://www.britannica.com/EBchecked/topic/77905/brassica
7. https://www.seedtest.org/upload/cms/user/S4.9.0900.Powell1.pdf
8. http://www.vegetableexpert.co.uk/GrowingBrassicas.html
9. http://www.ipm.iastate.edu/ipm/hortnews/1997/2-28-1997/cauliflower.html
10. http://tomclothier.hort.net/page11.html
11. http://baike.baidu.com/view/33958.htm
12. http://en.wikipedia.org/wiki/Brassica_oleracea
13. http://baike.baidu.com/view/147829.htm
14. http://lawrencehallofscience.org/foss/fossweb/teachers/materials/plantanimal/brassica.html
15. Bewley J.D, Black M. 1983. *Physiology of development and germination*. New York.
16. William E. Finch, Gerhard Leubner. 2006. Seed dormancy and the control of germination. New Phytologist journal 171:501-523.
17. http://211.143.182.157:8000/czpd/kczy/shang/sw/1/11/rj-kebiao/1/kzzl5.htm
18. http://4e.plantphys.net/article.php?ch=t&id=8
19. Taiz Lincoln and Zeiger Eduardo. 2006. Plant Physiology, chapter 23: topic 23.5
20. Joosen, R.V.L., Kodde, J., Willems, L.A.J., Ligterink, W., Plas, L.H.W.v.d., and Hilhorst, H.W.M., 2010. Germinator: A softeware Package for High-Throughput Scoring and Curve Fitting of Arabidopsis Seed Germination. Plant J. 62, 148-159.
21. Penfield Steven, Josse Eve-Marie, Kannangara Rubini, Gilday Alison D., Halliday Karen J., and Graham Ian A., 2005, Cold and Light Control Seed Germination

through the bHLH Transcription Factor SPATULA. Current Biology, Vol. 15, 1998-2006.

22. Hedden Peter and Graebe Jan E., 1985, Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of Cucurbita maxima endosperm andMalus pumila embryos. Plant Growth Regulation journal 4:111-122.
23. Yoshioka Toshihito, Endo Takashi and Satoh Shigeru, 1998, Restoration of seed germination at supraoptimal temperatures by fluridone, an inhibitor of Abscisic Acid biosynthesis. Plant Cell Physio journal 39: 307-312.

