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THE INFLUENCE OF THE RESERVE LIPIDS CONTENT ON SEED GERMINATION AT SUNFLOWER HYBRIDS

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Abstract

The sunflower species Helianthus annuus L. belongs to the group of oil plants and it is considered to be one of the most important sources of vegetable oil used in human nutrition. The analyzed plant material was represented by the following hybrids: Armada Clearfield, Duet Clearfield, Meteor Clearfield, DT5234 Clearfield, P64LE25, DT6004, EXS7016 and Pokora. There have been made determinations regarding germination capacity, seed vigor expressed by electrolyte leakage, reserve lipids content, lipase activity and root length increase. Duet and Armada achieved the highest germination capacity, the leakage of electrolytes during imbibition directly influencing seed germination. The lipid content of the analyzed hybrids did not similarly influence germination and the radicle development took place 48 hours after the induction of the germination process, which can be explained by a quick mobilization and biodegradation of the organic substances under the influence of the lipase's increased activity.

Key words: *germination, electrolytes, lipase, lipids, sunflower seeds.*

INTRODUCTION

Sunflower belongs to the Asteraceae family and is one of the most important oleaginous seeds crops in the world. Achenia accumulates 49.9-55.5% lipids, 16.4-18.4% proteins and 5-14% carbohydrates. Sunflower achenia lipids contain 84% of unsaturated fatty acids and 18% of saturated fatty acids (Burzo, 2005).

Imbibition is the first process that occurs when seeds are placed in optimal germination conditions. The penetration of water into the seed and the increase in mass happen very fast. Thus, Levari (1960) stated that at a temperature of 28°C a very obvious increase of the seed mass was observed during the first 2 hours of imbibition. The free ion content of sunflower seeds decreases during the germination period of 2.13 times as a result of their diffusion into the water of imbibition. The diffusion of cellular electrolytes into the soaking water causes the conductivity to increase to 4.468 μS/g seeds. After 24 hours of imbibition, the permeability drops to 50% and after 3 days reaches 38.2% of the initial value, which indicates the restoration of the structure and their semipermeability (Burzo, 2005).

Seed germination is the process by which they initiate a new ontogenetic development cycle when favorable environmental conditions are ensured. Germination begins with water uptake by the seed (imbibition) and the emergence of embryonic axis, usually the radicle, through the structures surrounding it (Bewley et al., 2013; Erbas et al., 2016). Sucrose synthesized during maturation of oil seeds is the source of carbon for triacylglycerol (TAG) synthesis (Bewley & Black, 1994). TAGs are hydrolysed by lipases, enzymes catalyzing the hydrolytic cleavage of the fatty acid ester bonds to yield glycerol and free fatty acids (Theimer & Rosnitschek, 1978). Almost all TAGs present in oleiferous seeds are lost during seed germination and seedling development (Muto & Beevers, 1974; Yaniv et al., 1998; Rabiei et al., 2007; Kim et al., 2011; Tonguç et al., 2012).

The purpose of this paper was to investigate the germination capacity of sunflower hybrids, seed vigor expressed by electrolyte leakage, reserve lipid content, lipase activity and rootlet growth in lenght.

The results obtained are useful for agronomists for the initiation of culture, but also for breeders, in order to characterize and support for homologation the created hybrids.

MATERIALS AND METHODS

The homologated plant material is represented by Armada Clearfield, Duet Clearfield, Meteor Clearfield, P64LE25 and Pokora, while the hybrids proposed for approval are represented by: DT5234 Clearfield, DT6004 and EXS7016. Of the 8 analyzed hybrids, 7 come from Fetesti, a small town from the southeastern area of Ialomita County. The agricultural enterprise which has cultivated them has got an experience of over 14 years in the production of cereals and technical plants. The P64LE25 hybrid, belonging to DuPont Pioneer, was cultivated in the southern part of Teleorman County, in Beiu Village of Ştorobăneasa.

Determination of seed vigor

Determination of electrolyte leakage during the seeds imbibition period is an indicator in the assessment of germination capacity.

The method is based on the determination of the electrical conductivity of the water in which the seeds of known mass have been maintained for 24 hours.

