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РАСТЕЖНА СИЛА, ТЕСТОВЕ ЗА НЕЙНОТО ОПРЕДЕЛЯНЕ И ПОСЕВЕН ПОТЕНЦИАЛ НА СЕМЕНАТА ОТ ЗЕЛЕНЧУКОВИ КУЛТУРИ
VIGOUR, VIGOUR TESTS AND SOWING POTENTIAL OF VEGETABLE SEEDS

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Abstract

Obtaining of the stable and quality agricultural production is directly related to the quality of the seed. Most vegetable crops are propagated by seed. Therefore the question about potential of the used seeds is essential in growing of vegetable crops. Besides traditional germination shift lately more attention not only in science, but in practice also is paid to their vigour. This is the indicator that gives an idea not only whether the seeds will germinate and sprout, but how they will develop normal seedlings in a wide range of the environmental conditions. This article presents a review of significant achievements in the world science in this scope. The main topics for the initiation of the idea and the essence of the vigour and the stages through which it has passed, the development to contemporary concept and methods for its establishment, achievements and growing importance are discussed. The particulars, positive aspects and disadvantages of the basic tests that apply that applied for determination of the vigour are followed. At the same time in the keys of the article are included many own research in this area as well as the method developed by author for determining the seed viogou named "Initial vegetative productivity of seeds". In conclusion are set out the important and significant achievements by unresolved issues as well as indicate the further direction in which it is necessary to develop this part of Seed Science.

Key words: seeds, germination, tests, vigour, germination.

INTRODUCTION

The seed is the product of a long historical and evolutionary development with great flexibility be disseminated and reproduce plants, which helped the seed plants to become dominant in terrestrial flora. The study of the properties and qualities of seeds is extremely complex and multilateral. The seed is the biological object in which most commonly manifests the relationship between theory and practice, but also fundamental laws of dialectics (Broniewski et. al., 1976).

The discovery of the seed of people is the basis for the establishment and development of civilization because after learning capabilities for growing, harvesting and mostly storage the seeds the human abandoned the nomadic lifestyle and began to build settlements for the long and sedentary living. (Daniels and Hysloop, 2003; Isbouts, 2009). From millennium farmers produce seeds and use them for propagation.

According to Copeland and Donald (2001), seed can be seen as a peculiar micro universe containing a live organism, able to display most of the processes inherent in older plants. Studying a lot of seeds we acquire new knowledge on almost all processes occurring in plants. At the beginning of the use of the seeds did not pay particular attention to nature and the ongoing processes in them. The first research on Seed Science can adopt the studies of botanists on organs and methods for propagation of plants and the long time it was part of botanical science. It was only when the seeds are commercialized for international trade, approximately 1870, appears necessary to assess their qualities for which is need to develop research to put on scientific bases the main process for assessment of the seeds (Broniewski et. al., 1976).

VIABILITY POTENTIAL

The features and properties of seeds can not be studied directly. For their life potential, the knowledge is obtained by the most important their characteristics - viability, vitality and vigour that determine the main sowing and productive qualities of seeds (Dencheva et al.,1985). It comes to the fore the need for significantly more detail determination of the vitality status of the seed, which is not only scientific seeking but has great practical importance. In order to better understand the difference in the nature of these terms and the necessity of introducing a wider range of analysis and indicators qualification, the seeds must briefly mention the definitions of the above-mentioned characteristics.

Dencheva et al., (1985) express the opinion that the viability indicates the amount of all seeds with alive embryo established by various methods, i. e. the so-called vitality seeds. Not all viability seeds, however, can develop normal sprout, which requires the introduction of another term vitality - the ability of seeds to germinate under certain favorable conditions, if overcome any of the possible statuses of dormancy or it is occurrence rate of viability. The viability of a seed lot is determined by normal germinated viability seeds, expressed as a percentage and indicated more often as germination. It is known that not always a high percentage of germinated seeds, accounted in laboratory conditions means that these seeds placed in a field environment would show the same germination and that the germinated seeds will produce normal seedlings from which to develop further normal plants. This requires searching of methods and tools to evaluate the potential of the seed. As an expression of these capabilities is perceived the strength of growth and accumulated biomass of seedlings within the species and variety of features. So are introduced the concepts of "vigour" or "growth biomatter".

Under the vigour of seeds be understood the sum of all the properties of the seeds that determine the potential level of their activity and field germination in the process of germination. While viability expresses the ability of the seed grows normally, the vigour shows the ability to develop normal plants.

The imagines of the viability of the seeds are well known, but regardless of that, there are some controversial moments and therefore is need clarification and precision. Depending on the viewpoint and line of the use of seeds there are different understandings in this direction. Seed producers and traders of seeds under viability understand the ability of the seed to germinate and develop normal sprouts and thus used as a synonym capacity of germination. In this way whether a seed is viable or not depends only on its ability to germinate and develop normal sprouts (Copeland and Donald, 2001).

In another aspect viability denotes the level to which the seed is alive, metabolically active, has an enzymatic activity to catalyze metabolic reactions necessary for germination and growth of sprout. In this context, a single seed may contain live and dead tissues and therefore may or may not germinate.

Therefore the presence of living tissue is particularly important for the viability of the whole seed.

In assessing the state of the seeds it is also necessary except for the determination of germination in a standard way, to be able to give information about the further development of sprout and subsequently the plant. The definition of germination - emergence and development of the embryo of the seed emphasize to these essential structures of the species of given seeds are indicative of the ability to reproduce a normal plant under favorable conditions, but as Copeland and Donald (2001) point and materials AOSA (1991) also it does not give full information on the behavior of the seed lot of the field due to the following:

- emphasis on "essential structures" that lead to receiving the normal plant without giving a relationship with the speed and power of growth, which is the initial criterion for successful development;

- despite the fact that this test is standardized and repeatable, due to favorable conditions in which it is performed - artificial standard, almost sterile and controlled thermostat, i. e. It is a synthetic medium that almost never can be found in the field. This ensures the expression of the maximum development of the properties of seeds, but the seeds sown in the field are under quite different conditions and therefore that of a regulated test usually greatly overestimated the field germination;

- in the standard test is performed two determinations - first (germination energy) and last (germination). In the first are accounted germination of the strongest seeds, and the second was after a considerable period, which is sufficient for germination significantly weaker seeds. Thus is the total germination, is a sum of both strong and weak seeds. Exactly this is one of the disadvantages that weak seeds rarely develop in this manner in the field;

- by definition, germination is without detailed basis or a scale for accounting, i. e. The seeds or germinated or not, and does not distinguish between weak and strong seedlings. So germination can vary from weak in somewhat better to fully developed and strong seedlings. Thus it is impossible to take account of the gradual deterioration in the quality of seeds, which significantly affects the overall manifestation of the seed.