APPENDIX

Photos

Photo1. Seeds germination in the transparent tray on paper

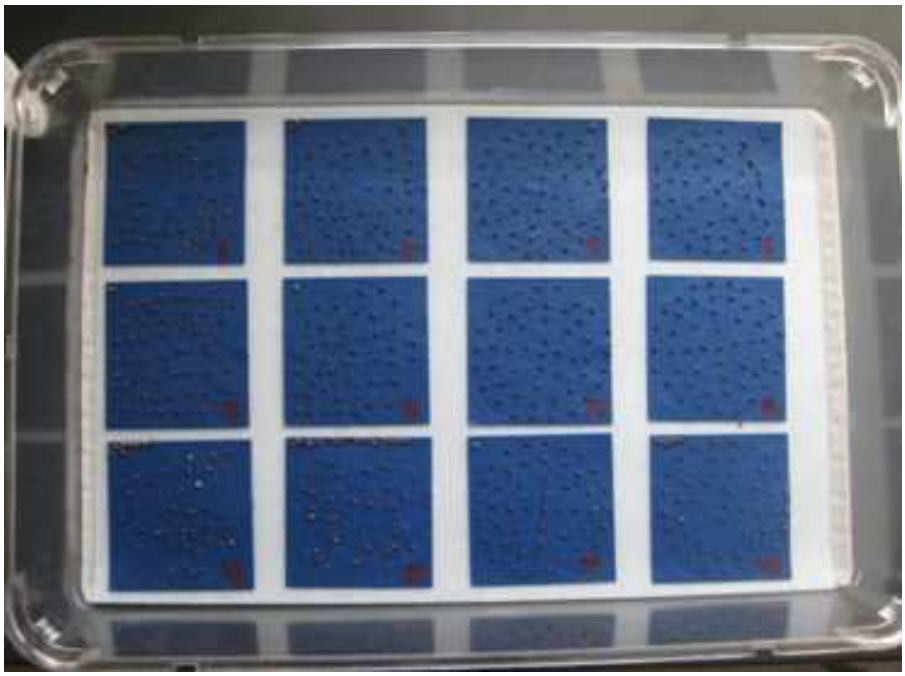


Photo2. Seeds germination in the grey tray

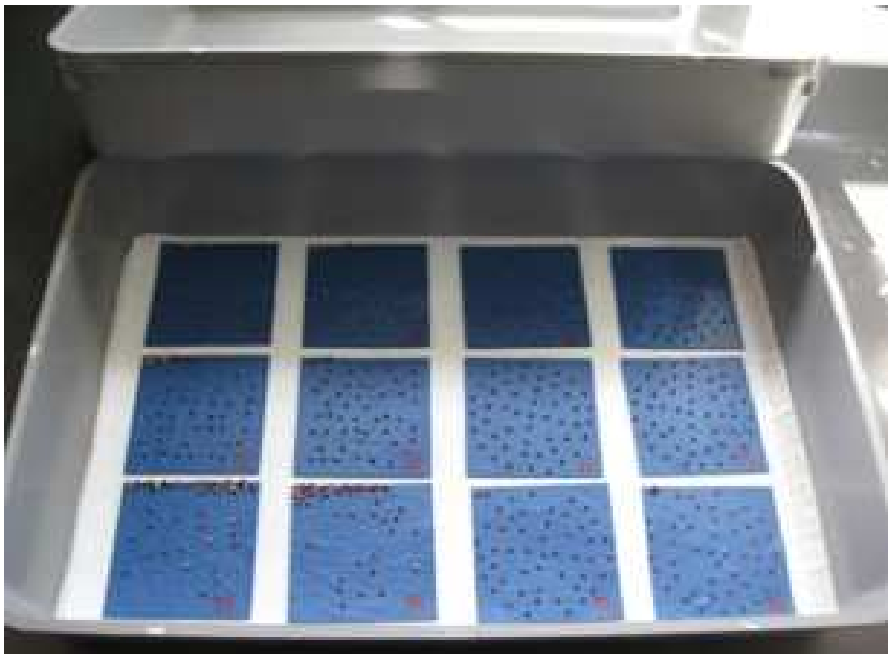


Photo3. The trays in the 10° C cabinet

Photo4. Seeds germination on soil in the plastic box (8cmx12cm)

Photo 5. Trays for decreasing evaporation for seeds germinate on soil.

Photo6. Seeds incubate with fluridone treatment on the shaking machine

Photo7. Another way for fluridone treatment

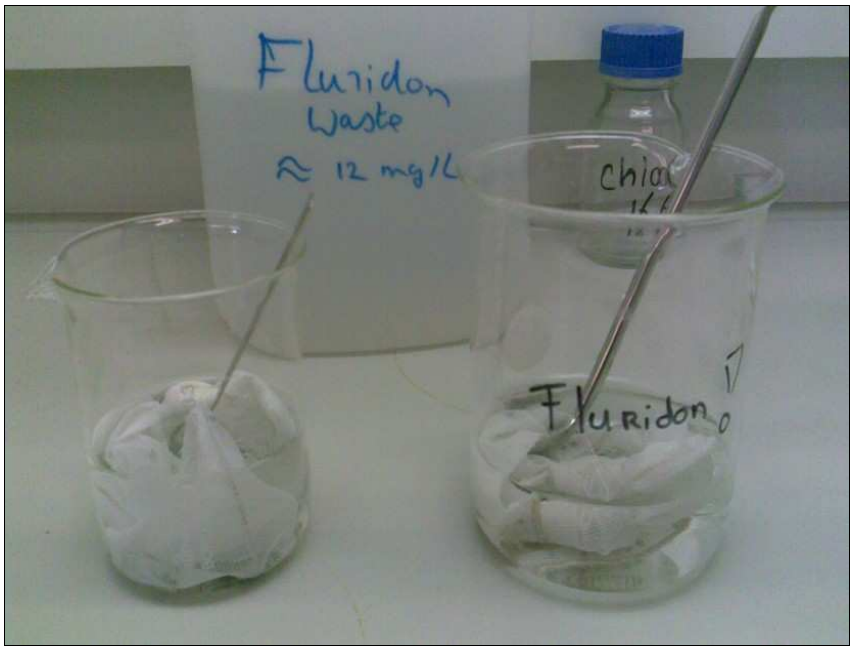


Photo8. Seed Germination in the Agar