

**IMPROVEMENT OF SEED GERMINATION PERFORMANCE OF STORED
COMMERCIAL PEPPER SEED LOTS WITH CHLOROPHYLL
FLUORESCENCE SORTING METHOD**

ABSTRACT

This work was conducted to investigate whether chlorophyll fluorescence (CF) sorting method enhanced the seed germination and vigor (mean germination time) of stored (aged) commercial seed lots of four different pepper cultivars. Seeds were stored hermetically for 6 and 12 months at -18, 5 and 25 °C with 8.0±0.2% of seed moisture. Laboratory germination and mean germination time were determined on stored and CF-sorted and unsorted seed subsamples. Results indicated that CF-sorting significantly increased laboratory germination ($p<0.05$), and reduced mean germination time ($p<0.05$). CF-sorted seeds from fractions with low CF had higher germination performance than unsorted ones. It can be concluded that CF as a non-destructive sorting technology has the potential to upgrade the quality of stored, aged pepper seed lots.

Key Words: *Chlorophyll fluorescence, seed gemination, Capsicum annuum L., vigor*

1. INTRODUCTION

The quality of a seed lot is the result of various pre-storage and post-storage factors (Powell et al., 1984). Even though seeds of a high quality are produced adverse post-production storage conditions reduce seed quality. Pepper seeds are considered to have a relatively short life-span and lose quality over a medium range storage period of over six months. This is the common case for sub-tropical regions where hot and humid environments prevail (Belletti and Quagliotti, 1988; Ozcoban and Demir, 2002).

High seedling stands and uniform emergence have become a necessity for saving cost and time due to the high proportion of hybrid seed use in vegetables. Late and variable emergence has been associated with the occurrence of seed ageing (Finch-Savage, 1986), and this is particularly prominent when carry-over (stored and aged) seed lots are used from a previous production season. Pepper seeds that were produced the previous year must be stored at least 12 months for use in the following year. Discarding seed of low germinability (more aged) from any stored lot (left over) would increase overall seed quality.

CF (Chlorophyll fluorescence) is a non-destructive seed sorting technique relying on measuring the amplitude of the chlorophyll fluorescence (CF) signals of intact seeds. The chlorophyll content of the seed coat decomposes as the seed matures. The technique detects chlorophyll in the seed coat, measuring chlorophyll fluorescence and linking it to the quality of seeds (maturation level). This has been performed in cabbages (*Brassica oleracea*) (Jalink et al., 1998; Dell'Aquila et al., 2002), tomatoes (*Solanum lycopersicum*) (Jalink et al., 1999), barley (*Hordeum vulgare*) (Konstantinova et al., 2002), pepper (Kenanoglu, 2013) and carrots (Groot et al., 2006). However, the method has not been used to determine whether more aged seeds are eliminated from stored seed lots. Della'Aquila et al. (2002) reported that CF sorting was found to be successful in enhancing the quality of artificially aged cabbage seed lots. The present study was design to test a) the possibility of enhancing seed quality in left-over seeds, b) enhancing the quality of seed accessions to be stored in gene banks or used for breeding purposes. In this work we investigated the extent to which CF-sorting enhanced the laboratory germination, mean germination time of commercial pepper seed lots by eliminating more aged seeds following storage.

2. MATERIALS AND METHOD

Four cultivars of peppers (*Capsicum annuum* L.) cvs. '11B-14', 'Demre Sivrisi', 'Corbacı' and 'Carlston' seed lots from Küçük çiftlik commercial seed company. Initial seed moisture contents were determined according to ISTA (2004), and ranged between 7.1 % and 7.8 %. The seeds were stored in sealed bags at 5 °C until the tests were performed.

Standard laboratory germination tests were conducted on three replicates of 50 seeds. The seeds of each replicate were placed between three moistened 20 x 20 cm² filter papers (Filtrak, Germany), two below and one above, which were moistened with 6 ml of distilled water each. These papers were then rolled up and placed in plastic bags in order to prevent water loss. Germination tests were carried out at 25 °C in the dark. Normal seedlings with developed shoot and root structures were evaluated after 14 days.