Therefore, except for germination data are looking different possibilities for establishing of objective criteria, what is the initial and subsequent development of the new plant. Therefore, unless germination in a comprehensive and detailed assessment should also include other parameters reflecting seed potential growth power, the energy of growth in sprout, expressions of the seed at other than optimal conditions. All this requires the use of different methods and tests to be answered about the status of the seed and the possibility of developing good sprouts and plant.

Vigour, as McDonald (2002) underlined, is a relevant test of the samples that show high germination under laboratory conditions of the standard test for germination. Over the past 30 years the discontent of seed producers and users of seeds from the results of a standard germination test as the only criteria for evaluating their potential increased. Therefore the share of laboratories applying tests to determine the vigour has increased. Particularly high is the share in the US, where more than 80 departments for qualifying seeds work and for establishing the vigour .The ISTA and AOSA Vigor Test Committees have been at the forefront of this development.

The development of uniform tests to determine vigour is a relatively slow process. It is good only be recalled that the first law on seeds is from 1816, issued in Bern, Switzerland and nearly 100 years after it published rules for qualification.

HISTORICAL OVERVIEW AND DEFINITIONS FOR VIGOUR

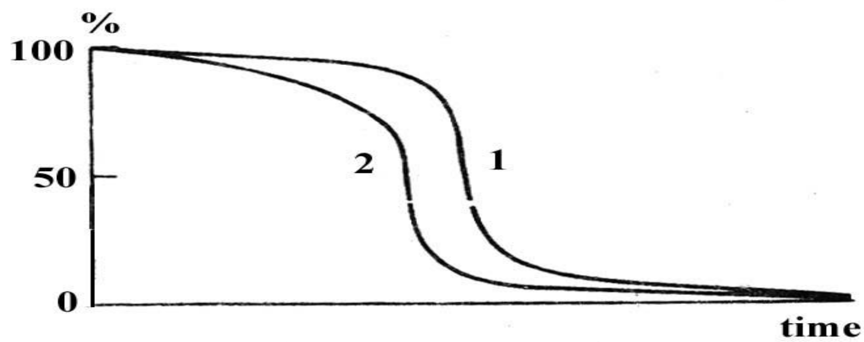
Copeland and Mc Donald (2001) reported that first, which makes a distinction between germination and seed potential is Fredrich Nobbe in 1876. He introduced the concept of "triebkraft", which means driving energy force or breakthrough (pushing) power. Especially important parameters for the seed quality, except germination, he adds another - speed and uniformity of emergence of seedlings.

In 1950 the International Congress of seed testing emphasizes once more on the concept seed potential and vigour. Highlighting the differences between the European and the American way of qualification and testing of seeds. Frank. WJ (1950) back in 1950 noted the differences in the comprehension of the concept of germination test between European and American laboratories. In Europe, the seeds are tested for germination under optimum conditions, to determine the quality especially in relation to international trade of seeds. American concept aims to determine the ability of seeds to so-called "Growth Productivity". To establish the possibilities of developing the capabilities of the seeds lots on the field the concept of vigour is introduced.

On the ISTA Congress in held Madrid in 1979, the official wording of the life force of seeds states: "The vigour of seeds is the sum of all the properties which determine the potentially level of their activity and field germination in the process of sprouting." It can also be added that the vigour is the sum of these properties that determine the activity and manifestations of the seed lot with acceptable germination for fast and simultaneous germination and development of normal seedlings under a wide range of environmental conditions.

Vigour is a very important indicator for the seeds of vegetable crops, as they are small-, with slow germination and emergence. Furthermore, they are sown in the upper soil layer where the variation of humidity and temperature is high. In these seeds are observed characteristic features, both in their chemical composition - the presence of essential oils and in their anatomical as well as morphological structure - fragile seed coat, leading to frequent breakdowns in harvesting, seed extraction, seed cleaning and storage. Very often it is necessary storage of vegetable seeds for different periods. Actual seed production requires high technology, very good agricultural knowledge and specific equipment, which reflected on higher prices.

Changes in vigour, as reported Dencheva, At. et al. (1985) occurred much earlier than those of viability and can be identified long before the changes occurred in the germination (Fig. 1). The curves represented the two indicators - germination and vigour are divided into three periods. During the first period, the function of the seed weakens slowly. This stage ends at 75% vigour of the initial, and then germination is still 90% and over 90%. In the second period, the deterioration is with much greater rate and stay alive only 10-25%. In the third period, perished also those seeds, but much more slowly. Both curves have the same course, but the loss of vigour preceded the loss of viability.



1- dynamic of the changes of viability; 2 – dynamic of the changes of vigour

Fig. 1. *Changes of viability and vigour of the seeds during the period of their storage (by Justice and Bass, according Dencheva, At. et al., 1985)*

It is obvious that not all of these seeds under field conditions will germinate and still less, even in the best agrotechnical, will be able to develop normal plants and will be able to realize the set genetically, maximum productivity and potential. This demonstrates that except to viability percent by sprouted seeds should pay particular attention to the strong vigour. In loss of vigour with 25% in the same time the germination is 90% and above 90%which makes these seeds to be referred to first class.

