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The survey of the effect of the acceleration aging and cold pretreatment on germination and other factors of the plantlet growth in two varieties of canola

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Abstract

One of the most important problems with oily plants like canola is their storage. Because if the storage of the seeds is not suitable the seeds will oxidize as they are oily and they lose their germination ability much faster than the seeds of the other plants. Therefore to assess the effect of cold and accelerated steers on germination and growth indexes of the plantlets of the two varieties of canola in a factorial experiment and in a randomized complete block design with three replication was conducted. In this research 3 factor including the first factor of two canola varieties (Hayloa -401 and RGS), the second factor of to 10 heat levels (1-30 C , 2- 40 C , 3-50 C ,4-60 C, 5,-20 C,6-30 C°+cold ,7-40 C° +cold ,8-50 C°+cold ,9-60 C°+cold ,10-control), and the third factor of the exposing time to heat in 4 levels (1, 2, 3, 4 days) were used. Finally the length of the plantlets, radicals, plumules, the percentage of germination, the rate of germination and the index of the seed growth were measured. The results showed that both the number of days of thermal treatment and different temperatures had significant effect on the measure factors. It also became clear that the more the temperature and the number of thermal treatment days, the more the effect of them on the reduction of germination of the two mentioned varieties. Furthermore the results showed that if the aged seeds (under the effect of heat) are exposed to cool, the germination will not change significantly and the seeds will have a low germination capacity, and the cold can't stop the negative effect of the heat and the humidity. So, it seems that suitable storage can be a good solution to maintain the germination capacity of canola seeds.

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Introduction

Oilseed rape is one of the most significant agricultural species of brassica genus the wild type of which is limited to Europe and North Africa (Anonymous, 2003).In a general classification, the oilseed rape is divided into two main categories. The first group is called industrial and the second one is called edible rapeseed. The plants of brassica genus are divided into two major groups based on the level of erusic acid (an unhealthy fat acid for animals and livestock). The first group is shortly called "HEAR" the oil of which has more than 5% erusic acid and it is regarded as inedible. The second group is called "LEAR" the oil of which has less than 5% erusic acid and it is regarded as edible. In addition to the above-mentioned unhealthy material, there is another material in meal and forage of the oilseed rape called "Glucosinolate". A study of elements of germination of 9 genotypes of oilseed rape suggested that temperature has significant effects on the time of germination start, rate, percentage and maximum germination of oilseed rape so that a decline of temperature from 20 to 4.5 °C quickly delays the start of germination and as a result, the uniformity and maximum level of germination reduce when the temperature reduces to less than 15°C. In comparison, the germination rate increases as the temperature increases from higher than zero to 4 and up to 16 and 20°C (Qasemi Golazani, K. 1995). One of the significant tests of quality and vigor of seedling is accelerated aging (AA) test. Accelerated aging is positively associated with seedling emergence rate (SER) and seedling establishment in the farmland (Hebert, *et al.,* 1995). The accelerated aging (AA) test can estimate the amount of seedlings that are able to grow in field conditions (Mir Mohammadi Mobayedi, S, M. 2005**)**. The oilseeds compose the second largest food reservoir of the world after crops. The oilseed rape is one of the most significant oil plants in the world so that it is regarded as the third significant oilseed after soybean and cotton. On the other hand, the environmental stresses are one of the most important factors contributing to reduction of agricultural products in the world (Harmam *et al.,* 1999). One of the major elements affecting the production of agricultural items in different regions of Iran is coldness (Behpuri *et al.,* 2010).

