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Vigor, vitality and seed dormancy of *Avena sativa* cultivars in a long-term experiment

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Summary

Vigor, vitality and seed dormancy of 14 Finnish cultivars of *Avena sativa* in room temperature were studied in a 22-year laboratory experiment. These parameters were studied by measuring morphological and physical characteristics of seeds and by basic germination and enzymatic tests 1, 4, 6, 11, 16, 21 and 22 years after seed harvesting in 1989. Methylene blue, Congo red and 2,3,5-triphenyltetrazolium chloride (TZ) tests were used to estimate seed quality and changes in vitality over time. Seed vitality clearly decreased in all cultivars during the experiment. The mean vitality declined from 96.3% (one year after harvest) to zero at the end. Vitality according to the TZ test was higher than indicated by the basic germination test. The mean vitality loss was 4.6% per year, but there were clear differences between cultivars. The decrease in vitality correlated with loss in seed weight. Clear signs of deepening dormancy were observed. Seed age is an important factor influencing vitality and dormancy. Vitality loss of seeds led to deep dormancy. The appearance, water uptake and imbibition of the seeds remained normal until the end. Ageing, vitality loss and dormancy are concluded to be expressions of genes. It is possible that in the future electronic simulation methods will be developed that will enable accurate estimation of oat seed quality without laboratory tests.

Introduction

Oat (*Avena sativa* L.) is an important food and forage cereal worldwide. It is one of the oldest crops and is still widely cultivated worldwide, including at high latitudes and in restrictive climates. Its importance in human nutrition is increasing following reports on the cholesterol-lowering, antioxidant and other health-related properties of oat and oat products and components (Othman et al., 2011; Huang et al., 2011; Liu et al., 2011; Kristensen and Buegel, 2011). Discoveries in the nutritional values of oat are also a challenge for the production and quality breeding of this crop (Nuutila, 2000). In Finland, oat is an important economic cereal with a long tradition of production and national breeding programs which have resulted in the development of several cultivars and of cereal marketing strategy programs (Pullinen, 1999). Generally, Finnish oat cultivars are relatively stable and well adapted to the local cultivation zones, and produce satisfactory yields. The vitality of oat seeds is almost perfect after full ripening and harvesting, although it is dependent on favorable weather. Harvest weather is often problematic, especially on the northern boundary of oat cultivation, where growing conditions, particularly the short growing period, temperature conditions and precipitation, are extremely difficult. Therefore, the vigor of seeds is important in seeking to improve the production and storage of oat. Vigor research, however, is challenging not only scientists but also for seed technologists and engineers. In the literature from 1960s, generally, the rate of ageing in seeds was reported to be dependent on three factors: temperature, moisture and oxygen pressure (Barton, 1961; Kozlowski, 1972; Bewley and Black, 1978; Mayer and Poljakoff-mayber, 1982; Roberts, 1981). Recalcitrant seeds are killed by low moisture content, and in orthodox seed, the percentage of viability depends on environmental conditions. Although from the beginning of 1970s literature followed optimistically a method for theoretical prediction of the percentage viability of seeds after any time over a very wide range of environmental conditions, this method of the prediction was not exact and, consequently, corrected by literature, and even by the same authors many times (Roberts, 1973; Ellis and Roberts, 1980,1981; Ellis et al.,1980; Gáspár et al., 1981; Roberts, 1981; Ellis and Wetzel, 1983; Vlk and Rogalewicz, 1986; Ellis, 1988; Filho and Ellis, 1992; Hong and Ellis, 1996; Walters et al., 2005; Pérez-garcía et al., 2008, 2009; Niedzielski et al., 2009; Nagel and Börner, 2010). The problem of long-term storage of seeds under environmental conditions (i.e. low input energy) with high viability is still unresolved. Long-term studies, where seed viability is monitored periodically, provide direct evidence of changes in germination percentage with storage time (Specht et al., 1988; Walters et al.,2005), although early stage of seed ageing is asymptomatic (Walters et al., 2005). There is currently no in-depth knowledge of the ageing of oat seeds or oat seed dormancy, although attention should be paid to these phenomena in seed technology and breeding to avoid possible losses in long-term storage, and especially in the case of long-term farm-storage. This kind of storage with low input energy may be possible from an ecological point of view and useful, as agriculture needs to be able to provide sustainable food security to meet new challenges (Peltonen-sainio et al., 2011). It is necessary to develop new methods of testing seed quality and thereby gain new knowledge and a profounder understanding of seed dormancy and ageing, and the mechanisms governing these phenomena. It is not known how changes in seed physical and biological parameters are connected with ageing and vitality loss, although it is known that seed size influences oat germinability in fully ripened seeds (Guberac et al., 1998). These changes, with 14 Finnish traditional oat cultivars, were studied in our laboratory for 22 years to determine how vitality loss is connected with the physical and biological parameters of the seeds. The following three questions were addressed: (1) How do the risks to the vitality and germination of oat cultivars develop during 22 years of seed ageing? (2) Are these risks connected with changes in seed parameters during ageing? (3) What differences are there in vigor, vitality and seed dormancy behavior between the different oat cultivars, and is it possible to find standards for these behaviors over the years?

