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FACTORS AFFECTING SEED VIGOUR

SUMMARY

The seed industry is essentially based on the production of quality seeds with high germination and vigour percentages and for that reason the aim of this study was to point out the most important factors that affect seed vigour. This study shows an overview of the development of seed vigour tests throughout history. Seed vigour is affected by many environmental factors, as well as genetic factors, of which the chemical composition of seeds, hardheadedness and resistance to disease-producing agents express positive effects. Special attention is paid to the role of phytohormones, such as abscisic acid (ABA) and gibberellin (GA), in regulating seed dormancy and their interaction with environmental factors, including temperature, light and moisture. The research results reveal a complex network of interactions among genetic factors, phytohormones and environmental conditions that mutually modulate the degree of seed dormancy and its impact on germination and seed vigour. Experimental data show that the application of certain agrochemical treatments can change the level of phytohormones and consequently alter the rate of seed dormancy and germination. Further research of these mechanisms will reveal prospects for the development of innovative technologies for the improvement of the seed industry as an inseparable part of agricultural production. This study provides a deeper understanding of the regulation of seed vigour and, also identifies new approaches to the improvement of plant production in accordance with the demands of contemporary agriculture.

Key words: seed, vigour, germination, dormancy, environmental factors

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INTRODUCTION

Seed is one of the most important links in agricultural production. It has an important role in the cycle of production of safe and healthy food of high quality. In terms of agriculture, seed means the renewal of plant production, i.e. its continuity, and is one of the most important factors of successful production (Popović, 2010; Ilin, 2014). That is why ensuring high seed quality is a priority of current seed production and a prerequisite for high yields of all plant species (Poštić et al., 2014). Seed production encompasses the establishment and cultivation of crops developed from all categories of seeds, control of this production, drying, processing, packaging, sampling, testing and certification (declaration) of seeds, their trade, storage, transportation and distribution or conservation until sowing (Ilin, 2014; Popović *et al.*, 2010; 2020a-b; 2021; 2022; Trung and McMillan, 2021; Zhang and Colak, 2022; Milunović *et al.*, 2022; Lakić *et al.*, 2022; Burić *et al.*, 2023; Stevanović *et al.*, 2023; Sekulić *et al.*, 2023; Stupar *et al.*, 2023; Miladinović et al., 2024). Production of field crops is of great economic importance, and seed quality is also of high significance, because errors following sowing can no longer be corrected (Velijević *et al.*, 2016; Filipović *et al.*, 2021; 2023; Rajičić *et al.*, 2023; Dimitrijević *et al.*, 2023; Vasileva *et al.*, 2023; Kosev *et al.*, 2023a-b; 2024). The task of seed production is to provide sufficient quantities of quality seeds of the appropriate assortment for all production regions. Therefore, all countries strive to achieve the highest degree of self-sufficiency in their own markets and create surpluses for export (Popović, 2010). Humans, since the beginning of their activities in crop growing approximately 10 millennia B.C. and especially since their recognition of the importance of seeds in plant multiplication have been interested in the physiology of seed, particularly germination and its effects on crop traits (Marcos-Filho, 2015).

The determination of seed quality is done by the application of standardised methods. These methods have been altered in accordance with scientific knowledge within the field of seed physiology. In addition, new technological procedures of seed processing can also influence the change of methods, in accordance with the requirements of certain plant species (Ilin, 2014).

Seed quality testing includes the determination of seed purity, germination, moisture and health condition and for certain plant species, the first count, 1000-seed weight and some other properties. This testing is performed in the manner and in accordance with the procedures established in seed testing methods. Seed purity, first count, germination, count of other species of cultivated plants and weeds in seed samples have to be within the tolerance limits (Regulation on Seed Testing Quality of Agricultural Crops).

Germination and seedling vigour are the most important parameters of seed viability, on which seed usability depends. Germination is expressed as the percentage of seeds capable of germinating into healthy, strong, undamaged seedlings, which can develop into normal plants under normal field conditions. In the case of a seed crop, effects of seed vigour are reflected on the initial growth, uniformity of the crop, uneven maturation and the level of seed yield (Knežević *et* *al*., 2014). High vigour seeds produce seedlings that will establish the best stand in the field, uniform emergence of crops, which will provide stable yields of outstanding quality (Poštić *et al.*, 2014). Since high-quality seed production is based on high-quality seeds, and production of such seeds depends on seed vigour, the aim of this study was to review the relevant literature in order to indicate the most important factors that affect seed vigour.

The aim of this study was to point out the most important factors that affect seed vigour, ie. production of quality seeds with high germination and vigour percentage.

MATERIAL AND METHODS

The aim of this study was to point out the most important factors that affect seed vigour, i.e. production of quality seeds with high germination and vigour percentage. This study shows an overview of the development of seed vigour tests throughout history. According to numerous studies, seed vigour is affected by many environmental factors, as well as genetic factors, of which the chemical composition of seeds, hard seededness and resistance to disease-producing agents express positive effects. This study presents mechanisms of seed deterioration and effects on seed vigour with the aim to comprehend internal and external factors affecting this process.

RESULTS AND DISCUSSION

Chronology of seed vigour testing

According to McDonald (1993) the first law on seed was enacted in Berne, Switzerland in 1816. Fifty three years later, the first seed testing laboratory was founded by Frederick Nobbe in Tharandt, Germany. In the same year (1876), Edward Hopkins Jenkins, agricultural chemists, established the first seed testing laboratory in the United States in Connecticut. The first "Rules for Testing Seeds" were written by Jenkins in 1917. The expression "seed vigour" was for the first time used by Nobbe in his “Handbuch der Samenkunde” in 1876. Furthermore, the same author suggested germination test procedures (Marcos-Filho *et al.*, 2015). The term *triebkraft* with the meaning "driving force" and "shooting strength" related to germinating seedlings was introduced by Hiltner and Ihssen in 1911. These authors wanted to draw attention to seeds that had produced seedlings with longer roots in contrast to seedlings developed from "weaker" seeds drawn from the same lot. In the 1930s, the US term "germination energy" related to the germination rate was broadly accepted. However, scientists and seed technologists of that time showed little or no interest in improving such studies. The lack of interest continued throughout the first half of the 20th century (Marcos-Filho *et al.*, 2015). The International Seed Testing Association (ISTA) was founded in 1924 with the idea to promote standard methods in seed testing all over the world. In 1931, ISTA adopted the first "International Rules for Seed Testing" (ISTA Rules) (Milivojević *et al*., 2018). During the 1940s, the tetrazolium (TZ) test, as a fast and reliable test, was developed for the determination of seed viability. This test is still one of the most commonly used seed tests in Brazil (França-Neto *et al.*, 1998). On the other hand, germination and TZ viability tests differ in relation to the emergence of seedlings in the field under unfavourable environmental conditions. Therefore, results obtained by these two tests have to be supported by results gained by more precise determinations. In order to be included into the ISTA Rules, the majority of test methods have to undergo interlaboratory test comparisons, because these comparisons provide reliable and reproducible results (Milivojević *et al.*, 2018).