20 seeds were weighed, immersed in 20 ml of distilled water and kept under these conditions for 24 hours. Using the automated conductivity meter, measurements of the conductivity of the solution were made 24 hours after the start of the experiment.

Determination of the lipid content

The lipid content of seed of sunflower hybrids was determined gravimetrically using the Soxhlet method.

The amount of lipids was calculated using the following ratio:

Lipids (%) = (a-b) * 100/m, where:

a is gross mass of the collector vessel;

b -collector vessel country;

m - mass of seed analyzed.

Determination of lipase activity from the studied sunflower hybrids

Lipase is part of the hydrolases class, the group of esterases, catalyzing the cleavage of the ester linkages between glycerol and lipid fatty acids. Lipase activity is determined by measuring the increase in acidity of some lipids as a result of the action of an enzyme preparation obtained from oilseeds. Free fatty acids resulting from hydrolysis of triglycerides under the action of lipase are titrated directly with sodium hydroxide solution. The ideal substrate for the study of lipase activity in some plant material are lipids from the same material.

0.2 g sunflower seeds were crashed and mixed with 3 ml sunflower oil. 2 ml 0.5 M acetate buffer (pH 4.7) was added and the pestle mill was placed in a thermostat at 30°C for 30 minutes. Subsequently, 15 ml 96% ethanol and 15 ml of petroleum ether were added. The fatty acids in the sample were titrated with 0.1 N NaOH in the presence of phenolphthalein as an indicator.

In parallel, a blank sample was made, which was immediately titrated with 0.1 N NaOH. The lipase activity was expressed in micromoles of oleic acid formed by the action of lipase per minute and grams of enzyme extract.

Observing rootlet growth

To observe the growth of the radicle measurements were made 24 to 24 hours after its occurrence for 3 days.

For this determination Petri dishes were taped with filter paper moistened with distilled water. A sample of 10 pre-germinated seeds was transferred to each flask. The measurements were performed for all analyzed hybrids and the data obtained is in the tables.

RESULTS AND DISCUSSIONS

Determination of imbibition and germination capacity of the analyzed hybrids

The research on the imbibition capacity of the 8 sunflower hybrids studied showed that the highest weighing pressure was achieved in the POKORA hybrid, where 70% of the seeds split the pericarp, followed by the DT6004 hybrid with 56% and the METEOR hybrid with 44%. The smallest values, of 1%, were recorded in the ARMADA and DUET hybrids. Intermediate values were recorded at the hybrids DT5234, EXS7016, P64LE25 at 1.9, 3.18 and 5.38 times smaller compared to the POKORA hybrid (Figure 1). It can be appreciated that the imbibition process was carried out at a different rhythm under the influence of the hybrid type.

The analysis performed on the hybrids proposed for approval, namely: DT6004, DT5235 and EXS7016 indicate a high rate of imbibition in the DT6004 hybrid, 1.51 times higher than that of the hybrid DT5234 CL and 2.54 times higher than the hybrid EXS7016.

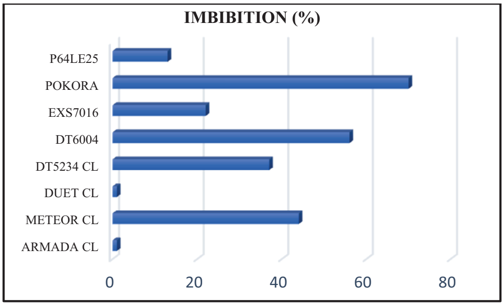

Figure 1. Imbibition capacity of the analyzed hybrids

In the next stages of the germination process, the germination was followed dynamically. After 48 hours from the start of the process, the following results were recorded: P64LE25 96%, EXS7016 79%, DT5234 75%, POKORA 93%, DT6004 88%, and METEOR CL 86%. Therefore, the germination capacity increased 7.38 times for P64LE25, 3.59 times for EXS7016, 2.02 times for DT5234 CL, 1.32 times for POKORA, 1.57 times for DT6004 and 1.95 times METEOR CL. However, a spectacular germination capacity can be noticed after 48 hours of optimum germination for DUET CL 47% and ARMADA CL 31%, given that during the imbibition period, in the two hybrids, a single seed (1%) presented the fragmented pericarp.