In conducted in the Department of "Horticulture" at the Agricultural University of Plovdiv, Bulgaria, a trial with ten years stored pepper seeds is observed the above-indicated dependence. When collating the percentage of changes, towards to the highest values adopted to 100% of those two indicators (vigour and germination), it was found that vigour decreases much earlier compared to germination, (Fig. 2). In Kurtovska kapia 1619, yet in the six-years old seed when the reduction germination of is 20%, the vigour declined by almost 58.2% for the whole lot and 33.1% for a seed. Weaker are the differences for Bulgarskirotund but here also the change of the vigour precedes that of germination, as of the fourth year germination is reduced by 18.9% and the vigour reduction is with 25.7% (for a seed) and with 24.4% (for lot) (Panayotov, 2010).

METHODS FOR ASSESSMENT OF VIGOUR OF SEEDS

The challenge to create a test to assess the vigour is that you need to determine one or more quantitative parameters, which usually cause deterioration of seeds. We test seeds because "a seed lot is composed of a population of individual seed units; each possessing its own distinct capability to produce a mature plant. A seed vigor test is an analytical procedure to evaluate seed vigor under standardized conditions. It enables a seed producer to determine and compare the vigor of a seed lot before it is marketed" (McDonald, 1999; Copeland, L. and M. Mc Donald, 2001, McDonald, 2001).

The hypothetical model developed by Delouche and Boskin (1973) describe some of the main parameters used in measuring the seed vigour (Fig. 3). The vigour of seeds is much more sensitive indicator than germination, so any event prior to the loss of viability, can serve as the basis for its determination. Earliest before a loss of viability can be measured degradation of cell membranes and therefore the most sensitive test for vigour are related to the integrity of the cell membrane. Another manifestation of the deterioration of seeds is losing the storability and resistance to diseases. Deterioration of seeds as a result of biological or environmental stress also decreases field germination. Finally, the number of abnormal seedlings and eventually loss of germination are increased. The tests for establishing the vigour measure one or several parameters, of changes, in the case of deterioration of seed, physiological and biochemical characteristics.

Depending on what and how to measure, Copeland and Mc Donald (2001) divide these tests on directly and indirect.

In the direct are create conditions similar to those in the field and study the ability of the seed to germinate under the simulated stress conditions

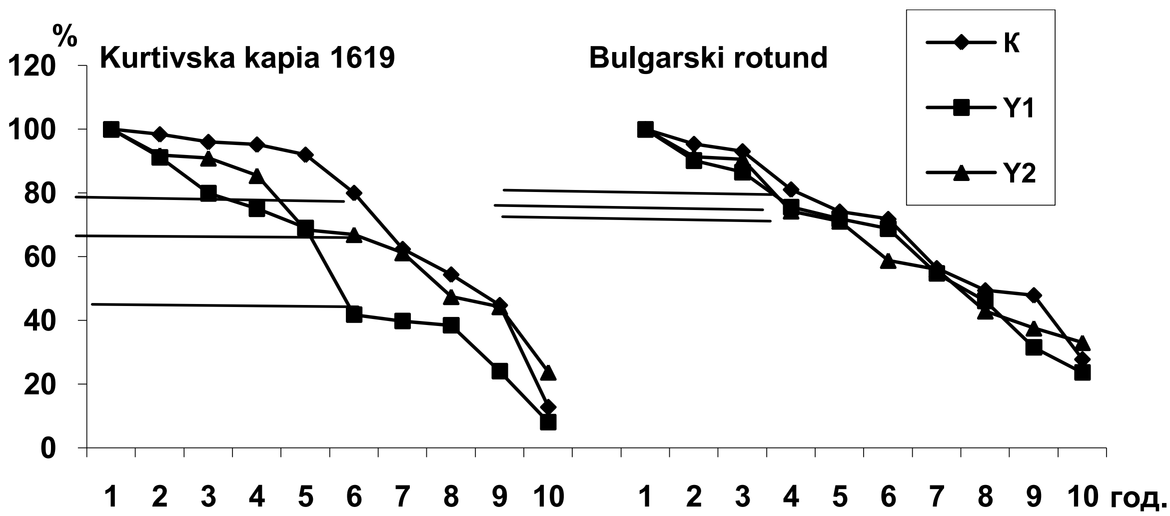


Fig. 2. *Changes of germination (K), vigour of the lot ($У_1$), vigour of one seed ($У_2$) (by Panayotov, 2010)*

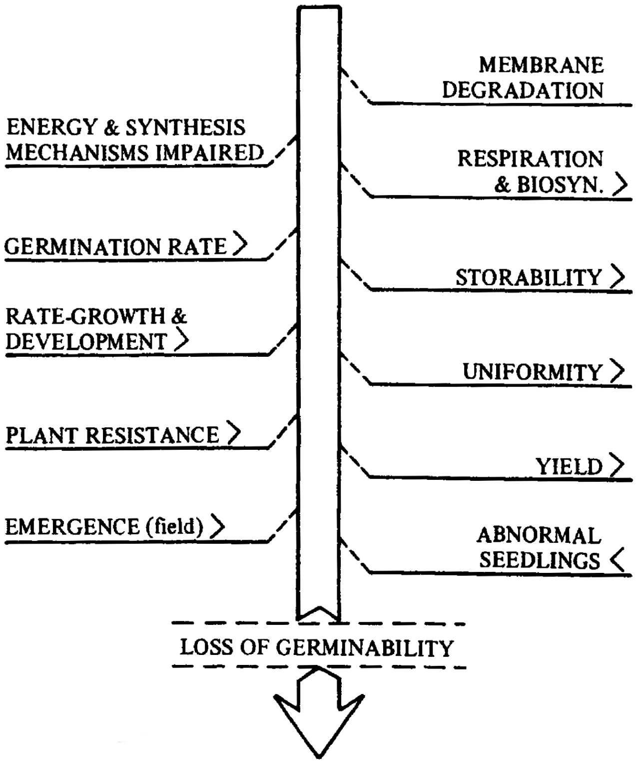


Fig. 3. *Probable sequence of changes in seed during deterioration (by Delouche and Baskin, according Copeland and Mc Donald (2001)*

in the field. The test at low temperatures belongs to this group since the seeds are placed under adverse conditions - low temperatures, wet soil and others. A critical feature of these tests is that they do not reveal the differences in quality when the seeds are exposed under favorable soil conditions.

