Chlorophyll Fluorescence Sorting Method to Improve Quality of *Capsicum* Pepper Seed Lots Produced from Different Maturity Fruits

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Abstract. This work was conducted to investigate the efficacy of chlorophyll fluorescence (CF) sorting to improve seed germination, seedling emergence, and vigor of seeds produced from different maturity fruits of four different cultivars. Four harvest dates from each cultivar were evaluated by harvesting orange (immature), bright red (half-mature), dark red (mature), and dark red and soft (overmature) fruits. Seeds were either sorted or nonsorted after harvesting and standard laboratory germination, seedling emergence, and controlled deterioration tests were conducted. CF sorting significantly increased laboratory germination, seedling emergence, and seed vigor. Maximum improvements were obtained from seeds harvested from half-mature and mature stages. Mean germination improvement among cultivars between CF-sorted and nonsorted seeds were 14% in the immature seeds, 11% in half-mature seeds, 6% in mature seeds, and 9% in overmature seeds. Improvements in seedling emergence were 21%, 17%, 9%, and 10% and 4%, 11%, 10%, 14% for seed vigor (CD germination) in the all maturity stages of seed lots, respectively. CF has the potential to upgrade seed quality in pepper lots as a non-destructive sorting technology.

The high initial cost of vegetable crop seeds, in particular hybrids, has led growers to use precision seeding and transplant production systems. Maximum plant stands have become a necessity for saving cost and time. The quality of a seed lot is the result of pre-storage and post-storage factors (Powell et al., 1984). Improving the seedling emergence potential (emergence percentages and uniformity) of a seed lot is important for crops that are produced from transplants such as pepper. Vigor may be enhanced through removing less mature seeds from the lot, thus obtaining higher emergence performance.

Peppers (*Capsicum annuum* L.) have continuous flowering that results in non-synchronous seed production because fruits that are produced from different flowering times mature at different periods resulting in

seeds of different quality. This affects seed quality in once-over harvesting (harvesting all fruits at the same time to produce a seed lot) systems. Multiple harvesting of fruits as they are mature may improve seed lot uniformity. However, this may not be practical and is expensive in large-scale commercial production. Therefore, pepper seed lots may consist of a mixture of less mature and fully mature seeds as a result of differences in flowering time. Such seed-to-seed variation in a single lot reduces overall quality. Less mature seeds germinate more slowly and produce smaller seedlings, whereas mature seeds emerge faster and produce larger seedlings (Demir and Ellis, 1992). This variation in seed maturity results in variation in plant growth and development. Therefore, separating out less mature seeds would enhance the overall quality of a seed lot.

Chlorophyll content of the seedcoat in many species decreases as seed matures (Steckel et al., 1989; Ward et al., 1992). Chlorophyll in white-seeded *Phaseolus vulgaris* seeds is visible and may be detected and sorted by color-sorting seed conditioning equipment (Lee et al., 1998). Another

non-destructive technique for assessing the maturity of seeds is based relying on measuring the amplitude of the CF signals from intact seeds. The technique makes use of laser technology, narrow optical bandwidth filters, and detection of chlorophyll *a* in the seedcoat, measuring the resulting chlorophyll fluorescence, and linking it with the quality of the seeds. This has been performed with seeds of cabbage (*Brassica oleracea*) (Dell'Aquila et al., 2002; Jalink et al., 1998), tomato (*Solanum lycopersicum*) (Jalink et al., 1999), barley (*Hordeum vulgare*) (Konstantinova et al., 2002), carrot (*Daucus carota*) (Groot et al., 2006), and pepper (*Capsicum annuum*) (Van der Burg, 2008).

The established CF sorting method analyzes the amount of chlorophyll in each seed by measuring the CF signal with sensitive detectors (Jalink et al., 1998). Using CF sorting, one measures the intensity of CF signal of each individual seed at high speed. The non-destructive methodology allows the analysis and sorting of dry seeds enabling the resulting sublots to be stored at their original moisture content without further redrying.

Vigor tests provide information on seedling growth and emergence over a wide range of environmental conditions. The controlled deterioration (CD) (Powell and Matthews, 2005) tests have been successfully used to rank and predict field emergence potential of seed lots in various crops (Powell and Matthews, 1981). In this work, we investigated to what extent CF sorting enhanced laboratory germination, seedling emergence percentages, and seed vigor (controlled deterioration) of differently matured pepper seed lots through eliminating less mature seeds.

