DOCTORAL DISSERTATION'S SUMMARY

"CONTRIBUTION TO *LOLIUM PERENNE L.* SEED'S BIOLOGY KNOWLEDGE"

Gramineous plants are highlighted by their special qualities concerning the biomass production for animal breeding, environmental protection, erosion control and aero-hydrous soil system improvement. Among these gramineous plants, a special position is occupied by the *Lolium perenne* (the English ray grass or lawn grass) considered to be one of the most renowned and appreciated lawn gramineous plants for the pastoral and ornamental economy. *Lolium perenne* has been studied from many points of view, in our country as well as in other countries, however the researches concerning this plant's seeds quality are scarce. In this context, the doctoral dissertation entitled *"Contribution to Lolium perenne L. seed's biology knowledge*" approaches a detailed study on the aspects concerning seeds quality, to obtain a clearer view of what seeds quality means for this variety.

The study is structured on nine chapters, and it ends with a set of conclusions and bibliographical references.

The first chapter is entitled "History and economic importance of *Lolium perenne* variety". In this chapter I presented a short history of cultivating fodder gramineous plants, and the economical importance of *Lolium perenne* variety.

Cultivating leguminous and gramineous fodder plants along with oat (*Avena sterilis*), Italian millet (*Setaria italica*), and millet (*Panicum milliaceum*), are related to massive animal breeding, in two centers in which they were domesticated: Turkish center – oriental Taurus, and Zagros center – eastern Iran.

The perennial ray grass is the first gramineous fodder plant that was cultivated, being inlaid in the temporary lawns in England in the XVII-th century. Originary from Europe, tempered Asia and Northern Africa, it is now worldwide spread, including Southern and Northern America, New Zeeland, and Australia. In Romania detailed researches were made after 1945, along with setting up I.C.A.R. experimental stations, that studied fields grassed with fodder plants within cropping-systems.

The economical importance of the perennial ray grass consists in the high agricultural output potential, fast regeneration after grazing, high fodder value, and remarkable resistance to animal downtrodden. It is used especially in complex mixtures composition, which provides better

outputs to the pure leguminous crops, with high nutritive quality through the proteins and digestive energy in balanced relations. Another use for the perennial ray grass is in lawn structure. Along with red bent grass (*Festuca rubra*), tall bent grass (*Festuca arundinaceaea*), meadow grass (*Poa pratensis*), etc., the perennial ray grass (*Lolium perenne*) is one of the most used variety, for its resistance to repetitive felling. Concerning its polyvalence, the perennial ray grass can be used for any kind of lawn, less for golf links.

The second chapter, "Varieties and systematics, morphological peculiarities of *Lolium perenne* variety", is presented the systematic placement of *Lolium perenne* variety, new varieties and their destination, as well as the plant's identification criteria, regarding its morphological peculiarities.

Lolium perenne belongs to one of the largest family, Gramineae (Poaceae), which contains 700 divisions and 8000-10000 varieties, according to some of the authors. Due to its capacity to form compacted and dense populations, with numerous individuals, it is broadcasted on large areas, from coast and depression to mountain regions up to 3600 m altitude. The *Lolium* division contains 40 varieties, and for agricultural purposes, the perennial ray grass (*Lolium perenne*) is of a great importance, next to the aristae ray grass (*Lolium multiflorum*), and the hybrid ray grass (*Lolium hybridum*).

According to the pollination method, *Lolium perenne* is a cross-pollineted plant.

Depending on the vegetation period, there are two types of plants found in the crop, the delayed type, for pasturage, and the semi-delayed type, for mechanical harvest, used as feed, fresh or ensilaged.

Concerning the large usage area of this variety, new varieties had to be created to correspond the multitude of destinations, environmental and soil conditions, use. In the official catalogue of Romanian crop plants the following varieties are registered: Basara, Barelan, Barlennium, Calibra, Danilo, Mara, Marta, Măgura, Merci, Sabor, Sakini, Score, Taya, Tetramax, Trivoli. These varieties are also registerd in The List of plant varieties cultivated in OECD system, worldwide, the list being more numerous. The Romanian *Lolium perenne* varieties registered in the official catalogue are: Mara, Marta, and Măgura.

The perennial ray grass, along with other perennial gramineous plants, can be recognized after its ear (inflorescence, ears, and seeds), or after the vegetative part (leaves, trunk, root). For an accurate recognition of the *Lolium perenne* seeds in comparison with *Lolium multiflorum*, the safest method is rootlet fluorescence determination. In

the first days after the germination, the Lolium perenne plantlet's rootlets are not fluorescent.

