

ASSESSMENT OF VITALITY POTENTIAL AND STORABILITY OF PEPPER SEEDS BY THE METHOD OF ACCELERATED AGEING

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Abstract

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The object of the present study was to investigate the possibilities for evaluation of viability, vigour and storability of sample of pepper seeds with different origin by accelerated ageing test and to optimize the regime for its implementation. The experiments were carried out with varieties Kurtovska kapia 1619, Bulgarski rotund and Delikates, produced in three different regions Vardim, Stryama and Plovdiv. Accelerated ageing test was conducted by described method of ISTA. Three temperature regimes - 35°C, 40°C and 45°C and three periods - 24, 48 and 72 hours were examined. After treatment, the seeds were subjected to standard germination test. The germination energy (first count), germination (final count), mean germination time, uniformity of germination, length of embryo root and hypocotyls, fresh and dry weight of seedlings and percentage of seedlings with deviation were analyzed. The optimum temperature regime about accelerated ageing of pepper seeds was established in temperature 40°C for 48 hours. The initial seeds with best characteristics were these with origin from Vardim. Higher sensitivity to accelerated ageing demonstrated the seed of Bulgraski ratund, Vardim. The highest vigor and storability were established about seeds from variety Delikates from Vardim.

Key words: germination, seeds, seedling, storage, seedling deviation, vigor

Introduction

Vitality status, qualitative characteristics, vigor and ageing of vegetable seeds there are species and very often-varietal character, determined mostly by their morphological structure, chemical components and relating to the conditions of harvesting year. The symptoms of ageing and of their deterioration affect on the morphology, ultra structure, cell membranes, loss of enzyme activity, restriction of respiration, increase of permeability and leaked substances from seeds, increase of free fatty acids, augmentation of lipid peroxidation etc. (Copeland and Donald, 2001). Alsadon et al. (1995) established the species response in deterioration of vitality parameters in accelerated ageing of tomato and cucumbers seeds for 0 – 72 hours in 45°C temperature in 24% moisture. The sowing qualities were retained longer for cucumber seeds, but in both species the mean germination time increase and this according to the authors makes the problem of accelerated ageing of vegetable seeds controversial.

Deterioration of pepper seeds quality in long-term storage according to Passam et al. (1997) depends on variety as well as

the conditions of seed production and storage. The differences between genotypes and between harvest years are reported. The ageing of vegetable seeds is accompanied with changes in DNA syntheses. Portis and Lanteri (1999) concluded that after detection of the clear signal for synthesis of β -tubolin during controlled ageing pepper seeds can be expected of DNA replications. One of the signs of seeds ageing, which correlated very well with deterioration of sowing qualities and reducing germination and vigour irrespective of ageing conditions is increase of the percentage of anaphases aberrations in roots tips and this can be used as indication for deterioration occurring of sowing quality (Pijlen et al., 1995).

Damages and degradation of cellular proteins in ageing of tomato seeds along with a reduction of germination and increase of mean germination time have observed Kester et al. (1997). Ageing of seeds changed drastically its viability and it is accompanied with several changes of their chemical compositions, mainly in content of carbohydrate and especially sugar (Horbowicz, 1997; Katagi et al., 1997).

Seed shelf-life or longevity according to Niedzielski (2009) is difficult to predict or to practical measurement through time

scale. Predictive models suggest that seed water content has the same effect on ageing rate for all seed lots within species and that initial seed quality is the dominant factor, by which it can explain the variations within species. These assumptions are used in accelerated ageing or controlled deterioration tests, which are commonly applied to predict seed longevity for commercial and research goals.

Bennet (2002) considers that by the results of accelerated ageing it can be ranked to seed lots by vigor and decisions can be made regarding the storability or planting of each seed lot. According to Marcos Filho (1998) the accelerated ageing and controlled deterioration are excellent opportunities to establish the seed quality and provide better accuracy and easily applicable.

Problems of application of accelerated ageing about assessment of sowing quality and storability of pepper seeds are studied limitedly and the specific regime for its implementation is debatable. Therefore, it is necessary to conduct researches in this scope with Bulgarian varieties, produced in our climate conditions.