Seed longevity of the lots was determined at 6 or 12 months and at -18, 5 or 25 °C. Seed moisture content of about 800 seeds in each cultivar was initially adjusted to 8.0±0.2 %. One sub-sample of 200 seeds of each lot was stored at either -18, 5 °C or 25 °C in airtight laminated aluminum foil packets. The samples were taken from storage after 6 and 12 months and CF sorting was performed. CF sorting was done using the methodology developed at Plant Research International in Wageningen, The Netherlands (Jalink et al., 1998). Seeds in each lot were sorted below 730 nm. Fluorescence occurs when some of the light absorbed by the chlorophylls is re-emitted at longer wavelength, typically between 650 and 750 nm (Jalink et al., 1998). Non-sorted but stored seeds were considered as controls. Then eight seed lots for each cultivar (two temperatures x two storage periods 4 sorted/4 control), a total of 32 lots (8 x 4 cultivars) were all tested for laboratory germination tests. All tests were conducted within 20 days of sorting, and during that period the seeds were kept at 5 °C in hermetically sealed aluminium foil packets.

The mean germination times (MGT) were calculated for CF-sorted and control lots of each species during germination, emergence and following controlled deterioration tests by the formula cited by Ellis and Roberts (1980) given below:

$$MGT = \frac{\sum (n \cdot T)}{\sum n}$$

where n = number of seeds newly germinated at time T (daily count),

T = days from the beginning of the germination test,

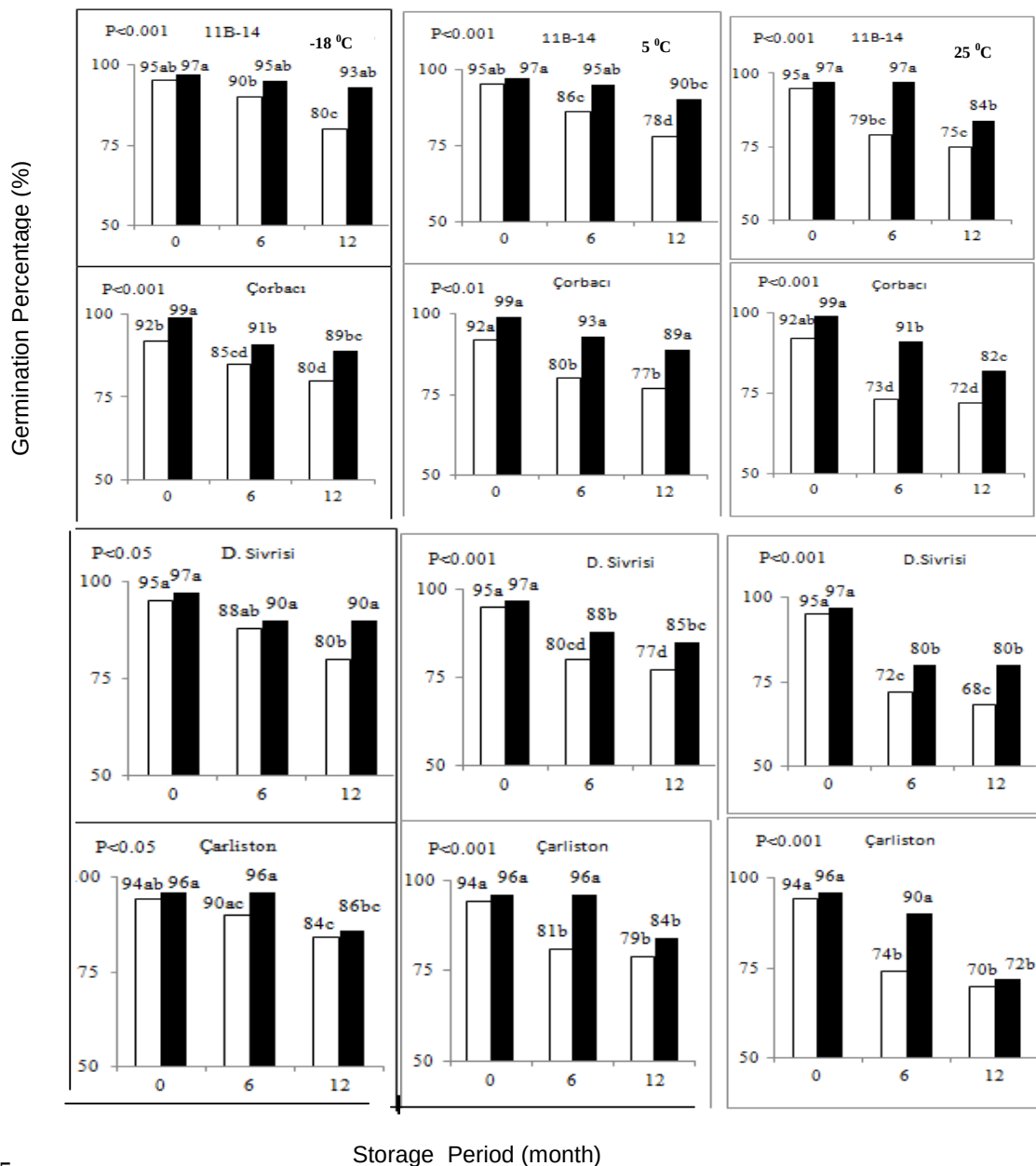
Σ n = final germination.