Material and methods

Seeds of 14 Finnish oat cultivars were harvested in 1989 in the former Hankkija Breeding Department and sent to the Department of Biology of the University of Joensuu (presently University of Eastern Finland), where they were studied and stored at room temperature 1990-2011. The first laboratory studies were performed in March 1990, and these were repeated several times over a period of 20 years. The origins, release year for commercial use and general characteristics of the cultivars are presented in Tab. 1 and Fig. 1.

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Tab. 1: Origins, release dates for commercial use and general characteristics of cultivars studied.

Cultivar	Parents	Release to market	On the list of official cult.	Additional info	Source
Esa	line 091 x Seger (SWE)	1922	N/A	white kernel oat***	Eviran tietokanta
Hankkija-773	Eho x Blenda*	1958	1974-1980	white kernel oat	Rekunen, 1975
Hankkija Valko	Pendek x Ta b 4137	1976	1977-1983	white kernel oat	Rekunen, 1976; 1980
Hankkija Vouti	Hannes x Astor (NL)	1982	1985-1992	white kernel oat	Rekunen, 1983
Hannes	Eho x Sisu	1964	1964-1980	white kernel oat	Ilola, 1965; Kivi, 1965
Kyrö	Ta a 3084 x Ta03370	1959	1959-1971	white kernel oat	Huttunen, 1960
Kytö	line 091 x Kultasade	1925	1935-1946	the first domestic cultivar, white kernel oat***	Sauli, 1925, 1926; Pohjakallio, 1937; Paatela, 1953
Nasta	Tiitus x Ryhti	1979	1972-1992	white kernel oat	Inkilä, 1978
Osmo II	Kuopio traditional x Guld	1922	N/A	black kernel oat, cultivated in Finland up to end of 1940s	Eviran tietokanta
Pellervo	Voitto x linja 091	1935	1938-1939	white kernel oat	Sauli, 1935
Puhti	Hannes x Ryhti	1978	1978-2004	white kernel oat	Inkilä, 1978; Rekunen, 1985
Ryhti	Sisu Rtg worked x Blixt**	1970	1970-1992	white kernel oat***	Inkilä 1970
Virma	Rosinante x Hja 30766	1990	from 1990	white kernel oat	Huttunen, 1955; Säynäjärvi, 1990
Yty	Ryhti x Tiitus	1989	from 1991	white kernel oat	Saastamoinen and Pärssinen, 1989

*bred as line Ta a 357
**bred from the cross Jo 50-2395
***observation, does not appear in sources

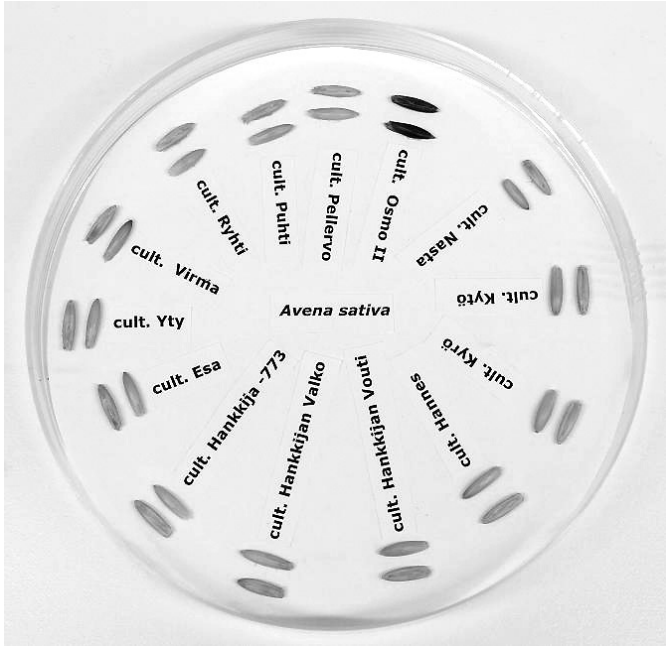

Fig. 1: Variability of seeds of *A. sativa* cultivars studied. Each cultivar is represented by two seeds taken at random and photographed from both sides.

Physical studies of seeds

Morphological and physical parameters of seeds were measured to monitor their possible changes over time. No disease, pathological deviations or strong seed polymorphism (ANISZEWSKI, 2001) were observed in the cultivars studied.

Seed and seed coat weight measurements

Four replications of samples of 20 seeds were taken at random from each cultivar. Each seed was observed using a series of magnifying glasses (diameters 10, 50 and 100 mm) and a stereo microscope Zeiss Stemi DV 4, and weighed on laboratory scales Scaltec SBC 41 (exactness 1 mg) each year of measurement. Four replications of five seeds were selected at random from each 20-seed sample, weighed, and the coats separated using a scalpel, a base glass and a stereo microscope Zeiss Stemi DV 4. The coats were also weighed separately.