The main goals of the present studies were to investigate the possibilities about assessment of viability, vigor and storability of different pepper seeds lots by the methods of accelerated ageing and to optimize the parameters of the regime for its implementation.

Material and Methods

The experiments were carried out in experimental laboratory of Department of Horticultural at the Agricultural University, Plovdiv with annual seeds, harvested in 2010 from three pepper varieties, belonged to different varieties - var. *capia*, var. *rotundum* and var. *corniforme*: Kurtovska kapia 1619 (K.K.), Bulgarski rotund (B.r.) and Delikates (D.), respectively by three lots each of them with different origin of seed production: village Vardim, district Veliko Tarnovo, village Stryama, district Plovdiv and Plovdiv (Institute of Vegetable Crops "Maritca"). The initial moisture content, measured with electronic moisture meter G-won type 503A, is as follows:

- for Kurtovska kapia 1619, origin: Stryama-8.9%, Vardim-9.2% and Plovdiv-9.0%;
- for Bulgarski rotund, origin: Stryama -9.4%, Vardim-9.3% and Plovdiv-9.4%;
- for Delikates, origin: Stryama - 9.4%, Vardim - 9.5% and Plovdiv - 9.5%. This suggests that the seeds with different origin are with similar moisture content.

The seeds of each varieties and lots were placed about accelerated ageing according to ISTA (2003) methodology. In order to determine the appropriate regime of artificial age-

ing of pepper seeds three temperature 35°C, 40°C и 45°C and three periods - 24, 48 and 72 hours were tested. The experiments carried out in four replicates. Seeds by 4.0 g of each varieties and lots were placed in special plastic box with dimensions 11 × 11 × 3.5 cm, length, width and depth with lid. At the bottom of each box 40 ml, distillate water was added. The seeds were put upon the pre-mounted mesh screen on plastic tray with dimensions 10 × 10 × 3 cm, length, width and depth. Thus, prepared boxes were set in water-jacketed ageing chamber in above mentioned temperatures and periods of treatment.

Immediately after finished the artificial ageing, within one hour, the seeds from each variant were set for standard germination test in four replications using method of ISTA (2003).

The germination energy (first count), germination (final count) by described method in ISTA Rules (ISTA, 2003); mean germination time (MGT) by Battle and Whittington (1969), uniformity of germination by Strona (1971); fresh weight of seedlings, length of embryo root and hypocotyls, at the moment of germination establishment were measured of 10 seedlings of each replication; dry matter of seedlings (Georgiev et al., 1980) and percentage of seedlings deviations according to prescription of ISTA (2003) and Welington (1970) were also determined taking into account of germination.

The statistical analyses were made by ANOVA. The data of the study were subjected to analysis of variance, and the least significant differences between means were calculated by the Fisher test at $p = 0.05$.

Results

From the experiments, it was found that the highest tested temperature of 45°C caused almost complete lethality of seeds of all periods and lots of each variety. This practically makes it inapplicable to evaluate the vigor and storability of pepper seeds. Therefore, further results are considered only two temperature regimes of 35°C and 40°C.

Germination energy of pepper seeds (Figure 1) was changed significantly in results of accelerated ageing. The seeds with the highest initial germination energy were of Kurtovska kapia 1619 and Bulgarski rotund from Vardim and Delikates from Plovdiv, while the lowest one was of Bulgarski rotund and Delikates from Stryama. The seeds of Kurtovska kapia 1619, originating from Plovdiv and Bulgarski rotund, Vardim, deteriorated most sharply - yet on 24 hours in 35°C they decreased its germination energy with 24.0% and 17.33%, respectively. Higher differences between individual lots began to account at 72 hours/35°C and at 48 hours/40°C, which is a prerequisite for assessing the vigor and storabil-

ity. Closer germination energy to the initial values in both regimes was established about Delikates from Vrdim and Stryama. The furthest of the results of baseline lots were these for Bulgarski rotund, Vardim and Plovdiv and for Kurtovska kapia 1619, Strima and Plovdiv. This indicates that about this characteristic they were with the weakest vigor and storability.