Materials and Methods

Pepper (*Capsicum annuum* L.) plants of the cultivars Demre Sivrisi, Çarliston, 11B-14, and Çorbaç were grown in open-field conditions in 2010. The maximum and minimum temperatures were recorded as 42 and 12 °C, respectively, during the growth season. The same plant cultivation practices were used as described in Demir (2002). Approximately 50 pepper plants in each cultivar were grown. The seeds were sown in March in plastic tunnels and seedlings were transferred to the open field of the Experimental Field of Department of Horticulture, Faculty of Agriculture, University of Ankara, Ankara, Turkey, in the beginning of June. Plants were irrigated as required. Average rainfall was 500 mm/month during the growing period. Flowering started the last week of June and immature harvests were taken at the end of July and mature harvest in mid-August.

The seeds were extracted from immature (orange, Stage 1), half-mature (pale red, Stage 2), mature (dark red, Stage 3), and overmature (dark red and soft, Stage 4) fruits. One hundred fruits were harvested and ≈10,000 seeds were collected at each harvesting time. The number of seeds in the fruit changed between 95 and 380 depending on the cultivar.

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Seeds were extracted by hand and dried in the dark at 25 °C for 24 h to 10% moisture content. The germination, seedling emergence, and seed vigor determinations were conducted within 1 month of completion of the final harvest. Seeds were stored in hermetically sealed aluminium foil packets at 5 °C in 24-h dark until the tests were conducted.

CF sorting was done using the methodology (CF seed sorter and analyzer; Model JS2001) developed at Plant Research International in Wageningen, The Netherlands (Jalink et al., 1998). Seeds in each lot were sorted below 730 nm. Nonsorted seeds were considered as controls. Eight seed lots for each cultivar species [four stage × two (sorted and control)], a total of 32 lots (eight × four cultivars), were all tested for laboratory germination, seedling emergence in plug trays (dimensions, 36 × 16 × 8 cm), and controlled deterioration vigor tests. All tests were conducted within 1 month of sorting and during that period seeds were kept at 5 °C in hermetically sealed aluminum foil packets (8 × 10 cm).

Standard laboratory germination tests were conducted on three replicates of 50 seeds each for the CF-sorted and control. The seeds of each replicate were placed between three moistened 20 × 20-cm filter papers (Filtrak, Germany), two below and one above, each of which was moistened with 6 mL of distilled water. These papers were then rolled up and placed in plastic bags (32 × 23 cm) 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 d.

Emergence tests were conducted with three replicates of 50 seeds for CF-sorted and control (nonsorted) treatments. Seeds were sown 2 cm deep in compost (Plantaflor; Verkauft, GmbH, Germany). Seedlings were grown in a growing cabinet at 20 ± 2 °C for 16 d. Photosynthetically active radiation was provided by cool fluorescent lamps (Philips) at a rate of 78 μmol·m⁻²·s⁻¹ for 12 h·d⁻¹. Relative humidity in the cabinet was maintained above 70% throughout the experiment to minimize water loss. Watering was done with an equal amount of water and at the same time of day. In the growing cabinet, boxes were rotated everyday to obtain uniform temperature during emergence. Appearance of the cotyledons above the compost surface was used as an emergence criterion, counted daily at the same time of day, and, finally, normally emerged seedlings were recorded as percentages. Treatments were arranged in a completely randomized design.

Mean germination time (MGT) and mean emergence time (MET) were calculated for the CF-sorted and control lots of each variety during germination, emergence, and the following controlled deterioration tests by the formula cited by Ellis and Roberts (1980) given subsequently:

$$\text{MGT and MET} = \frac{\sum (nT)}{\sum n}$$

where n = number of seeds newly germinated/emerged in each replicate at time T

(daily count),

T = days from the beginning of the germination/emergence test, and

Σ n = final germination/emergence.