Length and width of the seeds

The length and width of the seeds were measured from randomly chosen samples of 20 seeds using a Digimatic with electronic reader (exactness 1 x 10^{-5}m). The length of the seed was the longest vertical distance from base to top, and the width was the largest measure of the seed horizontally.

Germination studies

Basic tests

Laboratory germination tests were conducted each study year using Petri dishes (diameter 90 mm and of 15 mm deep), blotting paper with thickness of 2.5 mm, and extra clean (Milli-Q) water in standard room temperature. 20 seeds were selected at random from each cultivar seeds each study year and divided into four samples. Four pieces of blotting paper were watered and each sample was placed on the paper, covered well and put on a Petri dish. When covering the five seeds, special care was taken that none of them were in direct contact with each other. Every 24 hrs, the covers were opened for ventilation, and the seeds removed for observation. Next, the papers were watered, seeds returned, covered again and placed on the Petri dishes. Both the imbibition and germination of the seeds were recorded. The duration of the test was 14 days.

Special checking test

To determine whether seeds collectively placed on a single medium influence each other's germination, a test in order to check possible allelopathic interactions was carried out with 14 seeds of each cultivar. Three layers of watered blotting paper covered by small paper bag providing fresh air were used. Seed imbibitions and germination were observed and recorded every six hrs. The test was carried out 19 years after seed harvest. The tested seeds were checked again the next year. This checking test confirms that seed germination did not depend on interaction between seeds or on any kind of allelopathy of individual seed exudates in this research. The test proved that the germination test results are valid and depend on seed vitality.

Vigor and enzymatic studies

With the objective of checking imbibition and seed vitality and vigor the dye tests of Methylene blue (MB, Merck Darmstadt, art. 1283; molecular formula $C_{16}H_{18}N_3SCI$), Congo red (CR, Merck Darmstadt, art. 1340; molecular formula $C_{32}H_{22}N_6Na_2O_6S_2$), and 2,3,5-triphenyltetrazolium chloride (TZ, Merck Darmstadt, art. 8380; molecular formula $C_{19}H_{15}ClN_4$) were carried out. These dyes are commonly used in histo- and clinical chemistry and have also been used in seed tissue research (Aniszewski, 2006; Aniszewski et al., 2006). MB is a cationic dye, which binds to acidic groups such as pectins (Nari et al., 1991) and lignins (Fineran, 1997). CR is well known for its binding to some cell wall polysaccharides, such as cellulose (Verbelen and Stickens, 1995). TZ is used for detecting living cells by dehydrogenase indication (Serva, 2004; Oritani et al., 2004).

MB test

The MB test was used to study seed water uptake and water distribution in the seed. 0.1% Methylene blue dye was used in the same way as water in the germination test. Seeds were taken out after seven days, dried with blotted paper, photographed and observed under a stereo microscope. Five seeds were cut and the dyed areas within were observed with the naked eye and under a Zeiss Stemi DV 4 microscope, and photographed with a Canon Leica S8APO 10446339 microscope camera. Finally, all the photographs were analyzed.

CR test

The CR test was used to study seed imbibition. 0.1% solution of CR was used on moistened seeds in the same way as in the MB test. Five seeds were cut, the dyed areas were observed under a stereo microscope, photographed, and the photographs were analyzed.

TZ test

The TZ test was used to study vitality. 0.5% colorless water solution of 2,3,5-triphenyltetrazolium chloride was provided and placed on Petri dishes in the same way as in the germination test. After one week the seeds were taken from the solution and dried with blotting paper. Next, the seeds were cut in half vertically. Red color inside the seed was searched for using a magnification glass system and a stereo microscope, and photographed. Next, the photographs were analyzed.

Statistical methods used and presentation of results

The results were analyzed using SigmaPlot v.11.0 with Statistics component and Microsoft Office Excel 2007 on Windows^{xp} Professional. Statistical analyses and tests were carried out using descriptive statistics, normality test (NT), ANOVA, Student-Newman-Keuls method (SNKM) and Tukey's-test (TT). Results were presented using logarithmic scale, relative comparison, means with standard deviations, and on 3D graphs created with Blender v.2.62.

Results

Vitality losses and germination risk to lose germination capacity

Seed vitality, tested by the basic germination test, clearly decreased in all cultivars during the long-term experiment, while storage at room temperature and ageing constituted a risk to seed germination. In the beginning of the experiment, the vitality of all the cultivars was almost perfect, with an average germination rate of 96.3%; this showed a decrease after only four years of storage. Thereafter, vitality continued to decrease, reaching zero after 22 years of storage (Fig. 2). However, vitality measured by the TZ test was higher than vitality measured by the germination test in all the years of measurement. The greatest difference occurred in the final year of the experiment, when the TZ test indicated a mean vitality of nearly fifty percent (48.6), although no germination of seeds was indicated by the basic germination test (Fig. 2). In addition according to the TZ test, the standard deviations for vitality were very small up to the sixth year post harvest and clearly increased thereafter, in contrast to the standard deviations of the means for vitality obtained with the basic germination test, which also increased at a lower rate.