The third chapter, "Elements that influence the seeds production and seeds quality of *Lolium perenne*", is focused on highlighting the varieties requirements upon the environmental factors, the main elements regarding the crop technology and compulsory peculiarities in producing seeding seeds, as well as the official requirements for seed lots certification.

An important position in obtaining good quality seed outputs is held by the environmental factors (temperature, moisture, light) and the soil. By optimizing these factors, the output capacity of the specific variety is highlighted. The fodder output is obtained under the predominant influence of environmental factors (natural or man-modified), and detailed knowledge of the factors that race in its accomplishment, which can provide an useful tool in choosing working procedures that encourage an efficient usage of the land resources, as well as the financial ones. The plant contributes with 25–30% in the progress of the output, and the technological factor (soil preparation, crop rotation, fertilization, seeding) contributes with 65–70%. Maintenance activities have an important role in the complex of technological measures, implementing them on time contributes to a compacted culture, with no blanks, in obtaining a large seeds output of superior quality, prolonging the crop usage duration. These activities (irrigation for emergence, soil rolling, crust breakage, weeds and pests control) have a great importance for the whole usage period, but they have a special role in the first year, when the growth rhythm is slower and weeds may asphyxiate the plants. A special activity for the seeds production is the biological purification, activity that assumes the separation of atypical plants from the seed lot.

When establishing the best harvesting period, the fact that not all of the seeds reach maturity at the same time, must be considered. They reach maturity staggered, during 10 to 12 days. Generally, seeds reach physiological maturity in 25–32 days after pollination. The harvesting must be made when the moisture reaches 40-55%.

Seeds processing is regarding reaching the quality parameters provided by MAPDR Ord. no. 1263/2005, and Ord. no. 148/2007, similar conditions for this variety are also imposed in the European Union's countries.

For European Union, or Romanian marketing, the seeds are accompanied by the official seeds quality document - DOCS, and for the exported seeds the certification of varietal purity document must be released – an OECD document, and the document that certifies the seed

lot's quality – The international certificate for seeds quality, orange-colored.

The fourth chapter, "Seeds vigour and factors that influence upon it", presented the importance of the seeds vigour in establishing the seeds quality and factors that influence upon it. The notion of "vigor" for seeds quality was deepened to explain why various seed lots can have the same germination in good conditions, but the results can be different in bad laboratory or field conditions.

Seeds vigor, as an important practical trait, is influenced by a set of factors. They act directly, simultaneously and with different intensity. These factors' intensity upon the seeds vigour is strongly influenced by the specific conditions met during the seed shaping, maturation, and during storage. Important positions in varieties vigour behavior, are occupied by the following factors: genetic basis, seeds size, physical integrity, physiological quality, maturation stage in harvest, mechanical damage, microorganisms activity during storage, and physiological processes in the bulk seeds. The most important physiological processes are: breathing, post-maturation, seeds cincture and seeds germination, biological activity in the bulk seeds, seeds longevity and aging process.

The fifth chapter, "Research objectives, research method and the material", consists of the description of the experimental material and the methods used in the established tests to achieve the purpose of the doctoral dissertation - *Lolium perenne* seed's biology knowledge.

Eight varieties were used in the experiments: Mara, Calibra, Kaiser, Lorenz, Marta, Măgura, Summit and Tove. The samples have been taken after the seed parties from which they come from, have been processed and brought to the stage of final official certification, "Certified" biological category. The eight varieties were considered eight variants. In the harvest year 2005, analysis were made concerning the initial quality of the varieties, moisture, TKW, germination (first and final-count), vigour, soil germination and field emergency. The conditions ensured for seedlings development and their evaluations were in conformity with the ISTA Rules, except the field emergency.

After the harvest year determinations, each sample has been divided in three sub-samples. A set of sub-samples was stored in controlled environment (6 degrees Celsius and relative environmental moisture was below 50%) and a set was stored in uncontrolled environment, in a storage with oscillating temperature and moisture, depending on the season (in the summer- raised temperatures, sometimes higher than 40 degrees Celsius, and dryness, and in the

winter – low temperatures and oscillating relative environmental moisture). In the first, the second, and the third year of storage (2006, 2007 and 2008) on both sub-sample sets, same analysis have been made as in the harvest year (2005). The third set of sub-samples has been used in the harvest year to determine the influence of seed's size upon its quality. Each sub-sample was passed through a fine-meshed bolt, so that two sub-samples are obtained (small and large seeds) for each variety studied. The following tests were made on each category: TKW, seed size determination, germination (first and final count), and the average dimensions of the seedlings essential structures were determined.