In 1950, the ISTA Congress was held in Washington DC, USA, the first time outside Europe, emphasising the contribution of the US seed technologists. The then ISTA President W.J. Franck, being aware of rising international seed trade, pointed out the differences in results of germination tests between the US and the European seed testing laboratories. As stated by Franck, the main difference between the US and the European laboratories was in the purpose of the standard germination test. In the European laboratories, technologists believed that the most important point for the export of seed lots was reproducibility of test results, while the US technologist thought that the capability of seeds of a certain seed lot to produce plants was the essential aim of the germination test. Franck suggested that germination should be determined under favourable conditions in order to gain uniform test results. Furthermore, it was accepted that capability of seeds to produce plants under the field conditions should be defined by a new expression: vigour. During the same year, the ISTA Biochemical and Seedling Vigour Committee was founded and two primary goals were set up: 1) to define seed vigour, and 2) to develop standardised vigour test methods (McDonald, 1993). Standardisation of methods, also recognised as validation of methods, was implemented and developed at the end of 19th century. Unfortunately, the system was not reliable until the end of the 20th century. At that time, the procedure of method validation for seed testing was set up (Steiner et al., 2008; Milivojević *et al.*, 2018). The development of the ISTA Rules is a permanent procedure. ISTA members are involved in the constant process of developing methods for seed sampling and testing. The methods undergo appropriate validation to ensure that the test procedures provide reliable and reproducible results (Milivojević *et al.*, 2018).

During the 1960s, studies on the estimation and determination of seed vigour and its effects on seed traits were broadened, which resulted in important studies of seed physiology (Marcos-Filho *et al.*, 2015). The following year, the Vigour Test Committee was founded by the Association of Official Seed Analysts (AOSA) (McDonald, 1993).

The alteration of the ISTA Rules format started at the beginning of the 21st century (Jones and Kahlert, 2005). In 2001, yearly editions of the ISTA Rules were initially presented as replaceable pages (Jones and Allen, 2017) and then, the electronic annual version was introduced in 2015. ISTA accredited laboratories have to revise their technical knowledge and practice every year in accordance with the authorised edition of the ISTA Rules. This is assessed and verified by

Proficiency tests, so that laboratory analysts keep up with currently valid versions (Milivojević *et al.*, 2018).

Concept of the term vigour

Seed vigour is the most important biological trait of seed. In the broadest sense, it encompasses several inter-related traits (germination, viability, seed vigour, energy of growing, vitality, emergence force, longevity, germination capacity), i.e. properties determining the activity and performance of a seed lot of commercially acceptable seed germination under diverse environmental conditions (Lekić, 2009). The term vigour covers traits that are related to the following aspects of the performance of the seed lot (ISTA, 2014): rate and uniformity of seed germination and seedling growth, the ability of seeds to germinate under unfavurable environmental conditions, seed condition after storage, primarily retention of germination capacity. Seed vigour is defined as "the sum total of those properties of the seed which determine the level of activity and performance of the seed lots of acceptable germination in a wide range of environments" (ISTA, 2015).

Seed vigour does not only indicate the percentage of viable seeds in a sample, but reflects seed ability to produce normal seedlings under unfavourable growing conditions that may occur in the filed. Seed vigour is most simply evaluated through seed germination (Lekić, 2003). Vigour cannot be determined individually such as it is possible with seed germination. Van de Venter (2000) explained the phenomenon of vigour through a practical example of seed vigour differences (Table 1).

He hypothetically observed two seed lots (A and B) and their germination percentage and field emergence percentages on three farms. Germination percentages of these two lots were identical according to the standard germination test. Seedbed conditions (temperature, moisture, etc.) in the farm 1 were favourable and the stands of A and B were similar to their laboratory germination percentages. On the other hand, soil conditions in the farm 2 were slightly unfavourable (possibly cold and wet to some extent) and the seed lot B produced a stand which was less than that of the seed lot A. Conditions in the farm 3 were exceedingly unfavourable and the stand produced by the seed lot B was much less than that of the seed lot A.

The percentage of emergence was, in both cases, much lower than emergence percentage obtained in the laboratory. What can be a cause for the stand differences in the farms 2 and 3 regardless of the similarities between the two seed lots in the farm 1 and in the standard germination test? The answer can be found in different vigour of seeds: vigour of the seed lot A was higher than vigour of the seed lot B. Seed vigour is affected by many physiological and biochemical traits, including their complex interactions. Scientists are not able yet to entirely comprehend effects of these traits and interactions among them, thus it is not fully clear what seed vigour actually is.

Table 1. Hypothetical example of germination and emergence of two seed lots.

Seed lot	Germination (%)	Field emergence (%)		
		Farm 1 (favourable conditions)	Farm 2 (slightly unfavourable)	Farm 3 (very unfavourable)
A	90	88	80	70
B	90	87	60	40

Source: Van de Venter (2000)

Factors affecting seed vigour

It is known that genetic factors strongly affect seed vigour: chemical composition of seeds, hardseededness and resistance to disease-producing agents (pathogens) have positive effects on seed vigour (AOSA, 2002). Vigour is a promising trait of viable seeds in cropping practices and is affected by the complex interrelation between genetic and environmental factors (Figure 1.). Why properties of vigour vary it is insufficiently comprehended (Finch-Savage and Bassel, 2015).

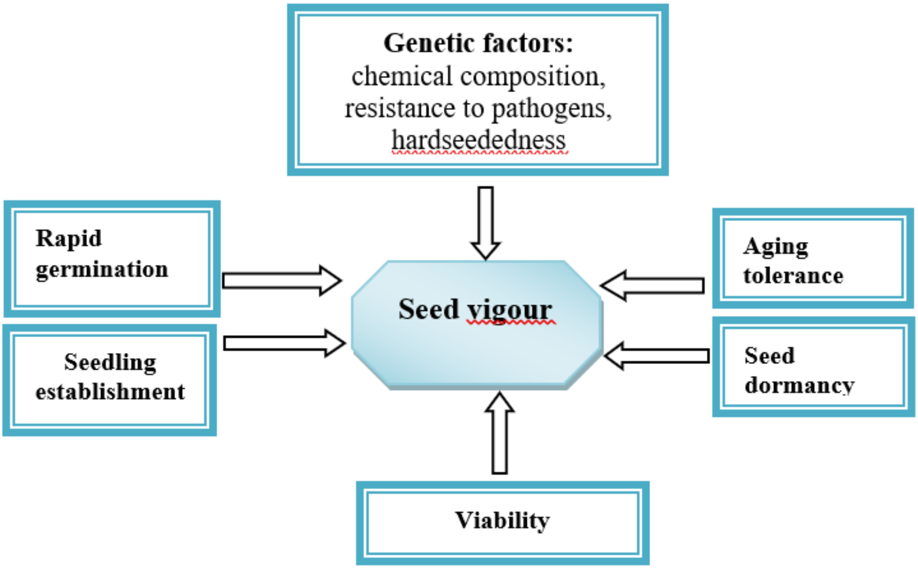

Figure 1. Factors affecting seed vigour

Seedling vigour is expressed by the length of radicle, stem and weight. Low vigour and pronounced seed dormancy are closely related to seed germination (Tomaz *et al.*, 2015), as well as to its size: larger seeds are inclined to produce more vigorous seedlings (Ambika *et al.*, 2014). Seed quality depends not only on purity, health, viability and vigour (Van de Venter, 2000), but also on many other factors, such as seed production locations, genetics among varieties, seed crop management, etc. (Velijević *et al.*, 2016). The seed production carried out under conditions of water stress, insufficient amounts of nutrients, extreme temperatures, often leads to the production of seeds with poor vigour, the so-called low vigorous seeds. Moreover, mechanical damages caused during harvest and processing, as well as improper storage, also adversely affect seed vigour (Jovičić, 2014). Even though numerous definitions of seed vigour accentuate field performance, seed vigour also has significant effects on seed storage. Low/poor seed vigour will result in low storage potential (Van de Venter, 2000).