The dynamics of mean vitality differed according to the length of storage time. The mean vitality loss during the first period was 5.6% per year and in the succeeding period near 9.3 %, with further decreases in the remaining periods. An exception to this was the last year of measurement, when the seeds did not germinate at all (Tab. 2). The tendency towards vitality losses between cultivars was similar in all periods. However, Hannes, Kyrö, Nasta, Pellervo and

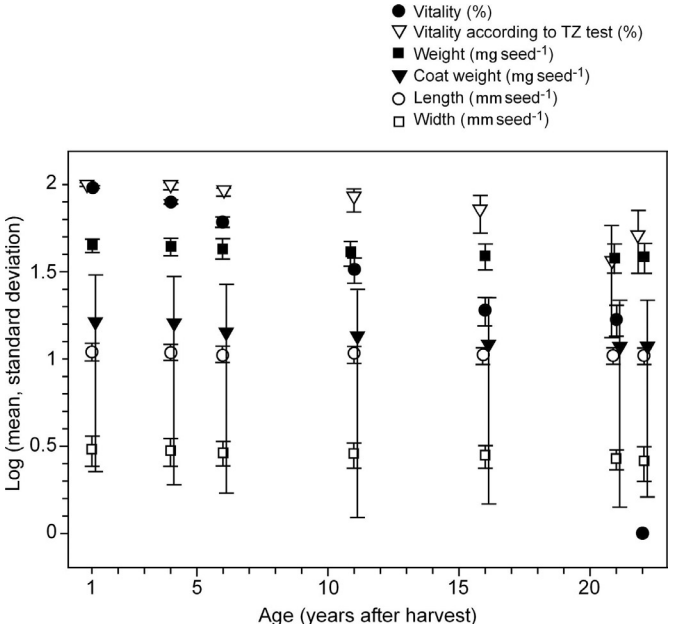

Fig. 2: The dynamics of mean vitality and physical parameters of oat seeds (means with standard deviations in logarithmic scale). The results of statistical analyses of data are as follows: vitality by basic test and TZ test and seed width NT ($P<0.05^*$), ANOVA ($P<0.001^*$), SNKM ($P<0.05^*$); seed weight NT ($P > 0.05^{ns}$), ANOVA ($P < 0.05^*$); coat weight and seed length NT ($P < 0.05^*$), ANOVA ($P > 0.05^{ns}$).

Tab. 2: Mean vitality loss per year for *A. sativa* species (average of all cultivars) and cultivars in different seed age periods. The results of statistical analysis of real data are as follows: NT (P<0.05*), ANOVA (P<0.001***), SNKM (all P<0.05*).

Cultivars	1.-4.	4.-6.	6.-11.	11.-16.	16.-21.	21.-22.	1.-22.
Esa	5.42	7.38	6.05	3.56	0.29	15.75	**4.58**
Hankkija-773	6.08	7.75	5.80	2.73	0.02	19.75	**4.58**
Hankkija Valko	5.75	8.75	4.90	3.10	0.05	20.75	**4.56**
Hankkija Vouti	6.08	7.38	5.20	4.00	0.85	13.75	**4.62**
Hannes	5.75	10.63	4.35	3.82	0.28	16.50	**4.63**
Kyrö	5.17	10.63	4.60	3.70	0.25	16.75	**4.58**
Kytö	5.83	9.63	5.15	3.06	0.39	16.25	**4.57**
Nasta	5.67	11.25	5.55	2.06	0.39	17.25	**4.61**
Osmo II	5.50	9.75	5.95	2.32	0.53	16.25	**4.58**
Pellervo	5.17	10.50	5.60	2.31	0.64	16.00	**4.54**
Puhti	5.27	8.10	5.65	2.99	0.76	16.50	**4.55**
Ryhti	5.83	11.25	5.65	2.47	0.43	14.75	**4.64**
Virma	5.50	9.00	6.20	2.56	0.69	13.75	**4.55**
Yty	5.75	7.63	7.25	1.59	0.46	17.50	**4.60**
All cultivars	**5.63**	**9.26**	**5.56**	**2.88**	**0.43**	**16.54**	4.59

Ryhti showed the highest losses in the second period, and Hankkija-773 and Valko in the last period (Tab. 2). The average vitality loss per year for all cultivars was 4.6 percentage points (pp), and the average for individual cultivars differed only little from this value (±0.05 pp) (Tab. 2; Fig. 3a).