The CD vigor test was conducted at 45 °C on samples of the seed lots at 22% seed moisture content (mc) for 48 h (Powell and Matthews, 2005). For each sorted and control group, a sample of 200 seeds of known initial moisture content was 8.5% placed on moist paper and allowed to imbibe to the weight calculated to achieve the appropriate (20%) mc. Achievement of this weight was determined by periodic weighing. Seeds were then kept overnight at 5 °C in laminated aluminium foil bags to allow moisture equilibration. The seeds were then sealed in laminated foil bags and incubated at 45 °C in a water bath and removed after 48 h (Powell and Matthews, 1981). Normal germination percentages were then tested as previously described. Statistical

analysis was performed by using SPSS 16.0 (Statistical Package for Social Science; Chicago, IL). Means were compared at the 5% level and Duncan multiple range test was conducted [four stages and two sortings (sorted vs. nonsorted)]. Percentages were angular transformed before analyses.

Results

CF sorting increased germination percentages for 10 of the 16 seed lots. Overall seed quality in the second, third, and fourth stages of fruit development were better than in the first (Fig. 1). There was an exception in cultivar 11B-14 in which seed germination and vigor declined significantly in overmature seeds ($P < 0.05$). In this final harvest, seed germination was reduced to 52% and 59%, respectively, in the nonsorted and sorted lots, and from 92% and 96% in maturation III.

Fig. 1. Percentage germination after controlled deterioration (CD) (45 °C, 20% mc, 48 h) of sorted (CF) and nonsorted (C) pepper seed lots harvested at different times of maturity of four pepper cultivars from sorted (CF) or nonsorted (C) treatments. Bars represent means (± SE). Means with a letter in common in the same cultivar and harvest are not significantly different ($P > 0.05$). mc = moisture content.

Fig. 2. Mean germination time (MGT) values of seeds from fruit harvested at different times of maturity of four pepper cultivars of sorted (CF) and nonsorted (C) treatments. Bars represent means ($\pm$ SE). Means with a letter in common in the same cultivar and harvests are not significantly different ($P > 0.05$).

Fig. 3. Seedling emergence percentages of seeds from fruit harvested at different times of maturity of four pepper cultivars of sorted (CF) and nonsorted (C) treatments. Bars represent means ($\pm$ SE). Means with a letter in common in the same cultivar and harvests are not significantly different ($P > 0.05$).

In the other three cultivars, germination values fluctuated slightly but remained stable above 78% in the second and subsequent harvests. For immature and overmature seed lots, the SDSA values (sort detected seeds above) were between 350 and 300 nm but then decreased in mature lots to 250 to 230 nm.

CD germination showed a similar trend among harvests to the germination tests. The

first harvests had the lowest vigor. Subsequent harvest remained stable, but the final harvest in '11B-14' had lower vigor. In that harvest, CD germination declined from 64% to 29% in nonsorted seeds and from 85% to 60% in sorted ones (Fig. 1).

Most of the CF-sorted lots germinated faster than the nonsorted ones. One exception was the third harvest of 'Çarliston'. Mean

germination time values varied between 3 and 6 d (Fig. 2). The differences varied between 0 and 2 d, and faster germination was obtained from mature seeds at the third harvest (Fig. 2). The maturation stage did not greatly reduce the time to germination in the Çorbacı cultivar. MGT values of the first three harvests in nonsorted lots were 6 d. '11B-14' and 'Çarliston' are relatively fast germinating cultivars, but in all cultivars, immature seeds (first harvest) were the slowest group regardless of sorting. These seeds showed 5 to 6 d for MGT values.

Seedling emergence percent was enhanced by CF sorting in all maturation stages in '11B-14', 'Demre Sivrisi', and 'Çarliston'. Sorting was not effective on the final two harvests of Çorbacı (Fig. 3). The first harvest had the lowest emergence percentages but CF sorting enhanced emergence to the same level as nonsorted treatments of subsequent harvests in 'Demre Sivrisi' with a 28% point improvement obtained in seedling emergence.

The overall improvements of CF sorting is summarized in Tables 1 and 2. The greatest improvements in all three criteria were obtained in the first and second harvests at 39% for both. In the third harvest, the improvement was relatively small. Comparing individual criteria, seedling emergence was the test that best reflected the improvement after CF sorting, because total CF sorting increased emergence to 57% (Table 2). Table 2 reveals that the number of superior quality CF-sorted seed lots was greater than nonsorted ones when all cultivars are considered. The number of high-quality lots (less than 90% in germination and emergence, less than 70% in CD germination) was 21 but five in nonsorted lots after three seed quality tests. The nonsorted group had 18 lots with lower quality, but this figure was 11 in the CF-sorted group.