Up to now, there have been numerous attempts for increasing the germination ability of the seedling. It seems that the germination ability of the seedlings is highly dependent on genotype, conditions of seed bed, plantation temperature and their mutual effects (MC Donald, MB. 1999). Certain pre- treatments such as application of external ABA in peanut which maintains germination in the stage before root emergence lead to increase of germination percentage of seedlings in dry conditions. In addition, dewatering pretreatments of rapeseed led to a condition in which the seeds grew with higher rate and uniformity compared with control seedlings and in the conditions of salinity, dryness and coldness (Zheng, *et al.,* 1998). In the present study, the role of cold pretreatments in developing resistance against coldness based on morphological characteristics of planted oilseed rape cultivars in the temperature of -20°C. Behpuri *et al.,* 2010 did a test on 2 safflower cultivars and 4 temperature treatments of 30, 40, 50 and 60°C to induce seed deterioration. They reported that seed deterioration resulting from applied temperatures influences percentage and rate of germination as well as rootlet and Caulicle. In a test on rapeseed, the effect of -20°C on different times was examined to clarify the effects of cold pre-treatment. It was reported that the cold pretreatment with temperature of -20°C increases the rate of germination of rootlet and Cauliclewhile no effect on germination percentage was observed (Fahimi Rad, Kimi Zade and Qanadi, 2010). The results of associated studies show that the rapeseeds lose their quality faster when exposed to higher temperatures compared with lower ones so that if the seedlings are stored in improper conditions (i.e. high temperature and humidity), the germination power and vigor of the cultivars significantly reduce. Therefore, for better storage of rapeseeds for plantation in the next year attention to maintenance conditions of the seedlings is highly critical and sensitive (Rabii and Bayat, 2010). The results of literature reviews point to reduction of germination rate due to deterioration (Qasemi Golazani, 1995).

The environmental conditions in which the seedling is stored determine the duration in which the germination potential and vigor of a seedling are maintained. Temperature, relative humidity of the environment and humidity of the seedling are the main factors affecting the storage capability of the seedling (Rabi & Bayat, 2010). The test on deterioration of sunflower seed shows that in the condition of seed deterioration, the germination percentage reduces compared with normal conditions (Latifi *et al.,* 2004). During a test on 6 rapeseed cultivars in spring and summer, the effects of two times of harvest (i.e. harvest on physiological ripeness and total ripeness) on germination and vigor of rapeseed cultivars were examined (Hebert *et al.,* 1995) The tests on measurement of seedling vigor such as standard germination test, accelerated aging test and cold test. The results showed that despite of the fact that the difference between germination capacity and vigor for seedlings harvested in total ripeness stage is little, the temperature and humidity stresses had insufficient and insignificant influence on seedling vigor in this stage as shown in accelerated aging test and cold test. However, the harvested seedlings in the time of total ripeness had higher vigor and survival probability compared with the time of physiological ripeness. In addition, the spring cultivars lacked significant difference with other ones in regard to standard germination test, accelerated aging test and cold test while the spring cultivars manifested significant differences in the above mentioned tests (Hebert *et al.,* 1995). The accelerated aging test and controlled deterioration test were used to evaluate the vigor of sugar beet and determine the alignment of results of tests with farmland growth percentage of seedlings (Silva *et al.,* 2006).

This paper aims at clarifying the unsuitable conditions of storing on germination like canola in a sense that it makes clear if per treatment has any positive effect on seed or not. Unsuitable stores cause a huge amount of seed is ruined. Because if the storage of the seeds is not suitable the seeds will oxidize as they are oily and they lose their germination ability much faster than the seeds of the other plants.

Materials and Methods

Design of tests and treatments

The present study was done in Agricultural Department of Islamic Azad University of Fasa. The seedlings of cultivar Hayola 401 were supplied from Center of Agricultural Services of Fasa Town because the above-mentioned cultivar is planted in Fasa. The cultivar RGB was supplied by Agricultural Organization of Darab because it is planted there. Both types of the seeds had been previously delivered by seed modification centers to the agricultural organizations of both towns. The test was done in a factorial manner based on completely random blocks with 3 iterations. In the present study, 3 factors including the first factor (i.e. two rapeseed cultivars of Hayola 401 and RGS), second factors with 10 temperature levels (3°C, 40°C, 50°C, 60°C, -20°C, -30°C, -40°C, -50°C, -60°C and control) and third factor (i.e. length of exposure to temperature in four levels of 1, 2, 3, and 4 days) were used with three iterations. It is noteworthy that the treatments examined in regard to both parameters of temperature and coldness were first maintained in the desired temperature and then transferred to cold conditions for 4 days so as to undergo germination test. In addition, the essential humidity similar to the one in the storage was obtained through placement of a water container in the heating machine.