a) Seed deterioration

Malik and Jyoti (2013) defined seed deterioration as "deteriorative alterations occurring with time that increase the seed's exposure to external challenges and decrease the ability of the seed to survive". High-vigorous seeds are seeds with good traits (Hampton, 1999). On the other hand, low-vigorous seed lots produces seedlings with smaller leaf area index, lower dry matter accumulation and the decreased growth rate of the crop. The main reason for the vigour loss is ascribed to seed deterioration during its development, harvest, drying and storage that begins when the seed is physiologically mature and is prolonged during storage (Basu and Groot, 2003).

Seeds with low vigour are mostly a consequence of seed deterioration processes. Seed deterioration results in the loss of seed quality in the course of time. The following factors are the essential reasons for seed deterioration: temperature, relative humidity, seed moisture content, and pest infestation (Akash *et al.*, 2022). It can be stated that the seed deterioration process is attributed to a series of interrelated "events", among which genetic damage, alternations in respiratory activities, changes in enzymes, proteins, hormones, the accumulation of toxic metabolites are included (Hampton, 1999). Moreover, lipid peroxidation, membrane disruption, DNA damage, impairment of RNA, and protein synthesis are also included (Akash *et al.*, 2022), as well as cytological, physiological, biochemical and physical modifications in seeds (Malik and Jyoti, 2013) (Figure 2). According to Akash *et al.* (2022), there are some other changes occurring in the process of seed deterioration, such as: breaking up of functional structures, biochemical changes that lead to ATP lower levels, the decrease in sugar content, the lack of ability of ribosomes to separate, degradation and inactivation of enzymes (amylase, dehydrogenase, oxidases, phospholipase), development and induction of hydrolytic enzyme activities, meristem exhaustion, intensification of leachates in seeds, the increase of the free fatty acid content, and reduced respiration.

There are the following three types seed deterioration (Farhadi *et al.*, 2012; Akash *et al.*, 2022):

1. *Field weathering.* Deterioration of seeds exposed to unfavourable air and weather conditions, i.e. to high air relative humidity and excessive temperatures in the period after seed maturation and prior to harvest is

considered to be caused by field weathering. According to Khatun *et al.* (2009) physiological changes can occur if seeds, even physiologically mature seeds, remain on maternal plants under adverse atmospheric conditions. This may result in the formation of hard seeds or off-colour seeds in legumes. Seed quality and viability are affected not only by weather conditions, but also by storage conditions (Jyoti and Malik, 2013).

2. *Harvest and post-harvest deterioration.* Inappropriate methods and procedures applied during harvest and handling of seeds can greatly alter seed quality. Mechanical damages are the most common and significant reasons for seed deterioration after harvest and during storage. Due to poor practices, microflora easily accesses seeds and seeds become susceptible to fungal infections, while storage potential is quite reduced (Akash *et al.*, 2022). If seeds are harvested under the most favourable conditions of seed maturation, and if post-harvest handling of seeds was appropriate, then moisture and temperature are the most significant factors influencing succeeding deterioration. If moisture is lost to the atmosphere, the deterioration rate rises considerably and reaches a maximum when the eRH is approximately 85-90% (Probert *et al.*, 2007).

3. According to Probert and Hay (2000) results of the seed deterioration rate was lowest when the eRH ranged from about 10% to 15%. Therefore, international standards prescribe that seeds for long term storage should be dried at the equilibrium relative humidity of 10–15% (FAO/IPGRI, 1994). Moreover, seed deterioration after harvest affects numerous biological, physiological and biochemical changes (Wang *et al.*, 2012).

4. *Storage deterioration*. Seed capability of being stored for certain time without loss of its quality is a seed trait largely genetically regulated. Storability of seeds is affected by seed quality during storage. It also depends on pre-storage conditions including environmental factors during pre- and post-harvest stages, as well as the seed moisture content and relative humidity and the temperature of storage buildings, the storage period and biotic agents (Kapoor *et al.*, 2011; Khan *et al.*, 2017). The degree of seed deterioration at the moment when seed lots are brought into storage affects storage potential. If the storage ambient is under any stress, such as alternations in temperature and relative humidity, high vigour seed lots in comparison to low vigour lots will endure stresses longer and their quality will deteriorate more slowly. Even if the low temperature of the storage room and the low seed moisture content are maintained, seed traits after storage depend on seed lot vigour (ISTA, 2009). High ambient temperatures and the high seed moisture content intensify deterioration of seeds (McDonald, 1999). These effects on life-span of seeds vary over species, and structural and biochemical composition of seeds (Shaban, 2013).