The sixth chapter, "Results of the research and their interpretation", contains the interpretation of the results obtained, concerning the influence of the storage conditions, storage period, and the influence of the variety upon the seeds quality.

By analyzing the results from the harvest year (2005), it is observed that, from the quality standards point of view, seven out of eight varieties respond to the standard norms for seeds final certification. The only variety that is below the standards is Calibra. This statement is based on the following important aspects:

- all varieties have the moisture level below the maximum limit admitted by the legislation for the fodder gramineous plants (13%), which ensures a good conservation during the storage period;
- the TKW has different values from a variety to another, as following: 1,300g – Mara, up to 3,860g – Marta variety. For the most varieties the TKW had values between two and three grams, which indicated their irregularity from this point of view. This indicator is determined optionally, and it is not set as a limit for certification;
- the first-count germination is the indicator that offers the first information about seeds vigor, and it has a limited values interval. The highest value of the first count germination was registered for the Tove variety (85%), the other varieties having lower and closed values (52%–73%);
- the final-count germination, limited by the law at minimum 80%, has high values, passing 90% for five varieties, and over 85% for two varieties. The Calibra variety, the final count germination is low (66%), making it impossible for this variety to be certified and used for seeding;
- the values for vigour determination are much closed to the ones of the final count germination. The seedlings essential structures are even developed, the majority having the average coleoptile size of 2/3 out of the root average size;

- seedlings weight has the lowest values on Kaiser (0,040g) and Mara variety (0,041g). The highest value per seedling has been registered to the Tove variety (0,131g). Calibra and Marta had the same average seedling weight (0,113g).Transitional and closed results were obtained at the Lorenz, Măgura and Summit varieties (0,052g, 0,055g and 0,058g);
- soil germination shows values between 80% on Lorenz variety and 95% on Măgura. The Marta, Mara and Tove varieties show values closed to the superior limit of the interval (89%, 90% and 93%) and Kaiser and Summit show lower values, closed to the inferior limit of the interval (83% and 86%). The only exception is the Calibra variety, which has the soil germination of 56%;
- concerning the field emergency percentage, five of the varieties (Calibra, Kaiser, Lorenz, Marta and Summit) have the identical value of the soil germination obtained in the laboratory. On the Mara variety, the field emergency is reduced by 4% and on the Măgura variety is 10% lower than the soil germination. These two varieties had frail seedlings and uneven field emergency. The Calibra variety had a low field emergency, which confirmed once more its low potential. Only one variety, Tove, had the field emergency percentage lower by 2% towards soil germination, being distinguished by an even and rapid growth.

Concerning the seeds size influence upon the determined parameters, by comparing the results obtained for small seeds with the ones obtained for large seeds, for all of the quality parameters studied, it can be stated that the large seeds are characterized by higher values, the differences being statistically ensured. The size of the seeds influences the first and final- count germination of the studied *Lolium perenne* seeds. The large seeds have higher first and final count germination values towards the values for the small seeds.

The differences between the first-count germination for small seeds and the first-count germination for large seeds, in the same variety, are bordered in a wide interval, from 3% to 22%, and the final-count germination for small seeds towards the final-count germination for large seeds are bordered in a limited interval, with values from 3% to 14%.

The larger the seed is, the average dimension of the root and coleoptiles is larger, and the seedlings are developed better. In all situations, the average size of the coleoptiles is reduced towards the one of the roots. To eliminate these quality differences between the seeds of the same lot, calibration is needed. This leads to accurate laboratory results and a rapid and even field emergency.

Storage period determinations results regardless of the storage conditions

Concerning the variety moisture, it has to be mentioned that regardless of the storage period and conditions, the moisture suffered statistically ensured losses, and the most significant loss was registered after the first year of storage.

The varieties that had initial moisture higher than others lost a higher percentage of water, so that at the end of the storage period, they reached much closed values. The varieties initial moisture has been between 11, 6% - 12,8%, and by the end of the storage period reached lower values, between 9,8% and 10,1% regardless of the environment conditions.

Regarding TKW, the varieties have different values, being enclosed in a wide interval. And concerning the TKW values of the same variety, they stay very closed during the storage period, regardless of the environment conditions.

For every variety, the TKW lowers during storage in controlled and uncontrolled environment, as we digress the harvest year, the diminution being accentuated in controlled environment. The Summit variety suffers a 0,27g TKW decrease, in controlled environment and 0,23g in uncontrolled environment, the Lorenz variety suffers the same loss of TKW regardless of the environment (0,12g), and Mara loses 0,18g in controlled environment and 0,03g in uncontrolled environment.