The indirect tests measure specific physiological properties of the seeds. For example, the test of conductivity is such as measured the releases by the cell. These tests can not determine all physical or physiological factors affecting field germination. Tests of vigour can be divided depending on the component of measuring respectively they are physiological, biochemical or physical (dimensions, etc.).

Physiological tests measure some aspects of germination and development of seedlings.

The biochemical studied mainly the changes in specific chemical reactions (enzyme activity, breathing, etc.), connected with the process of germination.

Physical are dealing with assessment the seed size, shape, texture, factors which determine the vigour due to the direct connection of these features with maturity.

The tests are divided yet into stressful and rapid.

The stressful include measuring certain aspects of germination, the length of hypocotyl under stressful conditions - high temperature and relative humidity (for accelerated aging test), the test of the low temperature, a test of cooling, osmotic stress.

In rapid tests are observed some chemical reactions associated with the vigour. They require less time. This is tetrazolium test, a test of conductivity and tests relating to the enzymatic activity.

CHARACTERISTICS OF METHODS FOR ASSESSMENT OF THE SEED VIGOUR

According to Copeland and Donald (2001) to be applicable the tests, they must comply with the following conditions:

1. Cheap. The test must be a minimum investment needed to equip the of the laboratory, for labor, for consumables.

2. Rapid. Each laboratory has a peak load and therefore the tests must be carried out quickly, with little time for analysis and space. We also need to quickly get the information to the users of test results.

3. Easy to implement. The procedures should be simple, does not require sophisticated equipment and staff with special training.

4. Objective. These tests should be easy to standardize, quantifiable measurable and digital expressible. Thus, the interpretation and use of the results will be easier.

5. Repeatability. Results should be able to repeat during the next determination. This is especially important for comparability of the results between the separate laboratory.

6. To have the connection with the manifestations of the seed under field conditions. Most definitions and studies emphasize of those particular characteristics - be capable predict how the seed will occur in field conditions.

TYPES OF TESTS FOR ESTABLISHING THE SEED VIGOUR

The germination tests are conducted under an optimal condition for germination of seeds of given species. Very often environmental factors in the field do not fit into the parameters of these requirements. So it needs to implement additional analyzes and tests to assess and predict how the seed would manifest in field conditions. Knowledge of essence of the various tests is the main factor that enables to select the most suitable method, depending on the seed species and the abilities of the given laboratory. Therefore it is good to describe briefly the advantages and disadvantages of the more widely spread technique of establishment of the vigour of the seeds. Several of tests, that are with more or less scientific and practical significance are developed and implemented (Copeland, and Mc Donald, 2001) as the most important of them, that will be described below, are following:

Cold test (Test the low temperatures)

This is one of the oldest tests using a stressful situation and it is used very often. The seeds are placed in soil or in a filter paper with a strip of soil and exposed to low temperatures for a given period, thereby causing stress from the temperature and from soil microorganisms on the water absorption and imbibition. After a period of low temperatures, the seeds are placed under optimal conditions allowing to germinate. The great difficulty in this test is the lack of uniformity in the soil, which differ in humidity, pH, content, pathogens etc., which lead to the obtaining divergent results. It can use perlite or vermiculite with the appropriate solution, but still better the soil substrate. Many laboratories consider that this is a useful test for vegetable seeds. Piana et al. (1995) reported that this test, in a comparative test of the variable low temperature (7 days at 10^0C, and 7 days at room temperature) is shown closest to the field results germination and viability of seedlings from an onion.

Accelerate ageing test

This test includes many of the features that should have another test of the vigour of the seed. Originally used as a method for evaluating of the seed storability. The object of this test is not imbibed seeds placed under high temperature and relative humidity (41^0C and about 100%) for a short period of 3-4 days, after which these seeds were placed under optimum conditions for germination.

It is realized in a plastic box (chamber) in the shape of a cube with dimensions of 11 x 11 x 3 cm, with a grill on which the seeds behave, placed in a thermostat with a water jacket. Seeds are arranged on a layer on the metal grill that hangs inside the plastic box containing 40 ml of water. The box is covered with a lid and kept in a thermostat at a high temperature, for example, 38-41^oC for different periods, for example, 48, 72 or 96 hours. Then the seeds are placed for germination. Except water, in the box can be placed a solvate saturated saline (40 g NaCl 100 ml^{-1} water) that achieves a relative humidity of 76%. This test is quick and cheap, easy and useful and can be used in different species.

In the above mentioned of Piana et al. (1995) also results that are close to field germination and viability of seedlings of onion were obtained in its application (48 hours at 42^0 C). However, from the standard test obtained results overestimate the life potential.

From the conducted experiments with three seed lots of pepper of various origins and subjected to artificial aging at temperature 35, 40 and 45^0C for 24, 48 and 72 hours, Panayotov (2014) indicates that the highest tested temperature of 45^0C, causes almost complete lethality of seeds at all exposures, varieties and lots. This practically makes it unsuitable for assessing the vigour and seed storability from the pepper. Higher differences between separately lots, which is a precondition for assessing the vigour and storability, begin to observed on a 72 h/35^0C and 48 h/40^0C (Fig. 4). Closer to the original values in these two regimes is established are those for variety Delicates originating from Vardim and Stryama. Furthest from the results of these baseline samples are those for variety Bulgarskirotund from Vardim and Plovdiv and for Kurtovska kapia 1619 from Stryama and Plovdiv, which shows that this indicator they are with the weakest vigour and storability. Reductions in germination are relatively weak and gradually for almost all exposures at 35^0C. A significant decrease was recorded in 40^0C regime for 48 hours and the differences between the samples are well developed. This allows emphasizing that appropriate regime for pepper seeds accelerate aging, which exhibits highest physiological sensitivity is 40^0C for 48 hours.

In the seeds of beans stabilized mutants (Table 1) germination energy significantly influenced as a result of artificial aging at 40, 45 and 50 degrees for 24, 48 and 72 hours. The most drastic changes, however, are in treatment for 48 and 72 hours. With the best physiological sensitivity and more pronounced differences are outlined two regimes of accelerating aging of beans seeds for a period of 48 hours - at the temperature at 40⁰C and especially at 45⁰C. The highest vigour and storability between tested stable mutants showed mutant, treated with 0.0125 M EMC in which even in regime 45⁰C for 48 h was accounted germination of 48.3% (Svetleva and Panayotov, 2014).