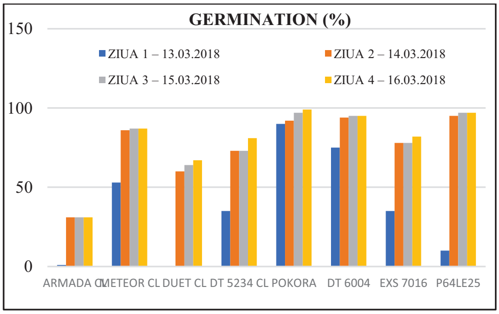

Figure 2. Germination capacity of the analyzed hybrids

Noteworthy is the fact that in the case of sunflower seeds, the formation of the rootlet takes place after 48 hours, which can be explained by rapid mobilization and biodegradation of the underlying organic substances under the influence of increased lipase activity. Thus, the optimal conditions for growth and development of the embryo are created, making possible the appearance of the first organ of the new plant. It is interesting to follow the germination process 72 hours after its initiation. Thus, the data presented in the graph indicates the completion of the germination, physiologically, of the analyzed hybrids.

The results obtained allow hybrids to be graded after the germination as follows:

- a first category is the P64LE25 and POKORA hybrids with over 95% germination;
- a second category is represented by hybrids whose germination capacity is between 75 and 94%, namely: DT5234, EXS7016, DT6004, hybrids proposed for approval, and METEOR CL, approved hybrid;
- in the third category is included the DUET hybrid, which is characterized by a germination capacity of 52%;
- the lowest germinating hybrid of 31% is ARMADA CL.

The determinations continued on the fourth day of germination, noticing that the hybrids ARMADA CL, METEOR CL, P64LE25 did not change their germline capacity compared to the previous day. With respect to hybrids DT5234 CL, DT6004, EXS7016, POKORA and DUET CL, the results indicated an increase of germination from 1.03 to 1.3 times (Figure 2).

At the end of the determinations, the non-homologated hybrids: DT5234 CL, DT6004, EXS7016 are considered to be of the germline capacity range of 80 to 95%.

Determination of the vigor of sunflower seeds expressed as electrolytes leakage

Dehydrated seeds have structurally damaged plasma membranes. Thus, in contact with water in the soil solution, some of the soluble substances dissipate by diffusion. The amount of soluble substances that diffuse into the soil solution is dependent on the rate of recovery of the structural integrity of the plasma membranes, being correlated with the germination capacity of the seeds (Figure 3).

Experimentally, the amount of ions that diffuse into the soil solution is conductively determined and constitutes an indicator in the assessment of germination capacity of the seeds.

Seed vigor was evaluated by conductometrically measuring the amount of ions that diffused in water after 24 hours of initiation of imbibition.

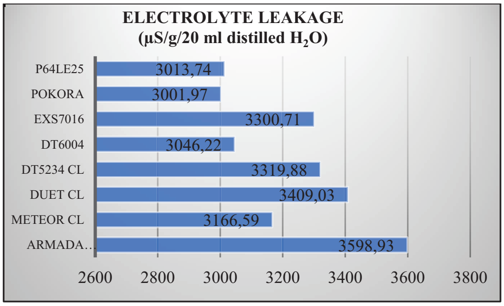

Figure 3. Values of electrolyte leakage of the sunflower seeds

The recorded data (Figure 3) indicates the highest values for the ARMADA hybrid, followed by DUET and EXS7016. The lowest values were determined at the POKORA and P64LE25 hybrids. Average values were obtained for hybrids METEOR, DT5234, DT6004. The results obtained and compared with the germination faculty indicated a direct influence between the two indicators, corresponding to the data from the specialty literature (Table 1).

Thus, in hybrids where the conductivity values were high, seed vigor by germinating capacity was reduced. Also, in the case of hybrids where the electrolyte leakage expressed by conductivity was low, the germination capacity was high.