Concerning the first-count germination of the varieties studied, it has different values from a variety to another and from a year to another. In the first year of storage, the values are closed regardless to the storage conditions, but in the following years the values are scattered, making the difference between varieties storage in uncontrolled environment. The Mara variety has a very good first-count germination of 93%, after the first year, 84% after the second year, and 33% after the third year of storage in controlled environment, and of 80% after the first year, 55% after the second and 19% after the third year in uncontrolled environment. The Marta variety has a very special behavior, it has a first-count germination of 92% after the first year, 86% after the second year, and 74% after the third year of storage in controlled environment and 61% after the first year, 16% after the second year, and 15% after the third year of storage in uncontrolled environment. The Tove variety is characterized by high values in both storage conditions.

The more we digress from the harvest year, the first-count germination decreases for most varieties, which indicates seeds aging, a process with different intensity depending on the variety. Compared with the decrease registered after the first year of storage, the first-count

germination decrease is highly accentuated after the second and the third year of storage.

The values of the final-count germination under the influence of the storage years and storage conditions are different from one variety to another and from one year to another. In controlled environment the Mara variety has a very good germination value (93%) after one year of storage, 90% after the second year and 72% after the third year. The Kaiser variety is highlighted by 84% after the first year, almost identical value of 83% after the second year, and 29% after the third year. The Măgura variety registers 92% after the first and the second year and 85% after the third year being a vigorous variety. In uncontrolled environment, the Mara variety, from the first year has a value decreased by 8%, and in controlled environment with 85% in the first year, 79% after the second year and 63% after the third year of storage. The Kaiser variety has 69% after the first storage year, 26% after the second and 24% after the third. The Măgura variety is a vigorous variety that loses in three years of storage in uncontrolled environment only 7%.

During the storage years the final-count germination decreases at most of the varieties, the decrease being accentuated after the third year of storage regardless of the environment conditions. If in the harvest year, the first and final-count germination of the same variety have closed values, the more we digress from the harvest year, the differences accentuate, being more significant in uncontrolled storage environment. At most varieties, after the second year of storage, especially in uncontrolled environment, the first count germination has values of 1/2- 2/3 of the final count germination's value.

The variety is an important factor, concerning the impact of the storage conditions and storage period upon the values of the soil germination. By the genetic heritage and seeds obtainment conditions, there are varieties with stability and high values of soil germination identified. As a proof of this statement lies the Tove variety, which, regardless of the storage period and conditions maintained its germination at 92% after the first year, 92% after the second year and 93% after the third year of storage in controlled environment, and at 91% after the first year, 90% after the second year, and 91% after the third year of storage in uncontrolled environment. Under the influence of the storage years, and storage conditions, the soil germination of Kaiser variety in the first, second, and third year of storage in controlled environment, registered values of 79%, 79% and 23%, and in uncontrolled environment 67%, 21% and 27%. The Marta variety had a special behavior, being an unstable variety, registering in the first year 86%, 86% in the second year and 90% in the third year of storage in

controlled environment, and 80%, 27% and 29% in uncontrolled environment.

Concerning the vigor, the varieties had different values under the years and storage conditions influence, the bigger differences being registered in uncontrolled environment. As we digress from the harvest year, seeds vigour decreases, the decline being more accentuated after the third year of storage.

The variety is an important factor concerning vigour maintenance under the influence of storage period and conditions. The Marta variety had a good vigour in controlled environment (92% after the first year, 91% after the second year, and 93% after the third year of storage), but in uncontrolled environment has lost its vigour after the first year of storage as following: 78% after the first year, 34% after the second year and 31% after the third year of storage. In comparison, the Tove variety maintained its vigour almost unaltered, regardless of the storage years and conditions. Most of the varieties gradually lost their vigor, by constantly reducing it year after year, regardless of the environment conditions. This was observed in the Lorenz variety's behavior (81% after the first year, 74% after the second year and 57% after the third year of storage in controlled environment and 73% after the first year, 65% after the second year, followed by 52% after the second year in uncontrolled environment).

As we digress from the harvest year, the growth rhythm of the essential structures is reduced, the reduction of the coleoptiles growth rhythm being decreased significantly. After the first year of storage, the average coleoptiles size is of 2/3 of the average root size, after the second year is of 1/2 of the average root size for most of the varieties studied, and after the third year it reaches about 1/3 of the average root size. In uncontrolled environment, the Lorenz variety had the average coleoptiles size of 1/4 of the average root size. The seedlings unevenness is also accentuated on the germination layer.