Test of controlled seed deterioration

This test is designed to direct differentiation of the vigour of lots of species with a small seed, as the vegetable. The main idea is the seed of all lots to be placed in exactly the same conditions which will deteriorate or age the seeds. Before starting the test, the humidity of seeds is increased to the same values for all samples. This is achieved as the seeds are placed on wet filter paper and periodically measured the moisture content by weighing method to determine when it achieved the required humidity. The seeds are then sealed in the pouch, allowing after staying overnight at a temperature of 5-10⁰C the moisture to equilibrate, after which they were placed in a water bath at 45⁰C for 24 hours. Following this period of deterioration, the seeds are tested by the standard test for germination (Black et al., 2008).

Conductivity test

Seeds with low vigour have reduced membrane integrity as a result of their degradation during storage or mechanical injury. Imbibed seeds

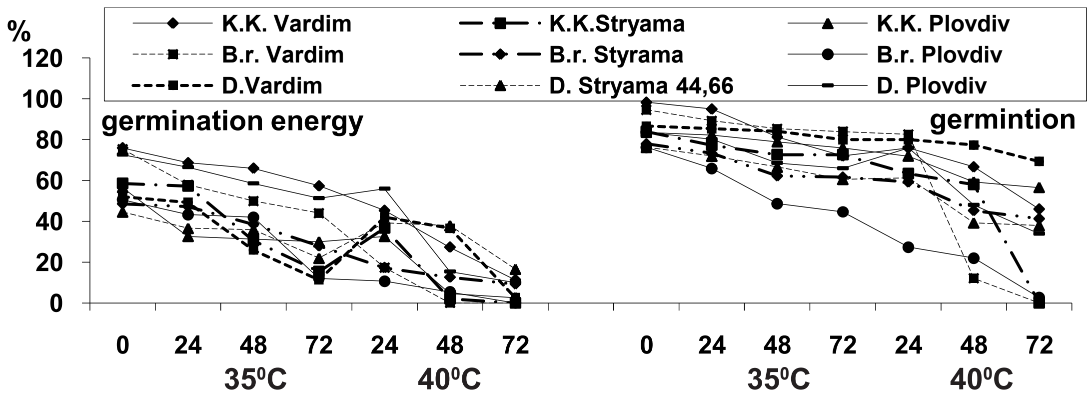


***Fig. 4.** Viability of pepper seeds after accelerated ageing (Panayotov, 2014)*

Table 1. Sowing qualities of bean seeds after accelerate ageing (Svetleva and Panayotov, 2014)

Variants / Mutant	Germination energy (%)			Germination (%)		
	1	2	3	1	2	3
Control	61.6	80.0	71.6	84.3	88.3	86.7
24 ч. /40⁰C	70.0	79.6	71.6	83.3	84.6	85.7
24 ч. /45⁰C	45.0	45.0	48.3	46.66	55.0	66.6
24 ч. /50⁰C	18.3	21.6	41.6	30.6	33.3	61.66
48 ч. /40⁰C	61.6	78.3	65.0	72.6	81.6	74.0
48 ч. /45⁰C	3.33	45.0	28.3	3.33	48.3	35.0
48 ч. /50⁰C	1.66	11.6	21.6	1.66	18.3	**28.3**
72 ч. /40⁰C	8.33	30.0	45.0	8.33	40.0	45.0
72 ч. /45⁰C	5.0	13.3	13.3	6.7	13.3	16.6
72 ч. /50⁰C	1.66	3.33	3.3	1.66	5.0	5.0

1 – Mutant with 0.0031 M NEC, 2; 2 - Mutant with 0.0125 M EMS; 3 - Mutant 0.0125 M EMS that there are poor membrane structures, emit substances from the cytoplasm into the middle where they absorb water or imbibed. These substances have electrolytic properties and carry electric charge, which can be identified by conductometric measurement (measure the electrical resistance). It is a fast, accurate, inexpensive and easy method. The moisture content and size of the seeds, however, can have an impact on the solution. This test, however, shows only the results for the tested seeds, i. e. 25 pcs. It is believed that all the seeds in the lot will deteriorate in the same way, but each seed is unique and has individual characteristics. Better results for the whole lot in µs $cm^{-1}g^{-1}$ to be presented on the basis of individual seeds.

It is often applied to the vegetable seeds, due to their small size, the strong variation in humidity, the need for long-term storage and rapid loss of viability.

Comparing the various methods applied, it was proved that the most significant correlation with carrot and peer seed field viability was observed when using electrical conductivity. This was a very suitable and applicable approach providing rapid and objective information about seed status (Andrade et al., 1995 and Torres, 1996). Other authors, however, reported the existence of pronounced cultivar differences (Prusinski and Borovska, 1993) and that the aforementioned tests had limited application for predicting seed status (Ratajczak and Duczmai, 1991). It is seen that the problem of using electrical conductivity as a parameter for characterizing seed status is still under discussion. Dong et al. (1998) established a negative correlation between the laboratory and field germination and electrical conductivity in onion seeds. With the increase of the storage period, the viability of onion seeds decreased, and the electrical conductivity increased.

A similar tendency was observed by Aladzhadjiyan and Panayotov (1994) in tomato seeds, stored for 2 to 15 years under room conditions - the increase in the storage period led to a decrease in electrical resistance, i.e. the electrical conductivity increased, and seed viability decreased. The authors, however, established a species-specific response - in pepper seeds, subjected to the same conditions for 2 to 8 years, the electrical conductivity decreased. In both species, this was accompanied by a decrease in the percentage of germination of seeds.

Ozoban and Demir (2002) established that in pepper and watermelon seeds stored at 10 and 20^{o}C, with 8, 10, 12, and 14% moisture contents for 18 months the conductivity tests did not give consistent readings with changes in viability and emergence.