Application of seed-deterioration treatments and cold pre-treatment

To provide seed deterioration treatments, the seedlings were placed inside a Petri dish and over the incubator meshes. Different temperatures and durations were applied on the treatments. To develop essential humidity, the water container was placed in the lower tiers of incubator so as to generate 80% humidity. For obtaining cold treatments, the seedling was placed in a freezer with temperature of -20°C for 4 days.

Disinfection steps and germination power test

Before doing the test, the seedlings were disinfected by hypochlorite sodium 5% for 5 minutes and washed for several times with distilled water. This was done to inhibit the contamination of seedlings (Halder *et al.,* 1993).

For evaluation of germination in standard conditions, 150 seedlings per iteration were placed inside Petri dishes and over filter paper in a temperature of 22°C and for 7 days after which the number of normal plantlets were counted (Hebert *et al.,* 1995). It is noteworthy that only the seedlings with 2mm length of rootlet were considered as germinated and normal ones (Elias & Copleland, 2001). In addition, the germination rate of seedlings was in proper level of 95%.

After the utilization of seed deterioration treatments and daily germination test, the number of germinated seedlings was counted and this resumed until the number of germinated seedlings got fixed. In the last day, the counting of germinated seedlings was followed by random selection of 5 samples and measurement of the length of their Caulicle and rootlet with a ruler. To measure the dry weight of Caulicle and rootlet, the number of Caulicle s and rootlets of each Petri dish was separately counted and placed inside small pockets. The number of Caulicle s and rootlets was registered on the pockets and they were maintained in the temperature of 60°C for 48 hours. After this duration, the content of each pocket was measured through a sensitive balance with accuracy of 0.0001g.

Seedling vigor index

The Seedling vigor index (SVI) was calculated through multiplication of final germination percentage by length of plantlet (Fahimi Rad *et al.,* 2010).

SVI=length of plantlet x final germination percentage (1)

Germination rate index

The germination rate index of seedlings was obtained through Maguire's method. It is equal with the ratio n/t in which n is the number of daily germinated seedlings and t is the number of days from the first day to the last day of germination after plantation.

$$G.R=\sum Ni/Ti \quad (2)$$

In the above relation, Ni refers to the number of germinated seedlings each day and Ti represents the number of days in which germination occurred.

In addition, the germination percentage of seedlings is calculated by division of the number of germinated seedlings by total number of seedlings in each Petri dish. In the end, the numbers were analyzed through MSTATC Software. The associated diagrams were drawn by Excell Software.

Results and Discussion

For determination of seedling vigor index, there are two relations

SVI=length of plantlet x final germination percentage (3)

SVI=weight of plantlet x final germination percentage (4)

In the present study, the first equation is used.

Table 1 shows the effects of days during which the heat treatments are used on different factors of cultivar Hayola 401. As the results show, the germination percentage and germination rate did not show significant difference. In regard to seedling vigor index and the length of rootlet and Caulicle, the heat treatments manifested higher and significant differences. This is not unexpected because the more days of application of heat is associated with higher influence upon germination factors. In fact, the effect of heat increases because the seedlings are exposed to heat for longer durations. In fact, the placement of seedlings in high temperature and for longer durations in conditions similar to those of storehouse influences the length of Caulicle, length of rootlet, seedling vigor index and length of plant all of which are presumed to be the main factors of growth of seedlings in the farmland. Table 2 shows the effects of days of using heat treatments on different factors of cultivar RGS. As it can be evidently observed, most of the measured factors do not show significant difference. Table 3 shows the effects of different temperatures on the intended factors of cultivar Hayola 401. As the results show, a significant influence is observed regarding most of the measured factors such as length of rootlet, length of Caulicle and seedling vigor index. The control treatment had better conditions. In this regard, (Behpuri *et. al.,* 2010) offered similar results about safflower cultivars. In addition, the germination percentage and rate was higher in lower temperatures.