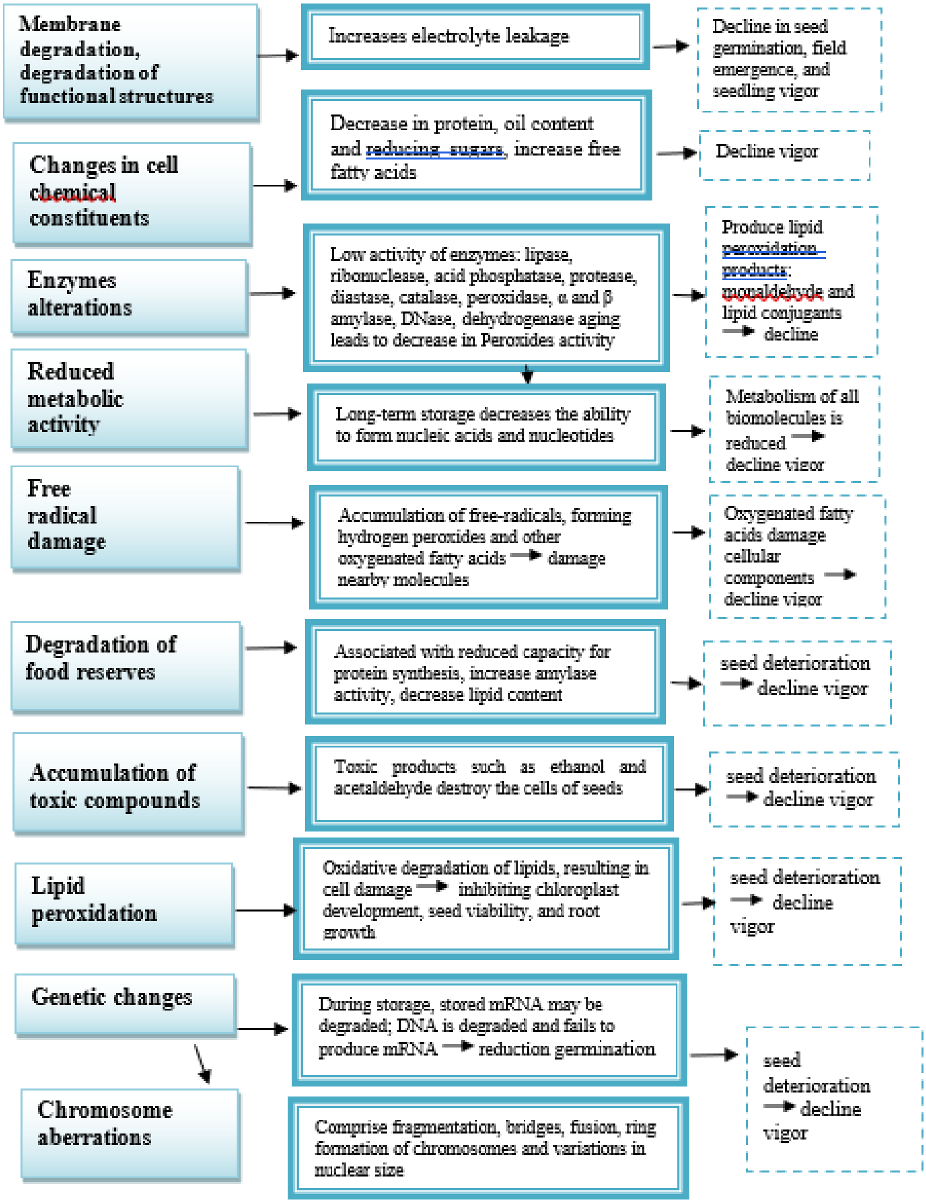

Figure 2. Major physiological and biochemical events of deterioration in seed

Marcos-Filho *et al.* (2015) give a schematic presentation of the relationships between seed germination and vigour in association to the progress of deterioration (Figure 3).

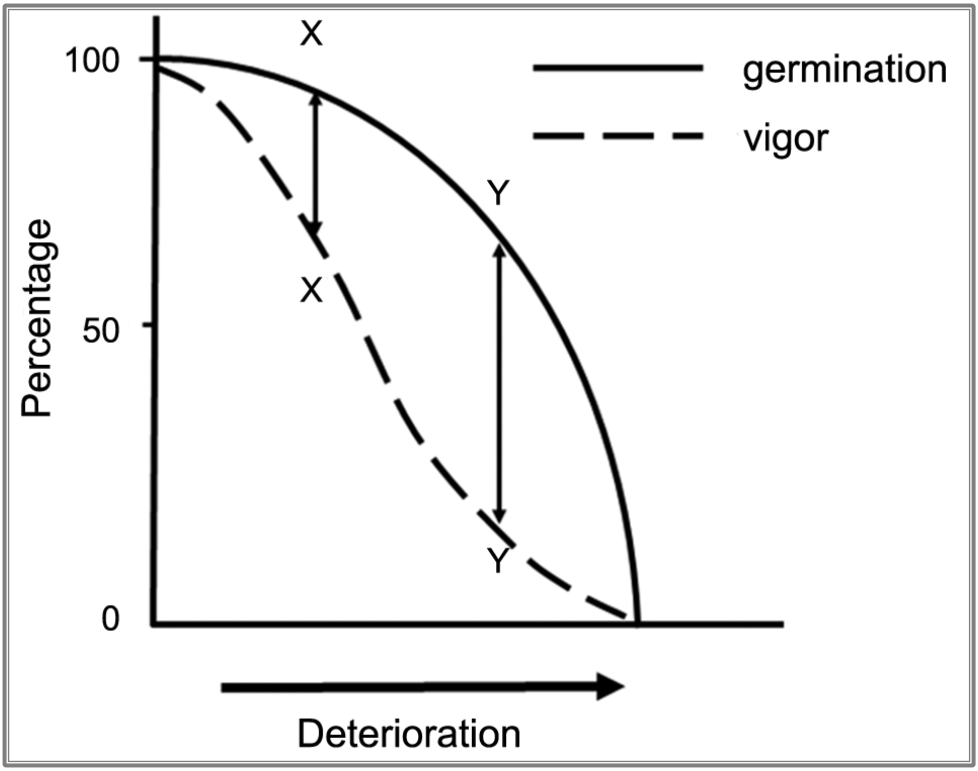

Figure 3. − Relationships between seed germination and vigour in association to the progress of deterioration d (?). The X and Y points on the germination and vigour curves correspond to different seed lots and illustrate the increasing difference between germinability and vigour as deterioration progresses. A high quality seed lot (X) that is less deteriorated will show relatively small difference in results from germination and vigour tests. However, a lower physiological quality seed lot (Y) with higher deterioration level will have higher germination performed under optimum conditions, but extremely low vigour.

Environmental factors affecting seed vigour

The longer seed life-span is the faster vigour is lost and seeds become progressively more susceptible to stress occurring between imbibition and radicle emergence (Bewley *et al.*, 2013).

Drought stress in the process of the seed development may cause serious reduction in seed vigour. Hydrolases (enzymes such α-amylase, β-amylase and α-glucosidase) have a crucial role during seed germination as they break down starch into sugar with water. Drought stress restrains the activity of these enzymes and adversely affects the metabolism of carbohydrates. In many plants, the accumulation of the osmolytes under conditions of drought stress is positively associated with tolerance to water stress. It is considered that these compounds (proline, glycine betaine-GB and soluble sugar) have an adaptive role in interfering with osmotic adjustment and protecting structures within cells in plants under stress (Biju *et al.*, 2017).

Seed vigour is susceptible to the increased level of the carbon dioxide concentration and varies over species (Lamichaney and Maity, 2021). Little work has been done to study impacts of elevated carbon dioxide (e[CO_2]) on seed vigour - the first reports were presented in 2017 (Hampton et al., 2013; Lamichaney and Maity, 2021). Given that e[CO_2] affects physiological performances of plants and the seed development, it is likely that such modifications will change seed quality. With this hypothesis Lamichaney *et al.* (2019) set up the experiment with rice and determined that atmospheric [CO_2] above 610 ppm decreased seed vigour of rice. These authors showed that seeds harvested from elevated [CO_2] up to 610 ppm did not have lower seed vigour. On the other hand, seed harvested at 720 ppm had decreased germination by approximately 10%. An increase in abnormal seedlings and dead seeds were characteristic of seeds physiologically deteriorated and it was attributed to the loss of seed vigour. Furthermore, e[CO_2] at 720 ppm decreased the content of seed nitrogen, substrate availability, and its consequent change in location.