The development of pea and pepper seeds and the increase of their viability induced some changes in their physical parameters and electrical properties, in particular (Table 2). Panayotov and Aladzhadjiyan (1999) and Aladzhadjiyan and Panayotov (1999) estimated that with the advance of growing and the increase of seed germinability, the values of specifically electrical resistance decreased and strong correlation being established. Authors pointed out that this parameter characterized the status of seeds and could be used as an indicator for determining the stage of their development.

Seedling growth rate test
(Test of dry bio - matter)

Vital seeds during sprouting can synthesize the new material, i.e. to accumulate biomass. These new products are transformed to the strong growth of the embryo root, which in turn affects the accumulated dry mass. Test on the value of dry biomass of the seedling is based on this concept and the results are reflected in mg of dry matter / sprouted seeds. This test is conducted under standard test conditions for germination. The growth elements of normally sprouted seeds were excised and placed at 80^{0}C for 24 hours in an oven, in order to measure the increase in dry mass. It is believed that there is a correlation with vegetative growth under field conditions. This test is very promising.

The disadvantage is that small differences in humidity and light affect the growth of the seedling. Should be standardized for different crops as the growth of the seedling is a genetically controlled trait.

Seedling vigour classification test
(Test by morphological behaviors)

This test extends the standard germination test as normal seedlings classified into two categories strong and weak, but also separate and abnormal seedlings. The test does not require additional equipment and used concepts and terms are similar to those of test for germination. There are serious difficulties. The separation of categories of normal seeds require good knowledge and is a prerequisite for further results vary.

In a ten-year storage of pepper seeds, percent of deviations to the 3rd and 4th year is relatively small. The increase in the percentage of seedlings with deviations Panayotov (2010) observed in variety Bulgarski rotund from the sixth year order to reach 96.67% in ten years old seed. A similar situation was also reported to Kurtovska kapia 1619. In this variety, however, still, in the starting age is observed deviations from the normal structure of the seedlings in the 10 to 13.3 percent. The maximum amount of abnormal seedlings is also in the seed at ten years (Table 3).

Table 2. Sowing quality and specific electrical resistance of pepper and pea seeds through different stages of development (Panayotov and Aladzhadjiyan, 1999; Aladzhadjiyan and Panayotov, 1999)

Days after flowering	Germination energy %	Percentage germination %	Specific electrical resistance (Ω. m)	Germination energy %	Percentage germination %	Specific electrical resistance (Ω. m)
Pepper cultivars						
Dzhulunska shipka 1021				Chorbadzzhijski		
20	0.0	0.0	44.1	0.0	0.0	30.0
30	0.0	0.0	20.5	0.0	0.0	28.4
45	2.2	2.2	14.8	0.0	0.0	18.8
55	14.7	36.0	17.1	8.6	11.3	15.5
70	54.6	87.6	11.0	37.0	77.3	10.1
Pea cultivars						
Ran 1				Prometei		
10	0.0	0.0	17.1	6.0	26.3	36.2
20	9.0	10.2	33.3	11.3	49.3	83.8
25	13.7	92.8	132.9	12.0	94.0	86.2
30	25.0	97.5	115.0	12.6	96.6	41.2
40	33.8	86.3	85.5	27.3	85.3	38.9

Table 3. Deviation from normal development of pepper seedlings after different period of storage (%) (Panayotov, 2010)

Period of storage (years)	Kurtovska kapia 1619		Bulgarski rotund	
	(%)	*Type of deviation and percentage to total number of deviation*	*(%)*	*Type of deviation and percentage to total number of deviation*
1	10.0	***K*** - 40.0; ***E*** - 60.0	0.0	
2	13.33	***E*** - 71.42; ***K*** - 28.56	3.33	***H*** - 40.0; ***K*** - 60.0
3	16.67	***H*** - 71.4; ***E*** - 28.6	10.0	***K*** - 33.33; ***H*** - 66.66
4	13.33	***E*** - 66.1; ***H*** - 22.1; ***K*** - 17.8	13.33	***E*** - 100.0
5	30.0	***K*** - 42.85; ***C*** - 57.15	16.67	***R*** - 91.0; ***K*** - 9.0
6	23.33	***R*** - 66.6; ***H*** - 33.33%	33.33	***E*** - 100.0
7	33.33	***K*** - 25.0; ***H*** - 75.0	30.0	***H*** - 50.0; ***K*** - 50.0
8	76.67	***E*** - 52.3; ***H*** - 37.3; ***K*** - 10.4	70.0	***H*** - 100.0
9	70.0	***E*** - 48.3; ***H*** - 32.3; ***K*** - 19.4	70.0	***K*** - 50.0; ***H*** - 50.0
10	90.0	***E*** - 68.3; ***H*** - 12.3; ***K*** - 19.4	96.67	***H*** - 88.12; ***E*** - 11.88

E - underdeveloped embryo root; ***H*** - without hypocotyls; ***K*** - without hairs ;
R - lack of root branches; ***C*** - unopened cotyledons

Tetrazolium (TZ) test

TZ test is one of the most valuable techniques for the assessment of seed quality. Based on the responses 2,3,5 - trifeniltetrazol chloride with compartments hydrogen as a result of the activity of the enzyme dehydrogenase in alive tissue. From this are formed insoluble red pigment called formazan that experienced analysts assess a staining intensity and separate the seeds of categories from strong to weak. Although the results have a good correlation with the vigour, the interpretation depends on the qualifications of the staff and in a sense is subjective. With its help, you can identify non-viable seeds, for example of carrots, for approximately three hours. While the conventional method of detecting the presence of a potential vitality takes three days, and for germination energy - seven days (Andrade, R.N.B. et al., 1996). The advantages of the tetrazolium test in determining the viability of seeds is that, along with reporting the initial appearance of embryo root about 4 mm, these are the two fastest ways to assess the seed quality. Difficulties are in establishing from analyzer staff whether the seed is vital, the manifestation of phytotoxicity and presence of seeds dormancy.