During the experiment, changes in seed physical parameters were observed (Fig. 2). In the end of the experiment, seeds of all cultivars revealed on average 14.5% reduction in weight than in the beginning. The estimated mean loss of seed weight varied between periods, but across the whole experiment was 0.24 milligrams per year. There were differences between cultivars. Their seed weight varied from 0.89 (Ryhti) to 1.14 (Kyrö) of the species mean in the first period and from 0.69 (Kytö) to 1.19 (Esa) in the last period. Some cultivars showed a considerable deviation from mean weight of all cultivars. Across the experiment, seed weight varied between 0.69 and 1.25 of species mean weight (Fig. 3c).

Loss of seed coat weight was also observed in every cultivar (Fig. 2; Fig. 3d), and was on average 29.2% across all cultivars during the experiment. The mean loss was 0.23 milligrams per coat per year. However, in the second period of the study, it was as high as 0.79 milligrams per coat per year. Seed coat weight varied significantly between cultivars, ranging from 0.49 (Yty) to 3.99 (Hankkijan Valko) of mean species coat weight. Cult. Osmo II showed a significant decrease in coat weight as the seed aged. Also, the standard deviations were greater than in the case of total seed weight (Fig. 2; Fig. 3d).

The other physical parameters, length and width, remained relatively unchanged throughout the experiment (Fig. 2; Fig. 3 e-f). However, there were differences between cultivars: the longest cultivar (Osmo II) was 11% above the species mean, and the shortest (Nasta) was 8% below the species mean. In turn, the widest cultivars were Kyrö and Osmo II, which showed fluctuations of 8-18% above the average width, and the narrowest were Puhti and Virma with fluctuations of 8-13% below average.

The results demonstrate that decrease in vitality correlates more strongly with loss of seed weight over the years than with changes in length and width (Fig. 2, Fig. 3 a-f). There is also a dependency between vitality and seed coat weight. Cultivar Hankkijan Valko had 25% higher vitality and a seed coat nearly four times heavier than the species mean after 21 years, although the length and width remained close to the species mean.

Vigor and seed dormancy

The results indicate that the MB, CR and TZ tests are very useful in seed vitality research. Methylene blue testing showed that all the cultivars' coats were dyed similarly (Fig. 4), with variation inside the seed. Some seeds were not dyed on the inside and some were dyed on the base and/or edge of the seed coat. A photograph of a typical MB result is presented in Fig. 5a.

The CR test resulted in partial coloration of the seeds. The endosperm and embryo regions were not dyed but the coat region was, as can be seen in Fig. 5b. Seeds that did not germinate were more extensively dyed by both MB and CR.

The TZ test resulted in a clearly visible red coloration in some regions of the seeds (Fig. 5 c-d). Red spots were dispersed on the inside of the seed (Fig. 5c) or located in tissues near the embryo or in other vital regions (Fig. 5d). Some seeds of the cultivars that did not germinate during the last experimental year were colored red in the TZ test and seemed to be dormant rather than dead. However, there were relatively wide differences between cultivars in the TZ test over the years (Fig. 3b). In the first year, cult. Puhti showed lower TZ activity in comparison to the other cultivars, which were very similar to each other. In the following years of measurement, the differences between cultivars increased. After 22 years, the cultivars could be divided into five different vitality groups according to the TZ test results. The first group (cult. Hannes and Yty) showed the highest coloration in comparison to the species mean (+58 pp). In contrast, the cultivars belonging to the fifth group (cult. Hankkijan Valko, Virma) were 61 pp below the species mean. The others were as follows: second group (cult. Hankkija-773, Hankkijan Vouti, Kytö, Nasta, Pellervo, Ryhti) +18 pp, third group (cult. Esa, Kyrö, Osmo II) -21 pp, and fourth group (cult. Puhti) -41 pp (Fig. 3b).

The vitality and signs of dormancy of cultivars after 22 years differed. After 16 years, germination stopped, but signs of dormancy were indicated by the TZ test. The results clearly show that seed vitality and dormancy depend on the age of the seed. Moreover, these seed phenomena are clearly connected with the changes in losses in seed and seed coat weight over the years. The other physical parameters are not clearly dependent on the age of the seed. Although there is no clear dependence between seed length or width and vitality, it cannot be ruled out that age has some influence on the small changes in these parameters. Furthermore, our results did not directly exclude the possibility that older seeds would germinate given a longer germination time.