The most important and significant index about assessment of quality and status of sowing material is the germination. Baseline samples of the three tested varieties with the highest germination were originated from Vardim, while with the lowest one were these from Plovdiv of Kurtovska kapia 1619 and Bulgarski rotund and from Stryama of Delikates. The decrease of germination was relatively weak and evenly for almost all expositions in temperature 35°C and normally it was the strongest in 72 hours. The highest differences in comparison with control in this duration of artificial ageing were observed in Bulgarski rotund, Plovdiv (with 31.67%) and in Kurtovska kapia 1619, Vardim (with 26.33%). A significant decrease was reported in regime 40°C for 48 hours and the differences between individual lots were well demonstrated. This allows pointing out that the appropriate regime of accelerated ageing of pepper seeds, in which the highest physiological sensitivity was observed, is 40°C for 48 hours. The previous exposition also caused deterioration, but the differences between samples were less distinct, while in the next exposition were occurred the extremely changes and lethality in part of lots and therefore did not allow accurate assessment between individual samples. In the above-mentioned regime 40°C for 48 hours the weakest vigour and storability were established of seeds of Bulgarski rotund

from Vardim (decrease with 82.66%) and from Plovdiv (decrease with 54.33%), followed by Delikates originated from Stryama and Plovdiv. The closest to the initial values preserved their germination the seeds from Delikates, Vardim (decrease only with 9.33%). That determine this lot with the highest vigour and storability, the second one was Kurtovska kapia 1619 from Plovdiv and Stryama, with decrease 5.33% and 26.0%, respectively.

The mean germination time (Figure 2) affected yet in the lowest temperature and exposition— 35°C/24 hours. More significant were the differences in this temperature in duration of 72 hours, as the highest deterioration was recorded in Kurtovska kapia 1619, Stryama - the period of germination of one seeds was increased with 4.25 days and nights. The highest differentiation between lots was observed in regime 40°C for 48 hours and the physiologically sensitive was very well defined. The deterioration towards to initial data was the biggest in Bulgarski rotund from Vardim, the necessary days for germination were with 4.76 more, followed from the seeds of Kurtovska kapia 1619, Stryama – 4.48 days more. The lowest differences were accounted in seeds from Delikates, Vardim – 2.49 days more for germination towards to the control and this determines the sample with the highest vigor and storability.

The uniformity of germination started to decline even though slightly in the lowest tested exposition and temperature 35°C/24 and 48 hours. Significant differences appeared in this temperature in 72 hours and clearer in 40°C for 48 hours. In the last regime the uniformity of germination kept better its data to the initial samples in Delikates, Vardim (with 3.93% lower), followed by this with origin Stryama, and the