Test of osmotic stress

In field conditions, the seeds often suffered from deficiencies of moisture or even subjected to dry stress. Such conditions can be created in the laboratory, using soil, soil solution or any other solution. The solutions are preferable to the soil as it is difficult to standardize soil conditions. The seeds are placed to germinate in a solution with a specific osmotic potential of NaCl, glycerol, sucrose, polyethylene glycol (PEG) and mannitol. Some osmotic substances, however, are with low molecular weight (sucrose, NaCl, glycerol, and mannitol) and entering into germinating seeds in some cases they are toxic. The high molecular weight PEG (4000 and up) is sufficient to create conditions resemble drought without any toxic effect. Germination under all conditions is markedly reduced and sprouts are heavily damaged. After having the vital seeds can tolerate large osmotic stress, this test can be used to evaluate the vigour. The advantage is that it requires no additional equipment and training. Under certain growing conditions, under stress for evaluation vitality status of tomato seeds can be used as closest to the real opportunities, and germination to take place in water potential 0,4 Mpa (Torres, S. B., 1998).

Respiration test

Germinating seeds using the energy released during respiration. The decrease in the intensity of respiration of germinable seeds prior shows deterioration of development of the seedlings. Respiratory rate is measured during the first 18 hours. There is a positive correlation between the rate (the value) of oxygen absorbed in the imbibition of seeds and growth of seedlings. This is a quick and quantification test, but it requires additional equipment and training, and, moreover, mechanical damage increase the respiration.

Test of "Initial vegetative productivity of the seeds"

By introducing the concept of initial vegetative productivity of the seeds is described more fully their. This is the accumulated biomass fresh after germination and development of seedlings on the basis of the use of the stored nutrients that are in the seed. Panayotov, N. (2013) offers this methodology by which eliminates the possibility of using nutrients from the substrate, the development of the seedling is borne solely by the nutritional components of the seed itself.

The basis of this test is the use of sand so that the seedlings to overcome the resistance of the coating layer them. Seeds are sown at one of the same depth in the well washed, physiologically clean, sterilized for 4 hours 105^0C for river sand with size 3,0 mm placed in disinfected containers, as well aligned, compacted and leveled. The depth of sowing is dependent upon the crop. The thickness of the sand layer is at least five times the depth of sowing. A hundred seeds in four replications were sown. Inter- and Interlinear sowing distances are three times the length of the seed (the largest size). The sand cover is the same thickness and also aligned, leveled and compacted well. Watering is with distilled water of pH 7.0-7.5 in equal amounts for all replications so as to maintain the humidity of 75-80% FWC. The conditions in which is placed the pots (temperature, humidity, etc.) are in conformity with the requirements of the species. The determination of the fresh biomass is carried out at a detectable reduction and cessation of growth and development of plants, according to the crops, low lightening of the leaves and down in the absence of growth in a sequential measurement of the height of the seedling on two different days. This state is most often seen in phase fully expanded cotyledons or appearance of a well developed first true leaf.

The number of seedlings were recorded and were divided into two groups - only with cotyledons and such that developed true leaves. Fifteen plants of each replication are removed carefully, using a spatula to the bottom of the depth of the pot so as not to disturb the root system. The roots are washed by adhering sand and dried with filter paper from surface water. the total mass of roots, stems and leaves for both groups of seedlings were measured separately and the data for a plant are averaged. The percentage of plants in a given group to the total number sown seed were determined. Plants involved in determining the vegetation mass must be at least a phase fully expanded cotyledons and/or in a phase of forming the first true leaves (the lots with stronger of vigour. Index of vigour (Y), determined by the initial vegetative productivity is calculated by the equation

$$Y = \frac{(m_1 p_1) + (m_2 p_2)}{100} ,$$

where

m_1 - mass of a plant in phase cotyledons

p_1 - the percentage of plants in the cotyledon phase

m_2 - the weight of a plant in the first phase leaf

p_2 - percentage of plants in the phase of first true leaves

Vigour, in this case, is a comprehensive assessment of the analysis of the data obtained for developed fresh biomass as well as phenological development of the seedlings. The test is based on the ability of better seeds to overcome more easily and more quickly the resistance of the sand covered and the seedling to appear on the top of the sand and when they there are more nutrients in the seed to develop more powerful biomass.

The advantages of this method are: in this method the vigour of the seeds is determined directly; based on the development of the seedling only on the basis of nutrients in the seed and its ability to overcome the resistance of the sand, thus is obtained more realistic picture of the vigour ; the determined index of the vigour of seeds is with high accuracy; does not require specific equipment; the applied means and materials are cheap; does not require a specific qualification.

In experiments with pepper seeds with different periods of storage Panayotov (2013) observed that after the 7th years old seeds of Kurtovska kapia 1619 and after 8 years old of Bulgarskirotund did not form the seedlings, as in these two variants they reach only to cotyledons stage and their percentage is respectively 10.3 and 11.2 (Table 4). With advancing age developed biomass decreases. The highest values of the vegetative mass and percentage of seedlings reached to a stage of first true leaves in both varieties are accounted in one-year-old seeds. First true leaf formed seeds to the third year. More significant are the differences between the samples developed seedlings to stage of cotyledons. Index of vigour , determined according to these two indicators also varies widely. For a one year seeds of Kurtovska kapia 1619 is 60.78, and for Bulgarskirotund is 65.67 to reach for the first variety at 7-year to 3.32, while for the other variety in 8-year-olds to 3.29. These great differences suggest that these parameters can successfully be used to estimate changes in the vigour. It is highly compliance and convergence in the trend between vigour indices by initial vegetative productivity and these by Elliot (2001) and for the one seed. The curves of these three indexes and germination (Figure 5) are almost similar and have the same way of amendment. This indicates that the proposed index can be used to assess the vigour of the seed. Index of vigour by initial vegetative productivity decreases much earlier than the other two tested index and especially to germination, which is a good prerequisite for the early detection of any deterioration in the vitality status of the seed.