According to Wang *et al.* (2012) stress induced by high temperatures and humidity (HTH) during the development and maturation of soya bean seeds in the field often led to seed deterioration. Seed deterioration occurring in the pre-harvest period has a stronger role than deterioration occurring in post-harvest period of the soya bean seeds. Wei et al. (2020) set the trial with two soya been cultivars Ningzhen No. 1 and Xiangdou No. 3. The seeds of the former cultivar are susceptible to pre-harvest deterioration under the HTH conditions, while the seeds of latter cultivar are resistant to pre-harvest deterioration under the HTH conditions. The authors compared effects of these conditions on the formation of seed vigour during physiological maturity. According to these authors, the more tolerant cultivars to HTH conditions were, the higher seed vigour was. Stress had an effect on seed vigour via adverse effects on signal and metabolic pathways, the ultrastructure of cells as well as physiology and biochemistry in soya bean leaves, pods, cotyledons, and embryos.

The higher moisture is the lower seed vigour is. Many cultivated plants were bred for fast imbibition after sowing to accelerate seed germination. Therefore, these plants are exceptionally susceptible to water stress, because oxygen accessibility is reduced. The activity of enzymes is also limited, which adversely affects carbohydrate metabolism, reduces water potential and soluble calcium and potassium, and changes the hormones of seeds (Abido *et al.*, 2020; Khaeim *et al.*, 2022). The objective of the study carried out by Khaeim *et al.* (2022) was to test maize seed germination and seedling development under effects of different abiotic stresses. The higher water level was the greater seed germination was. Nevertheless, when the optimum water level was achieved, seed germination decreased due to waterloging. Furthermore, water excess limited availability of oxygen for seeds. The temperature was another parameter that considerably impacted seed germination. Khaeim *et al.* (2022) applied temperatures in the range from 5 to 40 ^{o}C and determined that germination was to some extent higher at 20 ^{o}C than at 25 ^{o}C due to changes in enzymes that occurred at the higher temperature. Maize seeds begin to germinate at the average temperatures of about 20-30 ^{o}C. When temperatures exceeded this range, the status of cell energy and the activities of certain enzymes altered, because protein synthesis was thoroughly reduced, and the ATP content was raised extensively.

When seeds do not absorb water there is a lack of metabolic reaction, which furthermore obstructs activities of respiration and enzymes, synthesis of proteins, digestion of reserves, translocation and assimilation, embryo growth, breaking of the seed coat, radicle growth and, subsequently, the seedling formation (Carvalho and Nakagawa, 2012; Cardoso, 2012; Stefanello *et al.*, 2017).

Stefanello *et al.* (2017) set up two experiments to determine impacts of light, temperature and water stress on seed germination and vigour of linseed. The authors determined that the highest seed germination and vigour occurred at a constant temperature of 20 °C, regardless of light treatments. The lower osmotic potential of the medium was the more significant reduction in linseed seed germination and vigour was. Osmotic potentials corresponding to ≤ -0.30 MPa were detrimental to germination and no normal seedling developed starting at –0.50 MPa. Besides, lack of water in the medium can entirely hinders germination.

Seed filling is a critical developmental stage that essentially affects seed vigour, synthesis and accumulation of different components in seeds that develop. Various temperatures, especially temperatures that are elevated during the day, affect grain filling that subsequently results in poor or good grain quality (Abayawickrama *et al.*, 2017; Liu *et al.*, 2017). According to Wang *et al.* (2020), who observed effects of different temperatures during seed filling of hybrid rice seeds, properties of seed filling can affect seed vigour as a result of their effects on starch accumulation and structure. Moreover, these authors determined that contents of starch, amylose and, amylopectin, then relative intensity and the diameter of starch granules differed over various seed filling properties obtained under effects of different temperatures. Liu *et al.* (2017) revealed that the average starch granule size at the increased environmental temperature during the grain filling stage was considerably greater than those at lower temperatures.

Dormancy and seed vigour

Seed dormancy or seed inactivity represents the impossibility of maximum seed germination in a certain period of time immediately after harvest. This phenomenon usually occurs in wild species and not often in cultivated plants (Yogeesha *et al*., 2006). Seed dormancy is a complex trait encompassing dormancy components that are divided into physiological (dormancy of embryo, endosperm and seed coat), morphological, morphophysiological, physical and combinational (physical + physiological) dormancy (Finch-Savage and Leubner-Matzger, 2006).

Multiple authors report a key link between the content of ABA (abscisic acid) and seed dormancy. In the study carried out by Reed *et al.* (2022), effects of ABA on seed dormancy were well described. Throughout the development of seeds, biosynthesis of abscisic acid (ABA) in seeds is affected by the genotype and the environment, stimulating various depths of primary dormancy. Light, temperature, after-maturing, or chilling mitigate dormancy in these seeds Lower amounts of ABA can be accumulated in seeds in dependence on various genotypes or environmental conditions during the period of seed filling, which can result in non-dormant seeds and there is no need to break primary dormancy. Non-dormant seeds, under conditions when their needs for water and temperature for germination are fulfilled, change a lower gibberellin (GA) to ABA ratio to a higher one, thus advancing germination. Secondary dormancy can be induced in seeds in which primary dormancy is eliminated or in non-dormant seeds with relative dormancy under extreme conditions of temperature, lack of oxygen, light or ageing stress, in the prolonged period of time. Alleviation of secondary dormancy and relative dormancy is possible with time under suitable conditions of light, temperature, after-maturing, and/or chilling (Reed *et al.*, 2022).

Seed dormancy of grasses is an undesirable trait when meadows and pastures are established and often negatively affects the establishment of high-quality pastures. On the other hand, seed dormancy under natural environmental conditions can be a positive trait, if germination of seeds is delayed and seedlings are developed under favourable agroecological conditions (Stanisavljević *et al.*, 2010). For instance, not fully matured embryo, while the cause of red clover seed dormancy is in most cases the seed coat which is hard and impermeable to water and gasses. A high percentage of hard seeds causes reduced germination and the initial growth of seedlings, which affects the competitiveness of other component (grass/legume) in the mixuture (Kimura and Islam, 2012).

CONCLUSIONS

This study encompasses the analyses of key aspects related to seed vigour, which is a vital factor in the process of plant germination and growth under different environmental conditions. Issues related to factors that affect vigour stability and decline over time were also observed. Genetic, physiological and environmental factors that play a key role in seed vigor maintenance or disruption were analyzed. A special emphasis was placed on factors of the environment, such as temperature, moisture/humidity and light, which could significantly affect seed vigour and its ability to germinate and grow. Furthermore, the important state of seed dormancy was highlighted, as it is a state in which the seed cannot germinate even under optimal conditions. This can be a result of complex interactions between genetic and environmental factors. In particular, the temperature plays an important role in activating the seed dormancy process, while moisture and light affect the rate and efficiency of germination. Moreover, genetic variations that directly affect seed vigour were identified. These variations provide new insights into the adaptive mechanisms of plants to different environmental conditions. It was determined that elevated CO_2 concentration (720 ppm) adversely affected seed vigour, which resulted in the increased number of abnormal seedlings and the decrease in the nitrogen content in seeds, and its availability and translocation. These findings point out the importance of seed quality monitoring in order to identify areas susceptible to problems in the process of food production and high-quality seed production. The study provides a comprehensive overview of essential aspects related to seed vigour and points out the importance of an integrative approach to the determination of seed vigour of specified plant species. These findings provide a foundation for further studies and the development of strategies to improve production of food and seeds and agricultural products as a whole.