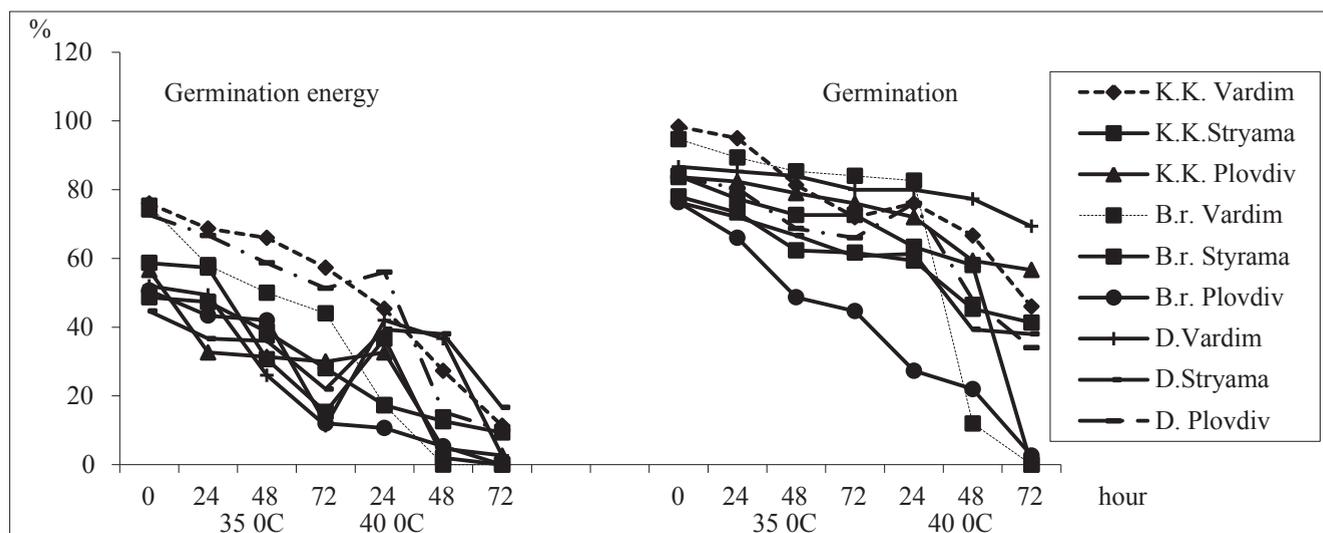


Fig. 1. Viability of pepper seeds after accelerated ageing

differences were more considerable in Bulgarski rotund and Kurtovska kapia 1619 seed, produced also in Vardim.

The length of embryo root (Figure 3) began to decline yet in the first period in both temperatures. In 35°C, however the differences of tested sample compared to initial, as well as between them were not very large and were less demonstrated. More significant they were observed in 40°C for 48 as well as 72 hours. In the last exposition for Kurtovska kapia 1619 and Bulgarski ratund zero values were accounted. Decrease in this temperature regime in 48 hours compared to obtained results for 24 hours was very well marked and allows comparison between different samples. Similar data

were reported about length of hypocotyls – slightly altered in temperature 35°C and better distinctness in regime 40°C for 48 hours. For these both indices, closely to the initial values were the measurements of the seedlings from seeds of Bulgarski rotund, produced in Stryama and the farthest - for the seedlings of seed of the same origin, but from Kurtovska kapia 1619.

Very important index about seed vigor is a fresh weight of seedlings. (Figure 4). Lightly course and relatively weaker changes, reported above for the other important morphological attributes of seedlings in temperature 35°C were maintained also about their fresh weight. The differences began to