Discussion

Seed vigor, vitality and dormancy depend on many factors, including genetic (Willenborg et al., 2005), environmental (Peltonen-Sainio et al., 2001; Biligetu et al., 2011), technological (Zielinski and Mos, 2009), and developmental (Farhoudi, 2011) factors. Our results indicate that the age of seeds is one of the most important developmental factors influencing fluctuation in seed vigor, vitality, and dormancy during storage at room temperature and allowing a germination time of two weeks. The seeds gradually deteriorated from showing nearly perfect vitality to complete loss of germination vigor in these conditions. However, the TZ test indicated partial metabolic and enzymatic activity, which proved that the seeds were alive but dormant. This strongly suggests that not all of the seeds studied died completely but instead went into deep dormancy,

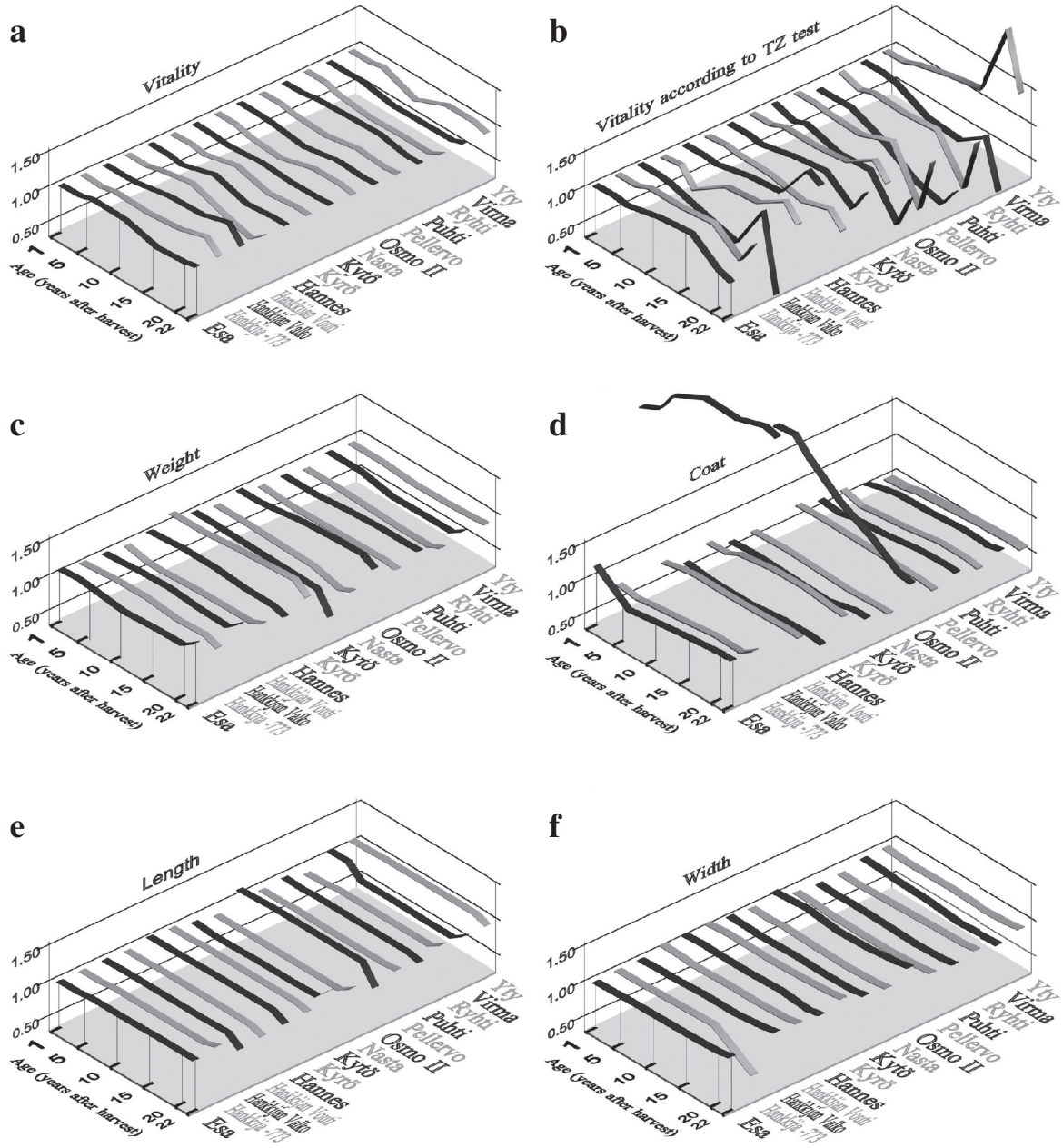

Fig. 3: Seed parameters in comparison to species mean (average of all 14 cultivars = 1.0) during the long-term experiment. 3a. Vitality according to basic germination test; 3b. Vitality according to TZ test; 3c. Seed weight; 3d. Seed coat weight; 3e. Seed length; 3f. Seed width.