Statistical analysis was performed using SPSS (Statistical Package for Social Science) to carry out Duncan.

3. RESULTS AND DISCUSSION

CF-sorting increased germination percentages mean germination time and enzyme activity of pepper cultivars in both storage periods with a very few exceptions (Figures 1 and 2). Germination percentages of unsorted seeds and CF-sorted seeds did not significantly (p<0.05) differ before storage 2 % in three cultivars and 7 % in one. After storage however, CF-sorted seed lots showed higher germination than unsorted ones. This happened regardless of cultivar, storage period and temperature. The difference varied between 2 % (12 months, Çarliston, 25 °C) and 18 % (6 months, 11B-14, Çorbacı, 25 °C). Seeds stored at the lower storage temperature of -18 °C had higher germination percentages than those stored at 5 and 25 °C in both the sorted and unsorted seed lots (Fig. 1). CF-sorted seeds present always a higher germination than the control except before storage (0 h). As CF-sorted seeds are fully mature seeds, it demonstrates that mature seeds are more vigorous (storability) than bulk samples within which there is heterogeneity in seed maturation.

Figure 1. Changes in standard germination and germination after CF-sorted and unsorted (C) lots of pepper seeds of the four cultivars after 6 and 12 months of storage at -18,5 and 25 °C. Means with a letter in the same cultivar at each temperature are not significantly different in the same test (P < 0.05). ■, it means CF- sorted; □, it means control group.