Table 1. Relationship between germination and the electrolyte leakage

Hybrid	Germination (%)	Electrolyte leakage (µs/g/20 ml h_2o)
ARMADA CLEARFIELD	31	3598.93
METEOR CLEARFIELD	87	3166.59
DUET CLEARFIELD	67	3409.03
DT5234 CLEARFIELD	81	3319.88
EXS7016	82	3300.71
DT6004	95	3046.22
POKORA	99	3001.97
P64LE25	97	3013.74

Determination of the seed's reserve lipids content

During the germination, the mobilization of the organic substances, which requires an enzymatic biodegradation, takes place. Thus, it results in more simple intermediate products that can be transferred from the endosperm to the embryo, ensuring growth. Considering the importance of seed organic substances reflected in the formation of the new plant, during the study, the lipid content was determined for each of the analyzed hybrids.

The highest lipid content was calculated for the DT6004 hybrid, followed by the POKORA hybrid at the 7.28% difference. The lowest content was recorded in hybrids DT5234 CL 43.4068%, METEOR CL 45.7996%, P64LE25 45.8924%, DUET CL 47.2989%. As for the POKORA and ARMADA CL hybrids, the recorded values exceed 50% (Figure 4).

The lipid content determined in the 8 hybrids does not affect the germinative capacity similarly. Thus, it is noted that the DT6004 hybrid, whose seeds have the highest lipid content, also has the highest 95% germination. At P64LE25, the lipid content was relatively low, of 45.8924%, but the germination capacity was 97%. Regarding ARMADA CL, even though the lipid content was not reduced (50.1016%), the hybrid recorded the lowest germination capacity, of only 32%.

Also, the hybrids METEOR CL, DUET CL, DT5234 CL, EXS7016 and POKORA do not find that there is a direct relationship between lipid content and germinative faculty.

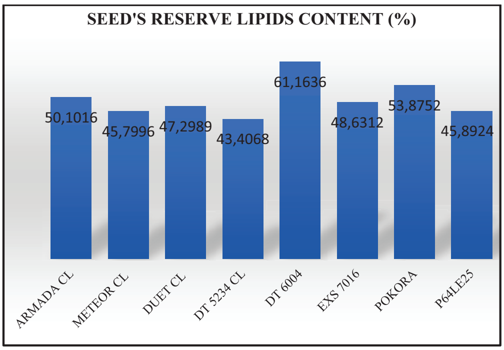

Figure 4. Values of the seed's content in lipids

Determination of lipase activity in sunflower seeds

Lipase is part of the hydrolases class, the group of esterases, catalysing the cleavage of the ester linkages between glycerol and lipid fatty acids. Regarding the activity of the lipase, the obtained results indicated that differences are caused by the characteristics of the hybrid and the physiological state of the seed (Table 2).

Table 2. Lipase activity

Hybrid	Lipase activity (µmole oleic acid/min/g seeds)		
	Non-germinated seeds	Seeds after a day of germination	Seeds after 3 days of germination
ARMADA CLEARFIELD	0.2	4.6	1.15
METEOR CLEARFIELD	0.26	2.9	0.7
DUET CLEARFIELD	0.065	0.9	0.2
DT5234 CLEARFIELD	0.05	0.5	0.12
DT6004	0.75	6.8	1.5
EXS7016	0.9	9.3	1.95
POKORA	0.45	2.1	0.68
P64LE25	0.47	1.9	0.62

Although the seeds were kept under the same temperature and humidity conditions, it can be observed that the non-germinated seeds of the DT5234 hybrid followed by the DUET hybrid show the lowest enzyme activity. The highest enzyme activity was calculated in the EXS7016 hybrid, its value being 0.9. For the other hybrids, values ranged between 0.2 and 0.7. With hydration of the seeds as a result of imbibition, the enzymatic activity intensifies at different rhythms, with high values being recorded in all studied hybrids.

The highest lipase activity was determined in the EXS7016 hybrid, followed by the DT6004 hybrid. It can be appreciated that, in sunflower seeds, the mobilization of reserve lipids is very rapid after only a day of germination, justifying the high activity of the lipase that caused the appearance of the rootlet.

Three days after germination, the enzymatic activity decreases remarkably, approximately 4 times, in all hybrids. The exceptions is the P64LE25 hybrid in which the enzyme activity decreases 3 times.

