**Vigor tests in Geranium*,* Salvia, Gazania and Impatiens seed lots to estimate seedling emergence potential in modules**

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**Abstract**

A study was made of the relationship between accelerated aging (AA, 100% r.h., 41 and 43 °C, 48 and 72 h), saturated salt accelerated aging (SSAA, 75 % r.h., 41, 43, 45 °C, 72h), mean germination time (MGT) and 2nd day germination percentage vigor tests and seedling emergence and mean emergence time in nine lots of (seven hybrid, two open-pollinated) geranium (*Pelargonium* sp.*)*, salvia (*Salvia splendens)*, gazania (*Gazania* spp.) and impatiens (*Impatiens walleriana)* seeds. Initial standard laboratory germination was above 70 % for all seed lots. Seedling emergence percentages ranged between 29 and 91 % in geranium, 2 and 89 % in salvia, 21 and 71 % in gazania, 6 and 66 % in impatiens and being higher in hybrids than open pollinated ones. Emergence time was the slowest (higher MET) in salvia and impatiens with a mean of 9.9 and 12.5 days and faster in gazania (4.3 days) and geranium (3.4 days) seed lots. Accelerated ageing was successful in predicting emergence of geranium (r=0.75-0.98, p<0.05-0.001) and salvia (r=0.67-0.95, p<0.05-0.001) seed lots. MGT was successful in geranium (r=0.74, p<0.05), salvia (r=0.91, p<0.001) and impatiens (r=0.92, p<0.001). 2nd day germination was highly correlated with emergence in geranium (p<0.001) but less significantly (p<0.05) in gazania. Combinations of 41oC/48h of AA, 45oC/72h of SSAA, 2nd day germination in pelargonium and salvia, and MGT in geranium, salvia and impatiens can be used in vigor testing. Standard laboratory germination is highly discriminatory in geranium and impatiens but less so in the other two species.

**Key words:** Emergence, seed vigor, saturated salt accelerated aging, accelerated aging

**INTRODUCTION**

High cost if flower seed has led growers to employ precision seeding and transplant production systems to maximize seedling stands (McDonald and Kwong 2005). High quality seed is required for maximum efficiency to obtain optimum seedling emergence and uniformity in plug production. Low emergence causes unproductive use of glasshouse space, resulting in heterogenous populations. Even in greenhouses conditions during seedling emergence are not always ideal. Deviations from optimal temperature or moisture availability can negatively affect seedling emergence, low quality seed lots are more sensitive to stressful conditions (TeKrony 2003).

Germination test results conducted in an ideal environment are used to evaluate the production of normal seedlings under optimal greenhouse growing conditions (Geneve 2008). However, these results may not always necessarily reflect the emergence potential of a seed lot under greenhouse conditions. Vigor tests help to identify the level of physiological aging of a seed lot and its potential for producing normal seedlings under a wide range of sowing conditions (McDonald 1975). Various vigor tests are widely employed in a number of agronomic and vegetable crops to determine the level of physiological aging (Hampton and TeKrony 1995). Vigor tests provide more information than standard germination tests, however, reports on vigor tests of flower seeds are rare.

Accelerated aging (AA) is an established vigor test used for large-seeded crops (TeKrony 1995; AOSA 2009). In this test, seeds are subjected to high temperature (41°C) and relative humidity (100%) over 24 -72h to induce aging, and subsequently evaluation is done by standard germination test. AA conditions may induce a rapid increase in fungal infection because high relative humidity (RH) in the chamber influences the aging level and variation among seed lots. These were eliminated by Jianhua and McDonald (1996) in a saturated salt accelerated aging (SSAA) test in which the relative humidity of the air was regulated by saturated salt solutions and seeds were aged at < 100%. They concluded that an aging environment of 41 oC for 48 h with KCl (93 %) and NaCl (75%) discriminated the seed vigor of impatiens seed lots. In our recent report on viola seed lots, we found that SSAA (41 oC / NaCl /72h) was a successful test in predicting seedling emergence along with mean germination time and 2nd day germination test (Demir et al. 2011). Time to radicle protrusion and seedling size were successfully used to rank petunia (*Petunia* sp.) and impatiens (*Impatiens walleriana*) seed lots (Dutt and Geneve 2007).