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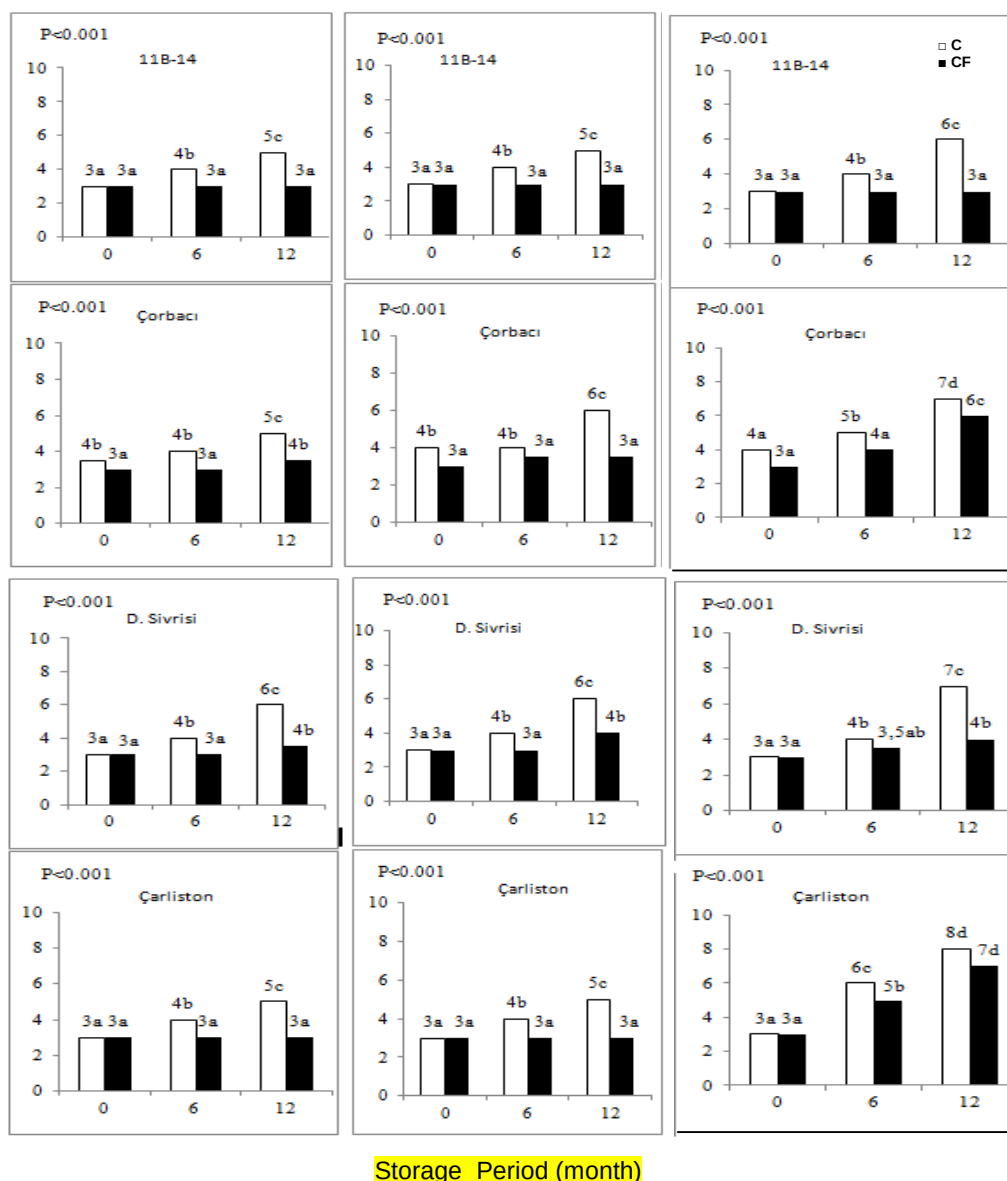
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107 CF-sorted seed lots germinated 1 and 3 days faster than stored and unsorted ones, respectively. This
108 is particularly prominent in the seed lots stored for 12 months. The MGT difference between sorted
109 and unsorted seed lots was around 1 day in 6 months storage at both temperatures. MGT went up to
110 3 days in some cultivars of seed stored for 12 months such as Çorbacı seeds at 5 °C and 11B-14, D.
111 Sivrisi seeds at 25 °C and Çarliston seeds at -18 °C (Figure 2).

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113 Figure 2. Changes in mean germination time (MGT) values of lots of pepper seeds of the four
114 cultivars following CF (Chlorophyll fluorescence) sorted and unsorted (C) after 6 and 12
115 months of storage at -18, 5 and 25 °C. Means with a letter in the same cultivar in each
116 temperature are not significantly different (P < 0.05). ■, it means CF- sorted; □, it means
117 control group.