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REFERENCES

Abido, W.A.E., Zsombik, L. (2020): Effect of water stress on germination of some Hungarian wheat landraces varieties. *Acta Ecologica Sinica, 38*: 422–428. https://doi.org/10.1016/j.chnaes.2018.03.004

Abayawickrama, A.S.M.T., Reinke, R.F., Fitzgerald, M.A., Harper, J.D.I., Burrows, G.E. (2017): Influence of high daytime temperature during the grain filling stage on fissure formation in rice. *Journal of Cereal Science*, 74: 256–262. https://doi.org/10.1016/j.jcs.2017.02.013.

Akash, A., Archana, H.R., Prathap, V. (2022): Seed Deterioration: Physiological and Biological Changes. *Just Agriculture*, 3(4), 1-5.

AOSA (2002). Seed vigour testing handbook. In: Handbook of seed testing, Association of Official Seed Analysts, NE, USA, *Contribution*. No. 32

Ambika, S., Manonmani, V., Somasundaram, G. (2014): Review on Effect of Seed Size on Seedling Vigour and Seed Yield. *Research Journal of Seed Science*, 7(2): 31-38. https://scialert.net/abstract/?doi=rjss.2014.31.38

Balešević-Tubić, S., Malenčić, D., Tatić, M., Miladinović, J. (2005). Influence of ageing process on biochemical changes in sunflower seed. *Helia, 28*: 107-114. https://doi.org/10.2298/HEL0542107B

Basu, S., Groot, S.P.C. (2023). Seed Vigour and Invigoration. In M. Dadlani, D.K. Yadava (Eds.). Seed Science and Technology: Biology, Production, Quality. pp. 67-89. *Springer*. https://doi.org/10.1007/978-981-19-5888-5_4

Bewley, J.D., Bradford, K., Hilhorst, H. (2012): Seeds: physiology of development, germination and dormancy. 3rd edition, *Springer Science & Business Media,* New York.

Biju, S., Fuentes, S., Gupta, D. (2017). Silicon improves seed germination and alleviates drought stress in lentil crops by regulating osmolytes, hydrolytic enzymes and antioxidant defense system. *Plant Physiology and Biochemistry,* 119: 250–264. https://doi.org/10.1016/j.plaphy.2017.09.001

Burić, M., Popović, V., Ljubičić, N., Filipović, V., Stevanović, P., Ugrenović, V., Rajičić, V. (2023): Productivity of black oats: *Avena strigosa* Schreb. on chernozem and its significance as food and medicine. *Selekcija i semenarstvo,* 29(1): 21-32. https://doi.org/10.5937/SelSem2301021B

Cardoso, V.J.M. (2012): Germinação. In: Kerbauy, G. B. Fisiologia vegetal. Guanabara Koogan: *Rio de Janeiro*, p. 386-408.

Copeland, L. O., McDonald, M.B., Copeland, L.O., McDonald, M.B. (2001). Seed vigor and vigor testing. *Principles of Seed Science and Technology*, 165-191.

de Carvalho, N. M., Nakagawa, J. (2012): Sementes: ciência, tecnologia e produção. 5. ed. Jaboticabal: *Funep*, 590.

FAO/IPGRI (1994): 'Genebank standards.' (Food and Agriculture Organization of the United Nations: Rome, *International Plant Genetic Resources Institute*: Rome.

Dimitrijević, S, Milić, M, Buntić, A, Dimitrijević-Branković, S, Filipović, V, Popović, V, Salamon, I. (2024): Spent Coffee Grounds, Plant Growth Promoting Bacteria, and Medicinal Plant Waste: The Biofertilizing Effect of High-Value Compost. *Sustainability*. 16(4):1632. https://doi.org/10.3390/su16041632

Filipović, V., Ugrenović, V.; Popović M. V.; Popović, S., Mrđan, S.; Dragumilo, A.; Ugrinović, M. (2021). Use and Agroecology Efficiency of Medicinal Plants in Plant Production. *An Introduction to Medicinal Herbs*. *Chapter in Book Emerald M. Ed.*, https://doi.org/10.52305/TKAL3430, p.1-365.

Filipović, V., Ugrenović, V., Popović, V., Dimitrijević, S., Popović, S., Aćimović, M., Dragumilo, A. & Pezo, L. (2023): Productivity and flower quality of different pot marigold (*Calendula officinalis* L.) varieties on the compost produced from medicinal plant waste. *Industrial Crops and Products*, 192: 116093. https://doi.org/10.1016/j.indcrop.2022.116093

Finch-Savage, W.E., Leubner-Metzger, G. (2006): Seed dormancy and the control of germination. *New phytologist*, *171*(3), 501-523.

Finch-Savage, W.E., Bassel, G.W. (2015): Seed vigour and crop establishment: extending performance beyond adaptation. *Journal of Experimental Botany,* 67(3): 567–591. https://doi.org/10.1093/jxb/erv490

França Neto, J.D.B., Krzyzanowski, F.C., Da Costa, N.P. (1998). The tetrazolium test for soybean seeds. Embrapa Soja, Londrina, PR, Brazil. *Documents*, 115.

Hampton, J.G., Boelt, B., Rolston, M.P., Chastain, T.G. (2013). Effects of elevated CO_2 and temperature on seed quality. *The Journal of Agricultural Science,* 151(2): 154-162. https://doi.org/10.1017/S0021859612000263

ISTA (2009): International Rules for Seed Testing. *International Seed Testing Association*, Switzerland.

ISTA (2014): International Rules for Seed Testing. *International Seed Testing Association,* Switzerland.

ISTA (2015): International rules for seed testing. Basserdorf, Switzerland: *International Seed Testing Association.*Switzerland.

Jyoti, J., Malik, C.P. (2013): Seed deterioration: a review. *International journal of life Sciences Biotechnology and Pharma Research,* 2(3): 374-385.

Jones, S., & Allen, E. (2017): Activity report on behalf of the ISTA Rules TCOM. *Seed Testing International,* No. 154: 20.

Jones, S., Kahlert, B. (2005): ISTA Rules Latest. Seed Testing International, 130: 45.

Jovičić, D. (2014): Antioxidant capacity, tolerance to oxidative stress and viability of rapeseed. University of Novi Sad, Faculty of Agriculture. Doctoral dissertation. In Serbian: Antioksidativni kapacitet, tolerantnost na oksidativni stres i životna sposobnost uljane repice. *Doktorska disertacija.* Univerzitet u Novom Sadu, Poljoprivredni fakultet.