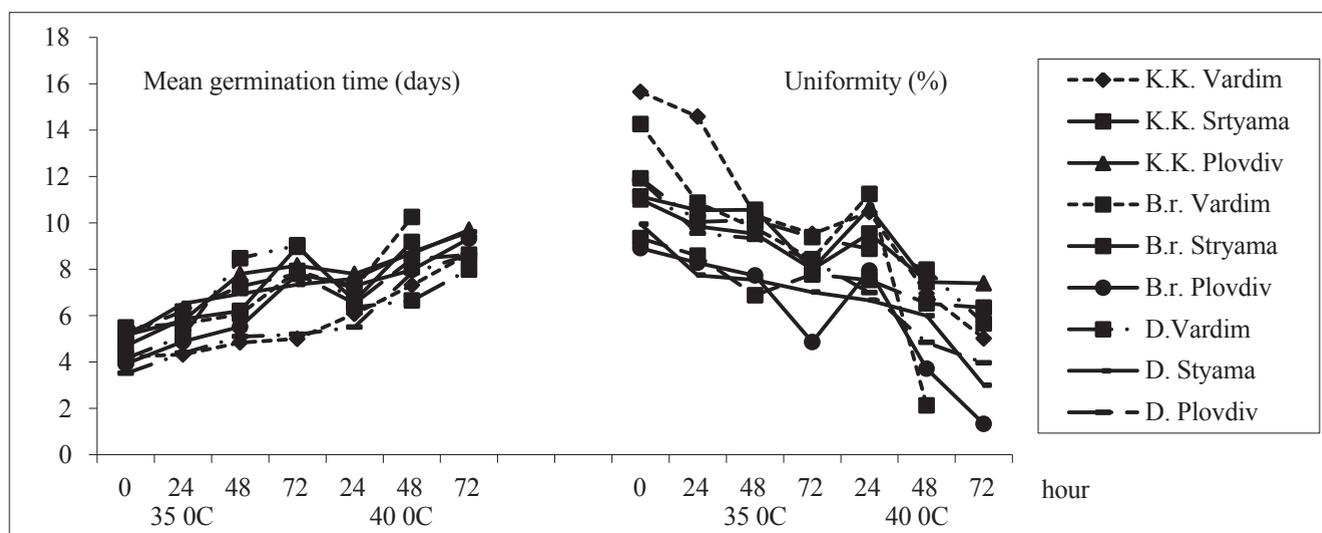


Fig. 2. Sowing characteristics of pepper seeds in accelerated ageing

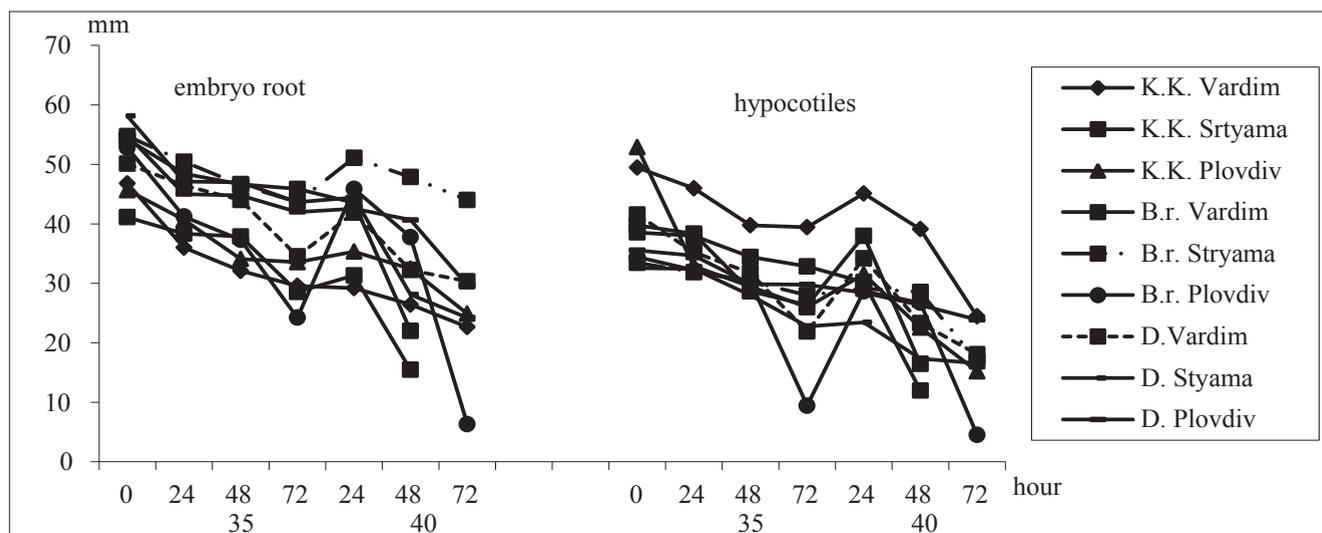


Fig. 3. Length of embryo root and hypostyles of pepper seeds in accelerated ageing

increase considerably in temperature 40°C mostly for exposition of 48 hours, followed by those of 72 hours. Decrease to the initial values was higher about the seedlings, developed form seeds of Bulgarski ratund and Kurtovska kapia 1619, produced in Vardim, while in this origin, but for Delikates the seeds developed seedlings with fresh weight similar to that of untreated seeds. This proves once again that seeds of Delikates, from Vardim are with the best storability and the highest vigor.

The effect of accelerated ageing on accumulation of dry matter of seedlings was less and in lower expositions, decrease was negligible. Changes that are more significant were observed in regime 35°C/72 hours and mostly in 48 hours in temperature 40°C. Comparatively high dry matter even in ageing period from 72 hours in the highest tested temperature of 40°C developed the seedlings from seeds of Kurtovska kapia 1619 and Delikates, produced in Plovdiv, of Bulgarski rotund from Stryama, followed by Delikates form Vardim. De-

crease about these variants towards to control was between 10.1 and 21.2%.