emergence from which would probably have needed a longer germination time. The deep dormancy is a status of seed vigor with reduced ability to establish the new generation, and it is the effect of a seed inside factor, such as ageing. The literature does not provide any data on long-term seed vitality behavior, seed death or dormancy of oat at room temperature. However, oat seed vitality deteriorated greatly after 10 years of storage in the cold chambers of a gene bank – about 35% of the samples presented significant differences between initial and final germination – and that the losses in vitality of oat seeds were significantly higher than those of barley or wheat (RUIZ et al., 1999). In other species, it is known that younger seeds have a higher emergence rate than older seeds (BELGACEM et al., 2006), and that the standard germination test generally displays poorer seed vitality than the other vigor tests (KHAN et al., 2010). The increase in the present vitality standard deviations in the sixth year post harvest is probably a direct expression of physicochemical changes in seeds and deterioration in the ability of particular seeds to germinate, as indicated by the TZ test results. These physicochemical changes in seeds over time also had a direct influence on the seed vitality dynamics. This is the main reason for the wide variation in the average annual rate of deterioration in oat seed vitality. The critical period for vitality loss was four to six years post harvest. At 11 years post harvest, seed vitality showed considerable deterioration and the physicochemical changes were probably slower. It seems that at that point, the seed protected its vitality by retarding the annual rate of deterioration, as shown by the lower annual rate of loss. The remaining vigor was lost and seeds became totally dormant 22 years post harvest. This phenomenon can be considered typical for oat and is based in the genome. Some diversity between cultivars is observed.

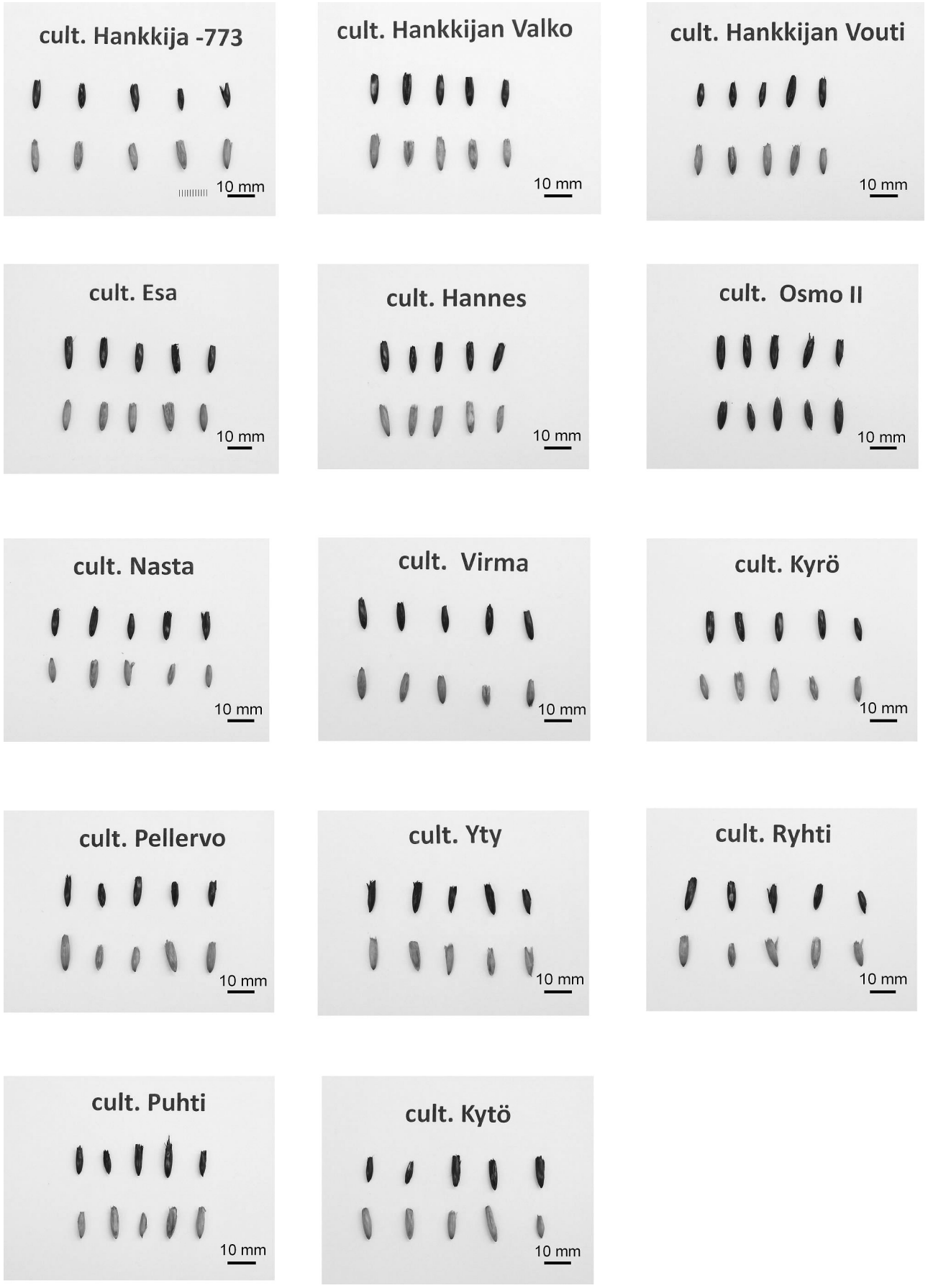

Fig. 4: Samples of seeds of different cultivars dyed by Methylene Blue (MB, upper row) and Congo Red (CR, lower row).