The weight per seedling, during the research period on both storage conditions is reduced, being more accentuated in uncontrolled environment. Among the vigorous varieties, weight differences were also registered in similar storage conditions. The Măgura variety had 90% of normal seedlings, with the weight of 0,050 g/seedling- dried substance, and the Tove variety had 93% of the normal seedlings with 0,118 g/seedling – dried substance after one year of storage in uncontrolled environment. After the third year of storage it reaches the level of 0,049 g/seedling and 86% normal seedlings for Măgura variety, and 0,089 g/seedling and 95% normal seedlings for Tove variety.

Concerning the field emergency, all the varieties suffered decreases during the storage period, not all of them being statistically

ensured. The most accentuated decreases registered after the third year of storage for Lorenz, Marta and Summit variety (20,50%, 22,77% and 24,63%). The Marta variety shows decreases of the field emergency, statistically ensured for the second and the third year (23,15% and 22,00%). For Calibra, Măgura, and Tove variety, field emergency's values decrease during storage years, is not statistically ensured.

The results lead to the conclusion that the *Lolium perenne* seeds can be used for two years after the harvest, counting on a good crop in technological conditions. The fact that varieties such as Tove and Măgura have high values of determined parameters, and they can be sowed regardless of the storage conditions, after three years of storage, highlights the need of research in this domain.

The seventh chapter presented the "Established correlations between the quality parameters determined during the storage years, under the influence of storage conditions".

Regardless of the storage conditions and storage period, the correlation between the first-count germination and the final-count germination of each variety studied is very significant (0,980*** in the first year and 0,936*** in the third year). In uncontrolled storage environment, the correlation has more reduced values than in controlled environment (0,930*** in the first year and şi 0,843*** in the third year).

In the first two years of controlled storage, the soil germination is very significantly correlated with the final-count germination, and after three years of storage the correlation is tighter, very significant, and it is established with the first-count germination (0,957***), while, in uncontrolled environment, only in the first year it is correlated with final-count germination, in the second and third year being correlated with the first-count germination (0,933*** in the second year and 0,843*** in the third year).

Field emergency in controlled environment after the first and second year of storage is very significantly correlated, and after three years significantly distinct with the soil germination (0,988***, 0,989*** and 0,691**) while in uncontrolled environment, it has the tightest correlation, very significant, with the studied varieties' vigour (0,987*** in the first year, 0,981*** in the second year and 0,922*** in the third year of storage).

The moisture and other determined parameters correlation is statistically ensured, being significantly distinct in both storage environments, after the third storage year. The tightest correlation are established with the soil germination 0,563** and the vigour 0,429**, and concerning TKW there are no correlations.

The eighth chapter is named “Influence of the storage years and storage conditions upon the Romanian and foreign varieties. Influence of the storage years and storage conditions upon diploid and tetraploid varieties*”.* It contains the most important conclusions obtained by grouping the varieties in the categories mentioned above.

The first and final-count germination, the soil germination and field emergency for the Romanian varieties have higher values, regardless of the storage conditions and storage period.

For the Romanian as well as for the foreign varieties, the first and final-count germination, the soil germination and field emergency decrease with seeds aging in uncontrolled environment, and the influence of the storage conditions and years of storage is stronger upon the Romanian varieties.

The first and the final-count germination, soil germination and field emergency for diploid varieties registers higher values in controlled environment, and in uncontrolled environment lower values towards tetraploid varieties.

For the diploid varieties, the final-count germination decreases with seeds aging in uncontrolled environment, and for tetraploid varieties increases in the same conditions.

The influence of the storage conditions upon the final-count germination and field emergency for the diploid varieties is stronger than upon the tetraploid varieties. The field emergency decreases with seeds aging for the diploid varieties and stays constant for tetraploid varieties.

The soil germination also in diploid varieties, decrease as the seed is aging in uncontrolled environment, while for the tetraploid varieties is almost constant, the amplitude being of 9% (the highest value is 75%, in controlled environment in the first storage year, and the lowest 66% in uncontrolled environment in the third storage year).

The results highlight the stability and the superior behavior of the tetraploid varieties towards the diploid varieties.

The **ninth chapter**, “General conclusions” sums, deepens and synthetizes the research, the analysis and the results presented in the doctoral dissertation.

The doctoral dissertation’s includes reference materials such as scientific researches with foreign and Romanian authors, and ISTA (International Seed Testing Association) testing rules, the Romanian legislation concerning seeds quality elaborated by the Ministry of Agriculture and Rural Development, along with published personal research which underlie the analysis.