Kapoor, N., Arya, A., Siddiqui, M.A., Kumar, H. Amir, A. (2011): Physiology and biochemical changes during seed deterioration in aged seeds of rice (*Oryza sativa* L.). *America Journal of Plant Physiology*, 6: 28-35.

Khan, F.A., Bhat, S.A., Narayan, S., Maqbool, R., Murtuza, I., & Khan, F.U. (2017). Seed deterioration and priming-An Overview. *SKUAST Journal of Research*, *19*(1), 12-21.

Khaeim, H., Kende, Z., Jolánkai, M., Kovács, G.P., Gyuricza, C., Tarnawa, Á. (2022): Impact of temperature and water on seed germination and seedling growth of maize (*Zea mays* L.). *Agronomy*, 12(2): 397. https://doi.org/10.3390/agronomy12020397

Khatun, A., Kabir, G., & Bhuiyan, M.A.H. (2009): Effect of harvesting stages on the seed quality of lentil (*Lens culinaris* L.) during storage. *Bangladesh Journal of Agricultural Research*, 34(4): 565-576.

Kimura, E., Islam, M.A. (2012): Seed scarification methods and their use in forage legumes. *Research Journal of Seed Science*, 5: 38-50.

Knežević, J., Đokić, D., Terzić, D., Poštić, D., Đukanović, L., Tošković, S., Tmušić, N. (2014): Comparative analysis of seed properties of different types of wheat. Komparativna analiza svojstava semena različitih vrsta pšenice. *Selekcija i semenarstvo*, 20(1): 55-62.

Kosev, V., Vasileva, V., Popović, V., Pešić, V., Nožinić, M. (2022): Ecological-genetic model in grass pea (*Lathyrus sativus* L.) breeding, *Genetika*. 54, 3: 1385-1397. https://doi.org/10.2298/GENSR2203385K

Kosev V., V. Vasileva, V. Popovic (2023). Yodai – a new variety of grass pea (*Lathyrus sativus* L.). *Genetika*, 55, 3: 10-20.

Kosev, V., Vasileva, V., Popović, V. (2024): New variety of white lupine Monica (*Lupinus albus* L.). *Genetika*. 56(2): 1-10.

Lakić, Ž., Popović, V., Ćosić, M., Antić, M. (2022). Genotypes variation of *Medicago sativa* (L.) seed yield components in acid soil under conditions of cross – fertilization. *Genetika*, 54, 1: 1-14. https://doi.org/10.2298/GENSR2201001L

Lamichaney, A., Maity, A. (2021): Implications of rising atmospheric carbon dioxide concentration on seed quality. *International Journal of Biometeorology*, 65(6): 805–812. https://doi.org/10.1007/s00484-020-02073-x

Lekić, S. (2003): Viability of seeds. In Serbian: Životna sposobnost semena. Društvo selekcionera i semenara, Association of breeders and breeders, Belgrade.

Lekić, S. (2009). Seed testing. In Serbian: Ispitivanje semena. *Book.* Belgrade.

Liu, J., Zhao, Q., Zhou, L., Cao, Z., Shi, C., Cheng, F. (2017): Influence of environmental temperature during grain filling period on granule size distribution of rice starch and its relation to gelatinization properties. *Journal of Cereal Science,* 76: 42-55. https://doi.org/10.1016/j.jcs.2017.05.004

Marcos-Filho, J. (2015): Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola*, 72: 363-374. https://doi.org/10.1590/0103-9016-2015-0007

McDonald, M.B. (1993): The history of seed vigor testing. Journal of Seed Technology, 93-100.

McDonald, M.B. (1999): Seed deterioration: physiology, repair and assessment. *Seed Science and Technology*, 27:177-237.

Miladinović, V., Popović V., Dajić Stevanović, Z., Delić., D., Kiprovski, B., Kolašinac, S., Mikulić-Petkovsek, M., Ugrenović, V. (2024): Effectivenes of *Bradyrhizobium japonicum* inoculation on nodulation dynamics in soybean roots. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. 52(2): 13627.

Milunović, I., Popović, V., Rakaščan, N., Ikanović, J., Trkulja, V., Radojević, V., Dražić, G. (2022): Genotype×year interaction on rye productivity parameters cultivated on sandy chernozem soil. *Genetika, Belgrade,* 54(2): 887-905. https://doi.org/10.2298/GENSR2202887M

Panković, L., Malešević, M., Damljanović, S., Čobanović, M., Savić, S. (1999): Current problems and established seed production of Novi Sad wheat and barley varieties in 1998/99. In Serbian: Aktuelni problemi i zasnovana semenska proizvodnja novosadskih sorti pšenice i ječma u 1998/99. godini, *Institut za ratarstvo i povrtarstvo, Novi Sad,* 31: 173-176.

Poštić, D., Momirović, N., Stanisavljević, R., Štrbanović, R., Gavrilović, V., Aleksić, G., Đukanović, L. (2014): Examination of the quality of seeds of English flax, Italian flax and red fescue. In Serbian: Ispitivanje kvaliteta semena engleskog ljulja, italijanskog ljulja i crvenog vijuka.*Plant protection. Zaštita bilja*, 65(2):70-76.

Vukeljić. M.V. (2002): Determination of residual amounts of heavy metals in a selected locality in order to protect the environment. Master's thesis, University of Novi Sad, FTN, PSUNS, Environmental Engineering, 1-88. Određivanje rezidualnih količina teških metala odabranog lokaliteta u cilju zaštite životne sredine. Magistarski rad, Univerzitet u Novom Sadu, FTN, PSUNS, Inženjerstvo za zaštitu životne sredine, 1-88.

Popović, V. (2010): Agrotechnical and agroecological influences on the seeds production of wheat, maize and soybean. Doctoral dissertation, University of Belgrade, Faculty of Agriculture Zemun, Agrotehnički i agroekološki uticaji na proizvodnju semena pšenice, kukuruza i soje. Doktorska disertacija, Univerzitet u Beogradu, Poljoprivredni fakultet Zemun, 1-145.

Popović, V., Glamočlija, Đ., Vučković, S., Mihailović, B.,Vidić, M., Dozet, G. (2010). Variation of oil content in soybeans depending on the variety and year, Variranje sadržaja ulja u zrnu soje u zavisnosti od sorte i godine, *51. Consulting the oil industry. Production and processing of oilseeds. 51. Savetovanje industrije ulja, Proizvodnja i prerada uljarica.* Herceg Novi, 27.6.-2.7.2010., 69-75.