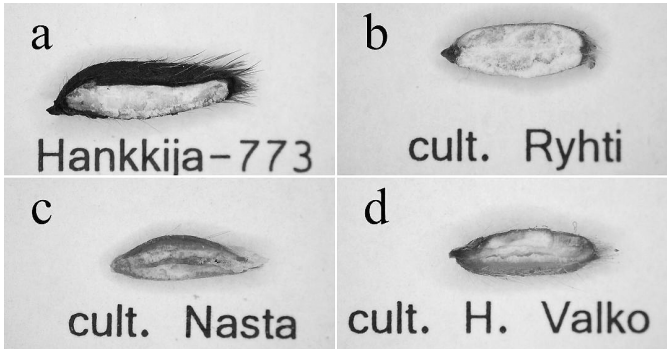

Fig. 5: Examples of cross-sectioned seeds dyed by Methylene Blue (MB) (a), Congo Red (CR) (b), Tetrazolium (TZ) (c-d).

Our results clearly showed that deterioration in seed vitality is connected with seed weight loss and an increased seed coat to total seed weight ratio 22 years post harvest. In spite of differences between particular cultivars, these phenomena were typical for the *A. sativa* species. In the literature, the relationship between seed weight and seed vitality is very diverse and species-dependent. In some species, seed weight has no influence on vitality (Toon et al., 1991; Bretagnolle et al., 1995; Wei et al., 2010); in others, heavier seeds have a higher germination rate (Roy et al., 1996; Hou and Romo, 1998; Myint et al., 2010), although the heaviest seeds have been reported to have the lowest germination rate (Hou and Romo, 1998). The changes in the other physical parameters of the seeds in the present study (length, width) were smaller than the changes in vitality and the changes in seed weight. These findings are new. According to the literature on laboratory tests, longer oat seeds have better vitality than shorter seeds (Guberac et al., 1998), and a decrease in seed size and osmotic potential increased median germination time and decreased the final germination percentage (Mut and Akay, 2010; Mut et al., 2010). Oat genotypes with large seeds appear to be better suited to germinate under large osmotic potentials (Willenborg et al., 2005), and larger seeds germinate faster (Farhoudi, 2011).

In spite of weight loss and other changes in physical and biotic parameters, their appearance of the seeds remained normal, and their water uptake and imbibition were non-defective after long storage. This was shown by the near perfect coloring of the seed tissues. MB-dyed plant tissues contained pectins and lignines, and CR tissues contained cellulose. Moreover, the live parts of the seeds were indicated by TZ, a chemical which, upon reduction, forms pigments of deep color, known as monoformazans and is used for histochemical detection of dehydrogenases in enzyme diagnostic and clinical chemistry (Aniszewski, 2006). It seems that histochemical tests, especially the TZ test, are more reliable in the detection of seed vitality than the standard germination test after long-term storage. The TZ test is stated to be one of the most reliable techniques for estimating seed vitality (Hosomi et al., 2011). It can be concluded from our results that the oat cultivars studied vary genetically in their ability to germinate after 22 years. It seems that the cultivars Hannes and Yty have genes which express higher levels of vitality and Hankkijan Valko and Virma have genes expressing lower levels of vitality. The vitality of the other cultivars studied appear to be located between these two extreme genetic groups. Germination ability might be expressed by genetic pool background (Lamia et al., 2012).

The ageing of oat seeds is associated with lack of ability to germinate and the secondary dormancy phenomenon. Both of these are rooted in a genetic mechanism influenced by the level of DOG1 and ABI3 (dormancy increasing factor) and KYP/SUVH4 (dormancy decreasing factor) in gene transcription (Zheng et al., 2012). These genes regulate seed dormancy, both primary and secondary. Seed ageing and loss of vitality, as well as dormancy, are expressions of genes and therefore, differences in germinating capacity between cultivars are also differences between genomes. Moreover, the expression of genes during ageing affects chemical changes in seeds, especially lipid degradation (Wang et al., 2012) and general enzymatic activity (Cackmak et al., 2010). This seems to be the basic reason for the deterioration in seed vitality and the deepening of dormancy over the years. Moreover, our results may help in paving the way for the possible future development of electronic simulation methods for the accurate estimation of oat seed quality without laboratory tests. Such applications are theoretically possible, because the behavior of seed vigor, vitality and dormancy are biological phenomena based on genetics and connected with ageing. However, to achieve this, more research is needed, not only on seed vigor, vitality and dormancy but also on *A. sativa* seed topography (a map of the seed coat and locations of seed cells, described by Aniszewski, 2009), seed biometrics and chemical changes over time.

Acknowledgements

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