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11'
Mean Germination Time (day)



Results of the present paper indicated that CF sorting increased germination percentages and seed vigour as determined by mean germination time in capsicum pepper seeds stored for six months or twelve months. These conclusions are in agreement with previous findings on tomatoes (Jalink et al., 1999), cabbages (Jalink, 1998), and barley (Konstantinova et al., 2002), that the CF method has the potential to enhance seed quality. Studies of efficiency of CF operation on aged seed lots are rare. Dell'Aquila et al. (2002) reported that CF sorting enhanced laboratory germination as well as seed vigour following artificial ageing (controlled deterioration) in cabbages. More recently, chlorophyll content has been associated with faster seed ageing during storage. The photo-oxidation process that occurs during storage in *Salix* seeds was shown to be accounted for by their high chlorophyll content (Roqueiro et al., 2010). This finding supported our findings that less mature (germinable) seeds with high chlorophyll content aged more, and CF enhanced seed quality by eliminating them. These findings have the potential to be used in fast regeneration of accessions in seed gene banks or genotypes stored by plant breeders for plant breeding purposes in place of laborious field experiments.

Seed ageing is a basic physiological phenomenon that delays emergence and reduces seedling size and uniformity (Finc-Savage 1986). Seeds that are left over from the previous year may be needed for

use in the next production season. In these circumstances, pepper seeds may lose quality (i.e. vigour) within a year when storage conditions are less than ideal.

This is the case in some subtropical regions of the world such as the south of Turkey where high temperature and relative humidity prevail. Reduced vigour is reflected in lower quality transplant production. The use of high value seeds such as hybrids necessitates the production of a plant for each seed in a seed lot in vegetable species. Otherwise seed loss increases total production expenses. Various methodologies are used to sort seeds and improve pre and post-storage seed quality. This process is known as priming. Priming technologies are required to get imbibed seeds up to a certain level, induce germination activity, then dried. Humidification, drying and storing primed seeds can potentially be time consuming and damaging to seed integrity (Tarquis and Bradford, 1992). However, CF sorting has the unique feature of non-destructiveness. CF is well suited to vegetable crops in which seed maturation on the mother plant occurs gradually due to continuous flowering and fertilization, so that seed lots consist of variously matured (high and low chlorophyll content) seeds (McDonald, 1999; Demir, 2002). Ageing extends the variability within deteriorated seed lots but CF sorting has the potential to effectively separate seed samples with more uniform germination. This was shown in the lower mean germination time values in CF-sorted sub samples (Figure 2). Lower mean germination time resulted in larger and more uniform pepper seedlings in the modules (Demir, 2002).

CF may not be effective on species in which there is little or no chlorophyll in the seed coat such as aubergine (*Solanum melongena*), and sunflower (*Helianthus annus*). Its use may also be limited in cases where the chlorophyll content or color of the seed may change dramatically among cultivars within the same species, as in *Brassica napus* (Zhang and Gusta, 2010). In our work, CF sorting successfully segregated high quality seeds in all cultivars as there is no difference among the pepper cultivars regarding chlorophyll content (Table 1).

Table 1. Advantages of CF sorting (mean of all cultivars) in terms of germination percentage and mean germination time for 6 and 12 month storage of pepper seeds

Storage period (month)	Advantage							
	Germination (%)			Total	MGT (day)			Total
	5 °C	25°C	-18°C		5 °C	25 °C	-18°C	
0	3.2	3.2	3.2	9.6	0.25	0.25	0.25	0.75
6	11.2	15	4.8	31.0	1.0	1.75	1	3.75
12	9.2	8.2	8.5	25.9	2.25	2.75	1.8	6.8
Total	23.6	26.4	16.5		3.5	4.75	3.1	

Mean of the all cultivars (CF-C / 4 cultivars)

In conclusion, CF can be a reliable tool to separate low quality (more aged) seeds from high quality ones. This may be usable for improving the seedling production potential of left over seeds, thus

170 increasing the overall vigor of the stored lots. Moreover, its use for the rejuvenation of gene bank
171 accessions can also be considered.

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