Popović, V., Ljubičić, N., Kostić, M., Radulović, M., Blagojević, D., Ugrenović, V., Popović, D., Ivosević, B. (2020a): Genotype x Environment Interaction for Wheat Yield Traits Suitable for Selection in Different Seed Priming Conditions. *Plants – Basel*. 9(12):1804. https://doi.org/10.3390/plants9121804

Popović, V., Vučković, S., Jovović, Z., Ljubičić, N., Kostić, M., Rakaščan, N., Glamočlija-Mladenović, M., Ikanović, J. (2020b): Genotype by year interaction effects on soybean morpho-productive traits and biogas production. *Genetika,* Belgrade, 52(3): 1055-1073. https://doi.org/10.2298/GENSR2003055P

Popović, V., Petrović, B., Ignjatov, M., Popović, B. D., Vukomanović, P., Milošević, D., Filipović, V. (2021): Economic Justification Application of Medicinal Plants in Cosmetic and Pharmacy for the Drugs Discovery. *An Introduction to Medicinal Herbs. Book Emerald M. Ed.,* p.1-365. https://doi.org/10.52305/TKAL3430

Popović, D., Rajičić, V., Popović, V., Burić, M., Filipović, V., Gantner, V., Lakić Z., Bozović, D. (2022). Economically significant production of *Secale cereale* L. as functional food. *Agriculture and Forestry,* 68 (3): 133-145. https://doi.org/10.17707/AgricultForest.68.3.11.

Pravilnik o kvalitetu semena poljoprivrednog bilja. "Službeni list SFRJ", br. 47/87, 60/87, 55/88, 81/89, "*Službeni list SRJ*", br. 16/92, 8/93, 21/93, 30/94, 43/96, 10/98, 15/2001, 58/2002

Probert, R.J., Hay, F.R. (2000): Keeping seeds alive. In 'Seed technology and its biological basis'. Eds M. Black, D. Bewley, *Sheffield Academic Press*: Sheffield, UK, pp. 375–410.

Probert, R., Adams, J., Coneybeer, J., Crawford, A., Hay, F. (2007): Seed quality for conservation is critically affected by pre-storage factors. *Australian Journal of Botany, 55*(3), 326. https://doi.org/10.1071/bt06046

Rajičić, V., Popović, V., Đurić, N., Biberdžić, M., Babić, V., Stojiljković, J., Grubišić, M., Terzić, D. (2023): Impact of agro-ecological conditions and fertilization on yield and quality of triticale on pseudogley soil. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 51(3): 13387. DOI:10.15835/nbha52113387

Reed, R.C., Bradford, K.J., Khanday, I. (2022): Seed germination and vigor: ensuring crop sustainability in a changing climate. *Heredity,* 128(6): 450-459. https://doi.org/10.1038/s41437-022-00497-2

Shaban, M. (2013). Study on some aspects of seed viability and vigor. International *Journal of Advanced Biological and Biomedical Research,* 1(12): 1692-1697.

Sekulić, T., Stupar, V., Stevanović, A., Živković, Z., Saulić, M., Blažić, M., Popović, V. (2023): Biodiversity of microbial populations as the indicator of biogenicity of soil under ashes and agricultural soil. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 51,1, 13115, https://doi.org/10.15835/nbha51113115

Stanisavljević, R., Simić, A., Sokolović, D. (2010): Proizvodnja semena višegodišnjih krmnih trava u Srbiji. *Biotehnology in Animal Husbandry,* 26(2): 159-172.

Steiner, A.M., Kruse, M., Leist, N. (2008): ISTA method validation 2007: a historical retrospect. *Seed Testing International,* 136: 32-35.

Stefanello, R., Viana, B. B., Neves, L. A. S. das. (2017): Germination and vigor of linseed seeds under different conditions of light, temperature and water stress. Semina: *Ciências Agrárias,* 38(3): 1161. https://doi.org/10.5433/1679-0359.2017v38n3p1161

Stevanović, A., Bošković, J., Zečević, V., Pešić, V., Ćosić, M., Šarčević-Todosijević Lj., Burić, M., Popović, V. (2023). Variability and heritability of technological characteristics of Amaranthus leaves and seeds. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 51(2): 13128. https://doi.org/10.15835/nbha51213128

Stupar, V., Živković, Z., Stevanović, A., Stojićević, D., Sekulić, T., Bošković, J., Popović, V. (2024): The effect of fertility control on soil conservation as a basic resource of sustainable agriculture. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 52(1): 13389. https://doi.org/10.15835/nbha52113389

Tomaz, C.A., Martins, C.C, Sanches, M.F., Vieira, R.D. (2015): Time reduction for surinam grass seed germination test. Cienca e Agrotecnologia, 39(5), 488-497.

Van de Venter, A. (2000): What Is Seed Vigour? *Journal of New Seeds,* 2(3): 67–72. https://doi.org/10.1300/J153v02n03_06

Vasileva, V., Georgiev, G., Popović, V. (2023): Genotypic specificity of soybean [*Glycine max* (L.) Merr.] plastid pigments content under sowing date and interrow spacing. *Genetika*. 55(2): 455-471. https://doi.org/10.2298/GENSR2302455V

Velijević, N., Simić, A., Vučković, S., Đukanović, L., Poštić, D., Štrbanović, R., Stanisavljević, R. (2016): Variability of dormancy, seed germination and seedling vigor of red date and Italian ryegrass cultivars. Varijabilnost dormantnosti, klijavosti semena i vigora klijanaca sorata crvene dateline i italijanskog ljulja. *XXI Savetovanje o biotehnologiji sa međ. učešćem*, Čačak, 11-12.3.2016. 21(23): 73-80.

Vujaković, M., Balešević Tubić, S., Milošević, M., Zlokolica, M., Nikolić, Z. (1999): Legal regulations in seed production and accreditation of seed testing laboratories, Zakonski propisi u semenarstvu i akreditacija laboratorija za ispitivanje semena, *Naučni institut za ratarstvo i povrtarstvo,* Novi Sad, 379-384.

Wang, L., Ma, H., Song, L., Shu, Y., Gu, W. (2012): Comparative proteomics analysis reveals the mechanism of pre-harvest seed deterioration of soybean under high temperature and humidity stress. *Journal of Proteomics, 75*(7), 2109-2127. https://doi.org/10.1016/j.jprot.2012.01.007

Wang, X., Zheng, H., Tang, Q., Chen, Q., Mo, W. (2020): Seed filling under different temperatures improves the seed vigor of hybrid rice *(Oryza sativa L.)* via starch accumulation and structure. *Scientific Reports,* 10(1): 563. https://doi.org/10.1038/s41598-020-57518-5

Wei, J., Liu, X., Li, L., Zhao, H., Liu, S., Yu, X., Shen, Y., Zhou, Y., Zhu, Y., Shu, Y., Ma, H. (2020). Quantitative proteomic, physiological and biochemical analysis of cotyledon, embryo, leaf and pod reveals the effects of high temperature and humidity stress on seed vigor formation in soybean. *BMC Plant Biology,* 20: 1-15. https://doi.org/10.1186/s12870-020-02335-1

Yogeesha, H. S., Upreti, K. K., Padmini, K., Bhanuprakash, K., Murti, G. S. R. (2006). Mechanism of seed dormancy in eggplant (*Solanum melongena* L.). *Seed Science and Technology,* 34(2): 319-325.