Geranium, salvia, gazania and impatiens are common flower species that are used in plug production. Predicting emergence potential of seed lots through vigor tests are valuable for estimating successful transplant production.

This study was designed to evaluate the use of AA, SSAA, mean germination time and 2nd day germination as vigor tests for estimating, correlating the seedling emergence potential of seed lots in these four species.

**MATERIALS AND METHODS**

Samples of nine seed lots of geranium (*Pelargonium hortorum*), salvia (*Salvia splendens*), gazania (*Gazania splendens*) and impatiens (*Impatiens walleriana*) (Table 1) were obtained from commercial seed companies. The first 7 lots were F1 hybrids and the last two (Lots 8, 9) were open-pollinated cultivars (Table 1). Initial seed moisture content was determined on 100 seeds of two replicates by using the low temperature oven method (ISTA 2001). Initial laboratory germination tests were conducted on four replicates of 25 seeds each. Seeds of each replicate were placed on filter paper (Filtrak, Germany) in a Petri dish (9 cm, diameter) with 4 ml of distilled water. The dishes were placed in polyethylene bags and placed in an incubator at 20 °C in the dark. Normal seedling percentages were evaluated after 14 days (ISTA 2009). Percentage of radicle emergence (2 mm long radicle) on the second day of the standard germination test was determined (2nd day germination).

For the accelerated aging test (AA), water jacketed aging chambers were used. The temperature range during ageing was ±0.1 oC of the set temperature. Forty ml of distilled water was added to each plastic aging box (11x11x4 cm), and 100 seeds were placed on a monolayer cheese cloth placed on the wire mesh tray (10x10x3 cm) inside the box (Hampton and TeKrony 1995). Seeds were aged at 41 oC and 43 oC for 48 and 72 h, using one box for each aging / time combination. The standard germination test was then conducted using normal seedling development for assessment (ISTA 2009) after the seeds had been dried on the laboratory bench (25 °C, 40 % r.h.) for 2 hours.

For the saturated accelerated aging (SSAA) test, the box was filled with 40 ml saturated NaCl solution. The seeds were evenly distributed on a monolayer cheese cloth placed on the wire mesh to prevent seeds dropping into the solution in the AA and SSAA tests. Trays were placed in self sealing plastic bags to prevent loss of relative humidity. SSAA was carried out at 41, 43, 45 oC for 72 h. After aging, seed germination tests were conducted after drying on the laboratory bench for 2 hours as described above.

Seeds of non-aged, AA and SSAA (four replicates of 25 seeds / lot) were sown 1 cm deep in peat moss (Plantaflor-Humus, Verkaufs-GmBH, Germany) in trays (32x20x6 cm) and placed in an incubator at 20±2 oC. Light was provided at seedling level by cool fluorescent lamps (72 µMm-2s-1) for 16 hours daily. The relative humidity in the cabinet was kept over 75 % in order to reduce evaporation from the surface. The number of emerged seedlings (unfolding cotyledons free of the growth medium surface) was counted daily up to 20 days. Finally percentages of normal seedlings (developed cotyledons, without any necrotic area and no missing upper soil organs) were calculated.

The mean germination or emergence time (MGT/ MET) was calculated using the formula

MGT or MET = ∑ n. t / ∑ n

where n = number of seeds newly germinated or emerged at time t;

t = days from planting,

∑n = final germination or emergence.

All experiments were conducted as completely randomized design. Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS). Data were subjected to analyses of variance and mean separation was made at the 5 % level by the Duncan multiple range test. Correlation coefficients (r) of vigor tests with seedling emergence performance were also calculated.