In general, the effects of temperature on the studied factors were completely significant. In relation to the length of rootlet, one could point to the similar results suggested by Fahimi Rad *et. al.*, 2010 in a study on rapeseed in -20°C. The germination rate of the plant increased at 30°C after which it declined. The same results were reported by different researchers (Qasemi Golazani, 1995). In fact, high and low temperature influences the main germination factors. Therefore, placement of seedling in the storehouse seems essential. The above table shows the influence of different temperatures on RGS cultivar. Although the control sample lacks proper situation in a number of factors but it is observed that the difference between the control and other treatments is relatively similar.

As one can observe, the rootlet and Caulicle of the plants were influenced by heat and they were different from control sample. These two organs are the first ones to grow out of the seedling. As a result, high and low temperature might reduce their growth. Of course, the results concerning the effects of temperature are different from the other cultivar and this is natural because different cultivars have different genetics and show different reactions to different factors. In regard to this cultivar, it should be noted that proper storage is essential and if the seedling is maintained in an unsuitable storehouse, the germination is reduces and exist of rootlet and Caulicle out of the soil faces complications (Rabii and Bayat, 2010). The results are aligned with growth level of farmland (Silva *et al.,* 2006).

Table 1. The effect of different days of treatments on the measured factors in Hayola variety 401.

Row	The days of treatments	Percentages of germination	Rate of germination	Vigor index	Rootlet length	Caulicle length	Seedling length
1	1day	67.33a	3.529a	384.5b	3.106ab	2.609b	5.712b
2	2day	88.00a	3.527a	535.3a	3.423a	2.661ab	6.084ab
3	3day	84.33a	3.486a	490.8ab	2.985b	2.835ab	5.820ab
4	4day	85.00a	3.564a	533.2a	3.396ab	2.878a	6.274a

Table 2. The effect of different days of treatments on the measured factors in RGS variety.

Row	The days of treatments	Percentages of germination	Rate of germination	Vigor index	Rootlet length	Caulicle length	Seedling length
1	1day	87.33a	5.237a	457.3a	3.351a	1.931a	5.237a
2	2day	85.67a	4.971a	425.8ab	2.923b	2.049a	4.971a
3	3day	84.67a	4.823a	408.3b	2.722b	2.110a	4.823a
4	4day	85.83a	4.876a	418.5ab	2.749b	2.127a	4.876a

The mutual effects of temperature and days of applying temperature treatments on cultivar Hayola 401 show that in most of the cases, the control treatment had better conditions. Because in most of the cases, day and different temperatures have distinctively significant influence on measured factors, the effects are mutual. Therefore, the higher number of days of exposure to a certain temperature and higher temperatures are associated with higher influence on germination and the factors affecting its. As a result, the seedling might not be able to properly grow in the farmland.

The results of a study by (Qasemi Golazani, 2010) show the reduction of germination rate due to deterioration effect. The environmental conditions in which the seedling is maintained determine the duration in which germination and its vigor is maintained. The study on deterioration of sunflower seedling suggested that in the conditions of seedling deterioration, the percentage of germination reduced compared with normal conditions (Latifi *et al.,* 2004). One of the significant test for determination of quality and vigor of the seedling is aging test.

The accelerated aging in most of the herbal species is associated with emergence of sprouts and establishment of plantlet on the farmland (Hebert, *et al.,* 1995). The accelerated aging test can estimate the amount of plantlets which might grow in farmland conditions (Mir Mohammadi Mobayedi, 2005).

Table 3. The effect of different temperatures of treatments on the measured factors in Hayola variety 401.