Discussion

New approaches to enhance seed quality include color sorting by differences in light reflectance in the ultraviolet, visible, and near infrared (Lee et al., 1998). The detection of CF signal amplified by laser technology is another non-destructive technique in evaluating seed physiological characteristics that may benefit farmers, seed industries, and seed genebanks (Dell'Aquila, 2009). Results indicated that CF sorting increased seed vigor reflected by CD. These conclusions are in agreement with the findings of previous papers on tomato (Jalink et al., 1999), cabbage (Jalink et al., 1998), and barley (Konstantinova et al., 2002) that the CF method has the potential to enhance seed quality. However, previous studies evaluated seed quality solely based on laboratory germination tests conducted under optimum germination conditions. In our work we tested the effect of CF sorting not only on laboratory germination, but also on emergence and physiological aging as indicators of seed vigor (Figs. 1–3). Our results indicated that CF sorting

Table 1. The average improvement at each harvest stage from chlorophyll fluorescence (CF) sorting on germination, controlled deterioration (CD), and seedling emergence percentages.^z

Harvest stage period	Improvement			Total
	Germination (%)	CD (%)	Emergence (%)	
I (orange)	14 ^y	4 ^y	21 ^y	39
II (bright red)	11 ^y	11 ^y	17 ^y	39
III (dark red)	6 ^y	10 ^y	9 ^y	25
IV (dark red and soft)	9 ^y	14 ^y	10 ^y	33
Total	39	39	57	

^zThe values were obtained by subtracting control values from CF-sorted treatments.

^yMean of all cultivars (CF-C/four cultivars).

Table 2. The number of total lots (total of 16) as high, medium, and low quality harvested at different times of fruit maturity of four pepper cultivars after CF sorting or nonsorting regarding germination, CD germination, and seedling emergence.

Seed quality range		Number of lots	
		CF-sorted	Nonsorted
Germination	Higher than 90%	10	2
	Medium 70% to 90%	1	9
	Lower than 70%	5	5
CD germination	Higher than 70%	8	3
	Medium 50% to 70%	5	8
	Lower than 50%	3	5
Emergence	Higher than 90%	3	0
	Medium 70% to 90%	10	8
	Lower than 70%	3	8

CF, chlorophyll fluorescence; CD, controlled deterioration.

not only affects germination, but also seed vigor. Our results are in agreement with Dell' Aquila et al. (2002) that reported that CF sorting enhanced laboratory germination as well as seed vigor after CD in cabbages.

Unlike pre-sowing hydration seed treatments, CF sorting is performed on dry seeds. CF sorting is well suited to vegetable crops in which seed maturation occurs over time as a result of prolonged flowering and fruit set (Demir, 2002; McDonald, 1999) on the mother plant. For this reason, variation within the same lot is likely to be higher than in field crops in which the seed are harvested at full crop maturity.

CF sorting is a non-destructive technique for assessing the maturity of seeds, relying on measuring the amplitude of the CF signals of intact seeds. CF sorting permits physiological and other assays to be conducted on the same seeds after sorting. According to Suhartanto (2003), the presence of chlorophyll after seed maturation is undesirable because it is associated with lower quality, particularly lower seed longevity, so this is important finding for seed storage conditions. However, there is a limitation to wide-scale use of CF sorting of crop seeds. The technique is not effective on species such as aubergine (*Solanum melongena*), maize (*Zea mays*), and sunflower (*Helianthus annuus*) in which there is little or no chlorophyll that can be detected in the seedcoat or pericarp. In addition, seeds are

removed from the seed lot resulting in a financial loss for the seed producer. In this experiment, we discarded roughly 25% to 35% for the different seed lots. This may be alleviated by better seed production practices, i.e., harvesting at maximum maturity, which would reduce the proportion of low-quality seed. Moreover, use of the technique may be limited in cases in which seedcoat chlorophyll content or color may vary dramatically among cultivars within the same species, like in *Brassica napus* (Zhang and Gusta, 2010). In our work, CF worked on the four cultivars examined, and this shows that the method may be well suited for *Capsicum annuum*.

In conclusion, CF can be a reliable tool to separate high-quality (i.e., more mature) seeds from low-quality (i.e., less mature) seeds in variously matured pepper seed lots. This may help improve seedling production and uniformity through enhancing the seed lot vigor.

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