**RESULTS**

Seed moisture content of seed lots ranged between 5.9 % and 10.9 % and varied among the species and lots. The highest seed moisture was observed in lots six and seven of impatiens and the lowest in lots one and four of gazania (Table 2). Initial laboratory germination values ranged between 84 and 97 % in pelargonium, 70 and 96 % in salvia, 80 and 97 % in gazania, and 70 and 100 % in impatiens. Standard laboratory germination percentages of lots of open-pollinated cultivars were generally lower than those of hybrids in all species.

Minimum and maximum germination values after each combination of AA and SSAA, MGT and 2nd day germination are given for each species (Table 3). The range of germination percentages after aging varied among the tests. Mean values after aging tests were the highest in pelargonium at 72 and 83 % and the lowest in impatiens at 40 and 25 % in AA and SSAA tests. Minimum and maximum MGT values of salvia lots were higher (6.4 days) than those of the other three species, which shows that salvia is a slower-germinating species than the others. Slow germinating behavior was also seen in impatiens seed lots (6.1 days). Geranium seeds (1.3 days) were the fastest germinators. Impatiens seed lots had not germinated on the 2nd day. Second day germination values varied between 0 and 52 % in salvia but reached 100 % in geranium and gazania.

With regard to the maximum seedling emergence obtained from different lots of each species, mean seedling emergence percentages were generally higher in pelargonium (75 %) than in the other species (Table 4), which had very similar mean emergence percentages (46-56 %). Hybrid lots (one-seven) had higher emergence percentages in all four species than those of open-pollinated (eight and nine) cultivars. Emergence percentages of open-pollinated seed lots were significantly lower (p<0.05) than hybrids in all species except gazania. Impatiens (MET: 12.5 days) were the slowest emerging seed lots followed by salvia (MET: 9.9 days). Geranium seed lots were the fastest emerging (3.4 days). Salvia and impatiens were not only the slowest germinators under laboratory conditions but also the slowest emergers in the modules (Tables 3 and 4).

Some seed vigor tests correlated with seedling emergence and mean emergence time, but vigor tests were found to be more related to seedling emergence than to emergence time. Twenty seven significant correlations were found between vigor tests and seedling emergence while 17 with MET out of 44 (Table 5). Aging vigor tests appeared to be more successful (with a higher significance level) in pelargonium and salvia seed lots than in the other two species. MGT gave the highest correlation values in salvia (p<0.001) and impatiens (p<0.001). 2nd day germination gave the highest correlations (p<0.001) in the pelargonium seed lots. It gave as high a correlation as some combinations of AA and SSAA. The standard germination test was successful in all species (p<0.05-0.001) except MET in salvia.

**DISCUSSSION**

The results of the present work showed that AA, SSAA, MGT, and 2nd day germination predicted seedling emergence and MET in seed lots of four flower species. However, optimum seed vigor tests varied among the species. For instance, AA and SSAA were successful for geranium and salvia, MGT was particularly good for salvia and impatiens, while 2nd day germination gave highly significant values in geranium. Laboratory seed germination percentages were also highly predictive of seedling emergence in geranium and impatiens. Obtaining differing results in different species is common in vigor test studies, and there is no one universal vigor test which is recommended as appropriate for all species (Geneve 2008).

Well developed seedling production is important to save glasshouse area and energy and to reduce the work load in bedding plants (Alderson 1987). Late emergence and non-uniform seedling development reduce overall market value. The use of expensive hybrid seed makes it necessary to obtain one plant from each module. As a result, vigor information regarding the seedling emergence potential of any seed lot along with laboratory germination has great value for transplant production purposes.