Row	The temperatures of treatments	Percentages of germination	Rate of germination	Vigor index	Rootlet length	Caulicle length	Seedling length
1	Control	90.00ab	3.658ab	620.82a	3.676ab	3.222a	6.898a
2	30˚C	86.25abc	3.758a	390.7e	2.155e	2.375e	4.530d
3	40˚C	91.67a	3.658ab	503.0bc	2.810cde	2.678cde	5.488c
4	50˚C	85.83abc	3.678ab	461.5cde	2.712de	2.665cde	5.377c
5	60˚C	82.08c	3.610abc	431.3de	2.407e	2.847bcd	5.255cd
6	-20˚C	87.08abc	3.510bc	560.1ab	3.262bcd	3.172ab	6.433ab
7	30˚C and -20˚C	88.33abc	3.517bc	520.6abc	3.430bc	2.465e	5.895bc
8	40˚C and -20˚C	90.00ab	3.433cd	571.5ab	3.703ab	2.674cde	6.350ab
9	50˚C and -20˚C	84.58bc	3.502bc	577.5a	4.328a	2.498de	6.827a
10	60˚C and -20˚C	83.33c	3.510bc	556.4ab	3.793ab	2.885abc	6.678a

Table 4. The effect of different temperatures of treatments on the measured factors in RGS variety.

Row	The temperatures of treatments	Percentages of germination	Rate of germination	Vigor index	Rootlet length	Caulicle length	Seedling length
1	Control	87.50ab	5.548ab	485.6ab	3.288a	2.760a	5.550ab
2	30˚C	89.67abc	5.015bcde	499.6abc	2.993ab	2.133bc	5.015bcde
3	40˚C	84.17bc	5.143bcd	432.8abc	2.850ab	2.293b	5.143bcd
4	50˚C	82.50c	4.938bcde	407.2bc	2.620b	2.318b	4.938bcde
5	60˚C	84.17bc	5.862a	493.3a	3.092b	2.770a	5.862a
6	-20˚C	89.58a	5.548ab	496.9bc	3.278a	2.270b	5.548ab
7	30˚C and -20˚C	87.50ab	4.518de	395.9bc	2.837ab	1.682d	4.518de
8	40˚C and -20˚C	89.17a	4.354e	388.2bc	2.655b	1.699d	4.354e
9	50˚C and -20˚C	84.17bc	4.745cde	413.6bc	3.142ab	1.603d	4.745cde
10	60˚C and -20˚C	84.17bc	4.472e	376.4c	2.607b	1.865d	4.472e

The mutual effects of temperature and days of application of heat treatments on measured factors of RGS cultivar show that in most of the cases, the control sample has better conditions or its difference from other treatments is negligible. Of course, the application of the temperature of -20°C offered statistically better conditions in regard to germination rate and length of rootlet and plant. This temperature exerted no significant influence upon germination percentage and similar findings were suggested by (Fahimi Rad *et. al.,* 2010). In a test on oilseed rape, they studied the effect of -20°C in different durations so as to clarify the effects of cold pre-treatment. Fahimi Rad *et. al.,* 2010 reported that cold pre-treatment in -20°C increased the germination rate, length of rootlet and Caulicle length but it had no influence on germination percentage. However, the results obtained from cultivar Hayola 401 was relatively different and as stated before, different cultivars represent different reactions to diverse conditions.

Conclusion

The tests on measurement of seedling rigor include standard germination test, accelerated aging test and cold treatment test. The results showed that despite of the fact that the difference between germination capacity and seedling rigor was insignificant for seedlings harvested in the stage of total ripeness and apparently

the temperature and humidity stresses designed under accelerated aging test and cold test did not sufficiently influence the seedling rigor but the seedlings harvested at the time of total ripeness had higher vigor and survival probability compared with the time of physiological ripeness. In addition, the spring cultivars manifested no significant difference with each other in regard to standard germination test, accelerated aging test and cold test while the differences among winter cultivars were evident (Fahimi Rad *et. al,* 2010). One of the significant tests on determination of quality and rigor of cotton is accelerated aging test. In most of the herbal species, the accelerated aging is positively associated with emergence of seedling and estabablishment of plantlets on farmland (Hebert, *et al.,* 1995). The accelerated aging test can predict the extent of growth for farmland plantlets (Mir Mohammadi Mobayedi SM, 2005)

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