Table 4. Vigour index of pepeer seeds with different age, established by initial vegetative productivity (Panayotov, 2013)

Years	Kurtovska kaipa 1619					Bulgarski rotund				
	Seedlings with cotyledons		Seedlings with first leaf		Y-Vig-our index	Seedlings with cotyledons		Seedlings with first leaf		Y-Vig-our index
	Weight (mg)	%	Weight (mg)	%		Weight (mg)	%	Weight (mg)	%	
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	29.4	11.2	0.0	0.0	3.29
7	32.3	10.3	0.0	0.0	3.32	32.2	20.4	0.0	0.0	6.56
6	38.2	40.3	0.0	0.0	15.39	36.5	41.2	0.0	0.0	15.03
5	42.5	42.8	0.0	0.0	18.19	42.0	45.6	0.0	0.0	19.15
4	46.7	48.2	0.0	0.0	22.50	52.6	48.9	0.0	0.0	25.72
3	51.0	51.2	110.8	6.3	33.09	60.6	55.9	137.2	7.0	43.47
2	57.5	59.8	132.2	10.8	48.66	67.1	60.0	156.3	10.0	55.89
1	62.8	63.8	153.5	13.5	60.78	74.5	60.0	170.5	12.3	65.67

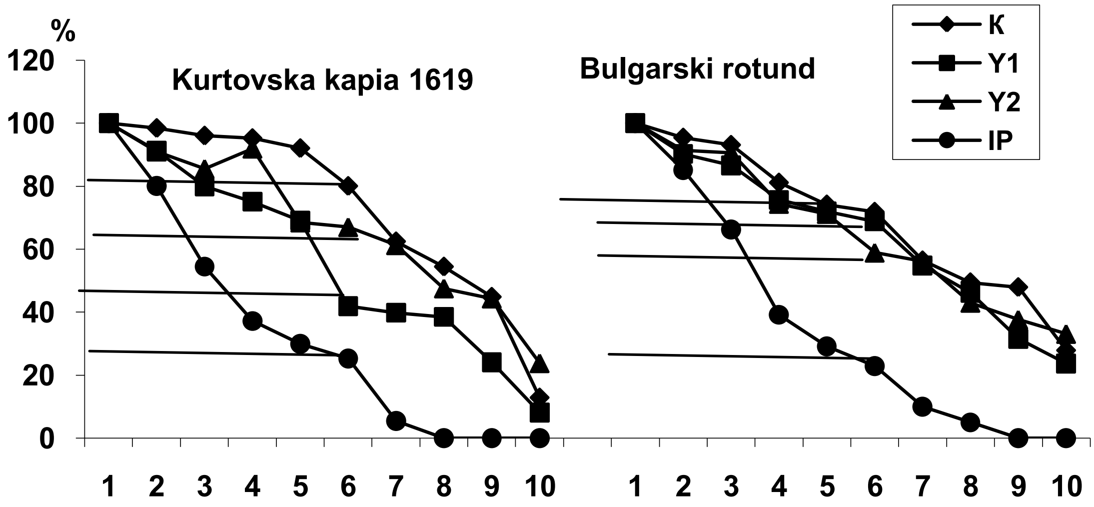


Fig. 5. *Changes of germination (K), vigour index of samples ($У_1$), vigour index of one seed ($У_2$) and vigour index by initial vegetative productivity (IP), 1-10 age of pepper seeds as Table 4 (Panayotov, 2013)*

All these tests have to be standardized so that they can be applied. Difficulties for standardizing occurs because part of the tests is related to subjective evaluation, the variation of humidity and temperature while within a smaller range is significantly affected in these tests, in contrast from the test for germination. Standardization of soil and presence of microorganisms in the test of low temperatures is also difficult. Should be considered and the existence of dormancy in some seeds.

NON-DESTEUCTIVE TEST FOR EVALUATION OF SEED VIGOUR

Particularly perspective and with important recently are so-called non-destructive methods in which the determination of the viability is performed without destroying the seed. Very often they used physical methods for the analysis of various properties and characteristics of seeds, which have a direct connection with their viability and vigour. They mainly provide consistently seed testing. At the same time, they are pretty quick, subject to automation and can be used to create a fast method for sorting of the seeds.

On the basis of signals from the chlorophyll fluorescence of whole seeds (Jalink et al., 1998) assess their ripening and quality. This is related to the direct correlation of a weakening of the green color and maturation. Vegetable seeds with low chlorophyll fluorescence have a high germination and develop normal seedlings. While in the seeds of higher chlorophyll fluorescence signal the loss of viability was much greater. This is a quick method.

Lee et al. (1995) and Lee et al. (1997) provide an interesting non-destructive test based on the fact that the dead seeds in soaking emit large amounts of amino acids, protein, sinapinic while from healthy, viable seeds these quantities are significantly lower.

Through an automated computer system of the images with help of the specific program are scanned three- and four-day seedlings of melon. Based on predefined data is achieved automatic assessment index of vigour. The effectiveness of the method is significant and the obtained results are similar to these occur from the previously accepted test for of vigour (Dell'Aquila, 2006, Marcos-Filho et al., 2006).

Other non-destructive tests are the use of x-rays and the acoustic analysis (Burg et al., 1994). Changes in the morphology of sprout of the seeds of tomato can be predicted by testing using of X-rays. Radiographic technique provides some data about the origin of the differences in the vigour of the seeds of tomatoes: morphological differences in the embryo and how teh content of endosperm is enough, which is crucial for the development of the potential of the seed

Quick checking on the viability of the seeds can be accomplished by subjecting them to UV light (Lee et al., 1997). Viable seeds that can develop normal seedlings do not fluoresce and in chinese cabbage and radishes, such seeds showed germination of 97-98%, and 84-87%, respectively. Dead seeds fluoresce and only 4-7% of them at a radish are sprouted.

CONCLUSION

1. Through the determination of the vigour of the vegetable seeds can achieve more accurate and more complete description of their viability and vitality status.

2. In Seed science the quality of the vegetable seeds are characterized by a significant number of signs such and the increasing the knowledge and information about their precise and fast establishment are crucial for both production of vegetables and for seed production of vegetables crops as well as in view of the enriching the tools used by science itself in this direction.

3. The main challenge in front of the researchers in Seed science is to discover and develop methods and ways of establishing _ of vigour in the direction of earliest possible prediction of changes in viability status of vegetable seed. These tests must meet the requirements, to be standardized and uniformity, so finds wide applicability and to become widely used means.

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