One of the indication of seed vigour, besides of above described morphological characteristics is their normal development of seedlings (Table 1). Without deviation are these seedlings from initial seeds of Delikates from Vardim and Stryama, but from Plovdiv the deviation was 3.3% only. Significant deviation indicated the seedlings from initial seeds from Kurtovska kapia 1691, Vardim and from Bulgraski rotund, Plovdiv. The seeds form the three varieties, produced in Stryama under the influence of accelerated ageing had developed the seedlings with most deviations and in Kurtovska kapia 1619, 40°C/48 it reached to 93.1%, while in the next exposition the seedlings even missed, that defines them that they were with lowest vigour. High vigour and storability presented the seeds from Delikates, Vardim where in this regime - 40°C/48 hours the lowest deviations from 29.9% were observed. In the next period, it reached to 48.6%, while for the

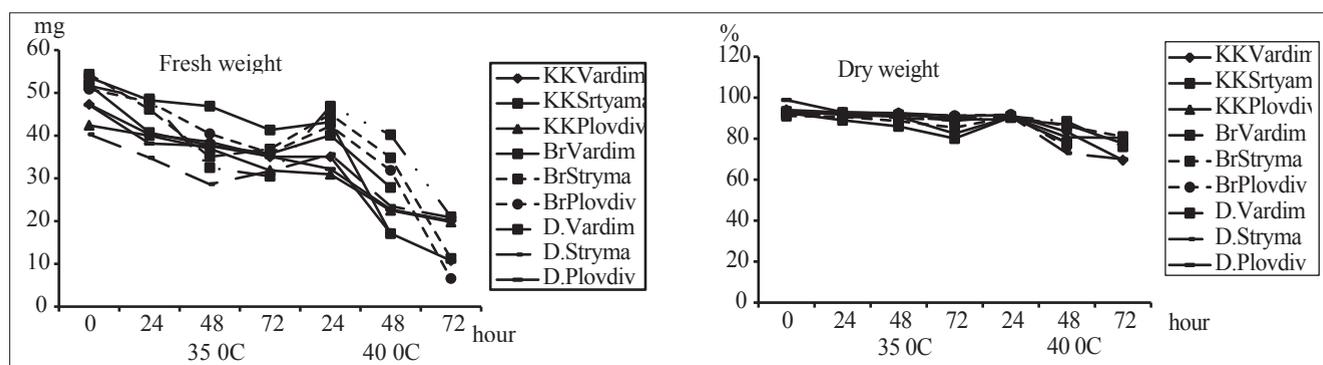


Fig. 4. Fresh and dry weight of pepper seedlings in accelerated ageing

Table 1
Deviations from normal development of seedlings of pepper seeds after accelerated ageing

Variants	Control	35°C			40°C			LSD p=0.05 %
		24 h	48 h	72 h	24 h	48 h	72 h	
Kurtovska kapia 1619								
Vardim	16.7	19.9	22.6	23.3	23.3	53.2	83.3	4.2
Stryama	9.9	26.6	13.3	28.8	63.2	93.1	-	6.6
Plovdiv	6.7	10.3	13	24.4	35.4	40	52.3	3.1
Bulgarski rotund								
Vardim	9.9	13.3	16.6	25.6	41	45.5	-	3.8
Stryama	9.9	19.3	22.6	26.3	31.1	52.2	66.5	8.6
Plovdiv	16	19.4	23.3	55.5	53.3	53.2	53.3	4.7
Delikates								
Vardim	0	0	3.3	23.3	19.9	29.9	48.6	2.1
Stryama	0	6.7	8.3	10.9	37.6	69.9	73.2	4.2
Plovdiv	3.3	9.9	13.3	16.6	29.9	53.3	73.2	5.1

other variants this index varied between 52.3% for Kurtovska kapia 1619 from Plovdiv to 83.3% about this variety from Vardim. Statistical significance was established. The main deviations that were observed in seedlings were associated with lack of root branches, unopened cotyledon leaves, undeveloped cotyledons or embryo root and lack of hypocotyls. Less often observed presence of lesions and lack of root hairs.