Measurement of the rootlet length in sunflower seeds

Of the total amount of hybrids analyzed for the rootlet extension, the hybrids DT6004 and POKORA have been noted (Table 3). In these, the growth of the rootlet on day one is 8.6 and 8.3 mm, respectively. There is a direct relationship between the lipid content and the roolet growth. Thus, the high lipid content has positively influenced the prolongation of the rootlet in the DT6004 and POKORA hybrids.

Noteworthy is that the DT6004 hybrid maintains its high growth rate on days 2 and 3 when it records the longest length of the rootlet compared to the other hybrids. It is estimated that the growth rate is almost constant from day to day, being 2.1 and 2.2 times higher respectively.

Table 3. Rootlet length (mm)

Hybrid	Day 1 14.03.2018	Day 2 15.03.2018	Day 3 16.03.2018
ARMADA CLEARFIELD	3.8	9.9	26.1
METEOR CLEARFIELD	7	13.8	24.1
DUET CLEARFIELD	3	9.3	26
DT5234 CLEARFIELD	5.7	13.1	32.5
DT6004	8.6	17.9	39.3
EXS7016	7	14.2	39.2
POKORA	8.3	14.3	30.4
P64LE25	4.8	10.6	26

Regarding the POKORA hybrid, the growth rate is not constant. Thus, on the first day, the extension of the rootlet is comparable to that of the DT6004 hybrid, the growth rate decreases on day two and registers a new increase on the third day. From the analysis of the data obtained from the 8 studied sunflower hybrids, the DT6004 hybrid shows a positive relationship between lipid content and lipase activity, these indicators favorably influencing rootlet growth throughout the research period.

It is also interesting to look at the EXS7016 and DT6004 hybrids, at the relationship between the lipid content, the lipase activity and the rootlet length, as it is found that in the EXS7016 hybrid, although the lipid content is lower,the lipase activity is the highest, causing a rapid mobilization of the reserve lipids. Thus, the rootlet growth was favored. This explains the similar value of the rootlet length to the two hybrids. Within 48 hours, the length of the EXS7016 hybrid rose 5.6 times, from 7 mm to 39.2 mm.

Regarding the characterization of the ARMADA CL, DUET CL and P64LE25 hybrids, it was found that the rootlet length did not exceed 4.8 mm on the first day. On day two, although the rhythm of growth is high, the rootlet length of these hybrids remains the smallest. The next day, the growth rate remains constant, the rootlet stretching to 26 mm in all 3 hybrids studied. Following the rhythm growth rate of all the analyzed hybrids, it is noted that on day two, the highest growth rate is recorded for DUET and the lowest for POKORA and METEOR hybrids. On the third day, in all analyzed hybrids, the growth rate was between 2.2 and 2.6 times, except for the METEOR hybrid at which the growth rate was the lowest, 1.75 times. Indeed, throughout the determinations, at the METEOR hybrid, the growth rate was the lowest, 1.86 times.

Also, in the analyzed timeframe, in the case of the DT5234 hybrid, there is an increase of the radicle from 5.7 to 32.5 mm, the elongation being 5.7 times. Thus, it is considered that the rhythm of growth was the highest of all the analyzed hybrids.

CONCLUSIONS

The properties of the hybrids have influenced the imbibition process. Thus, the highest imbibition pressure was achieved at the POKORA hybrid, and the lowest pressure was recorded at the DUET and ARMADA hybrids. The dynamics of the germinating faculty 48 hours after the start of the process indicated an increased germination capacity of 7.38 times for the P64LE25 hybrid, 3.59 times for the EXS7016 hybrid, 2.02 for the DT5234 hybrid, 1.32 times for the the POKORA hybrid, 1.57 times for the DT6004 hybrid and 1.95 times for the METEOR hybrid. At the analyzed hybrids, the electrolyte leakage during imbibition directly influences the seeds germination. The lipid content determined in the 8 hybrids does not affect the germination degree similarly, and the rootlet formation in sunflower seeds takes place 48 hours after the germination process is induced, which can be explained by a rapid mobilization and biodegradation of organic back-up substances under the influence of the increased lipase activity.

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