Discussion

As results of the experiments was found that the accelerated ageing caused a progressively decrease of germination and of mean time of germination depending on the one hand of origin of samples and the other side from variety, as well as of applied regime – temperature and period of ageing, associated with their falling into stressful conditions. This indicates that by this test is possible to implement very good separation and classification of seed samples by their vigor and storability. The principle of the method, as emphasizes Marcos Filho (1999) is based on the artificial accelerated deterioration of seeds, through exposing them to high temperature and relative humidity levels, which are considered as the most important environmental factors in relation of intensity and speed of this process. In this situation, low-quality seeds deteriorate more rapidly than more vigorous ones, presenting a differentiated decrease in viability. Black et al. (2008) also argued that the test is appropriate to distinguish the seed lots that have a high germination percentage in the laboratory but varied field emergence. Authors reported that the assessment of different samples is based on the assumption that their tolerance to the stress in which the seed has been subjected (temperature and humidity) is an indicator of seed vigor.

According to Kaewnaee (2011) one possible explanation for decrease of germination in accelerated ageing, where the seeds are in stressful condition, is lipid peroxidation, cell membrane damage and loss in membrane integrity. The researcher said that especially for pepper seeds this lipid peroxidation during accelerated ageing influences much more strongly to degradation of cell membrane causing disturbances to the ability of transport of proteins.

Similar conclusion reported also other scientist. Zhang et al. (1999) pointed out that the damage of integrity of biomembranes is the main reason for the changes in accelerated ageing seeds. Tang and Song (1999) also shared the opinion about significance of peroxidation of lipid membranes about deterioration of seed status during their accelerated ageing.

Besides of decrease of germination, in conducted experiments was found that the accelerated artificial ageing also affected on the morphological development of seedlings, re-

sulting on decrease of their fresh and dry mater, on the length of embryo root and hypocotyls. At the same time, the percentage of seedlings with deviation of normal development increased. In this connection, Joao-Abba and Lovato (1999) considered that namely this abnormal development of seedlings reduced the resistance to unfavorable conditions, which is an indicator about low seed vigor.

The condition under the accelerated ageing performed are of paramount importance, especially the temperature and ageing period (Tores and Marco Filho, 2003). According to Tomes et al. (1988), duration of ageing period proved less depressing influence than the temperature and recommended in specifying of the ageing regime to pay special attention to determination of the temperature, allowing assessment of seed vigour and storability.

In this regard, Hyatt and Tekrony (2008) are of the opinion that in small-seeded crops such as vegetables needs especially careful identification of appropriate conditions for artificial ageing, because it may not always coincide with those described in ISTA and AOSTA rules. There are contradictions regarding temperature and period of accelerated ageing of pepper seeds. Some researches recommended the accelerated ageing of pepper seeds, produced in Brazil (TeKrony, 1995; Panobianco and Marcos Filho, 1998) applies to working temperature 41-45°C for period of 72 hours. According to other scientists for seed with same origin appropriate temperature is between 38-45°C, but within 96 hours (Bhering et al., 2006). Kaewnaee (2011) considered that the seed ageing of this crop, produced in India, was effectively in temperature diapason 45-50°C. The conducted experiments, however, proved that about the investigated Bulgarian varieties good physiological sensitivity, allowing objective assessment of vigor and storability are fulfilled in accelerated ageing of peepers seeds in temperature 40°C, but for significantly shorter period of 48 hours, with which it can be achieved maintenance of lower temperature and lower energy consumption and the time for conducting the analysis are also shortened. This indicated that artificial accelerated ageing depends very much on one side of a genotype, and on the other on the origin of the seeds and that is necessary to determine the appropriate specific regime for ageing.

Conclusions

Through application of seeds, accelerated ageing test is achieved objective assessment of vigour and storability of pepper seeds and descending classification of individual lots by these indexes.

The initial seeds with the highest germination were these, produced in Vardim - Kurtovska kapia 1961 and Bulgarski

rotund. The highest vigour and storability indicated the seeds of variety Delikates, of the same region - Vardim.

Accelerated ageing, depending on temperature and period of treatment, progressively decreased not only the sowing qualities, but significantly deteriorated the morphological development of seedlings and increased the percentage of deviation that directly relates to the vigor.

Appropriate regime for accelerated ageing of pepper seeds, providing good physiological sensitivity and objective assessment of vigor and storability of individual lots is with temperature 40°C for period of 48 hours.

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