The AA has been more recommended for large-sized crop seeds such as soya beans (AOSA 2009), while SSAA has been recommended for small seeded species such as onions (Rodo and Marcos Filho 2003), onions and tomatoes (Hyatt and TeKrony 2008) and flower seeds (McDonald 1997). Our results showed that both tests are more successful at predicting seedling emergence in geranium and salvia. An aging environment of 41 oC for 72 h under SSAA conditions has been recommended for use on pansy seeds to rank seed lots (McDonald 1997; Demir et al. 2011). One of the main advantages of the SSAA technique compared to AA is that it reduces proliferation of seed microflora due to lower RH levels when using a NaCl solution. We observed microfloral proliferation in AA in this study. Our earlier experience in other species (melon) showed that adding fungicides to the water or dusting the seeds with fungicides during AA aging may reduce the microfloral growth rate in the chamber. However, not much is known about how this affects the aging process and subsequent germination test results. The threshold level of microfloral growth determined during seed storage is 70-75 % relative humidity. The final seed moisture content reached at a certain RH varies among lots in the same cultivar, and between cultivars and species. Jianhua and McDonald (1996) reported that the moisture content of impatiens seed lots was 8.7-9.2 % at 41oC after 72 h at 75 % RH in SSAA. Such variation between lots may be due to various factors such as chemical structure (oily, starchy, etc.). Various saturated solutions such as NaBr (55 % RH), NaCl (75 % RH) and KNO3 (88% RH) (Jianhua and McDonald 1996; Hyatt and TeKrony 2008) are used to control relative humidity in the chamber in SSAA. NaCl is relatively cheap and provides a consistent relative humidity over a wide range of temperatures (0-50 oC) (Copeland and McDonald 1995). Very high relative humidity i.e. above 85 % induce repair mechanism in AA tests. We have not measured how long the seed took to equilibrate above the water. Our earlier experience in some other crop seeds i.e. pepper and some lots in this study indicates that repair mechanism can operate during AA ageing tests. In both aging tests, one important aspect which should be considered is that the flower seeds used may drop into the liquid. Flower seeds are very small and the mesh in the AA apparatus may not be small enough to prevent it for all species. This is why we put the seeds on cheese cloth. Manufacturing of finer mesh AA inner trays may be considered for testing flower seeds in future.

The seed lots in this work were derived from different cultivars (Table1) for each species. Although laboratory germination percentages were similar, hybrid lots performed better (higher emergence) than open-pollinated seeds regardless of species. Hybrids have naturally higher quality features than open-pollinated seeds. This indicates that genetic constitution affects vigor. Differences among the hybrid lots even though initial germinations are the same indicate that vigor is also influenced by seed production procedures such as harvest time or drying (TeKrony 1995).

Studies on various crop seeds such as onion (Ellis and Roberts 1980), maize (Matthews and Khajeh Hosseini 2006) and cucurbits (Mavi et al. 2010) indicated that time to germination relates to seedling emergence and size: later emerging seeds had smaller seedlings. A similar relationship has been reported in flower seeds. Dutt and Geneve (2007) and Oakley et al. (1994) correlated mean germination time and seedling size in impatiens and petunia seeds. MGT was found to be highly predictive of viola seedling emergence potential (Demir et al. 2011). Aging increases MGT and seeds need a longer time to protrude the radicle (Dell’Aquila 1987; Matthews et al. 2011). The longer the time between imbibition and radical protrusion means late seedling emergence and lots with lower vigor. In this work, 2nd day germination and MGT were found to be successful in pelargonium and in salvia and impatiens respectively (Table 5). Standard laboratory germination also correlated with emergence. A decrease in the number of normally developed seedlings (a criterion in laboratory germination) is one of the first signs of aging in any lot (Ellis and Roberts 1980). Standard laboratory germination was not successful at detecting emergence in viola lots (Demir et al. 2011). Determining seed vigor on the basis of a germination test can be advantageous. Germination conditions are internationally known and established. The second day germination count is even quicker than MGT, which involves counting radicle emergence throughout the test and entails a more intensive work load and time. Problems that may arise in vigor tests based on germination tests can be a possible requirement for dormancy breakage in some cases, or the inability to get any value in the early stages of germination, as in the 2nd day of germination in impatiens lots in this study (Table 3). In this case, the time of an ideal single count may be arranged according to the germination behavior of the species and by employing dormancy breaking treatments.

In conclusion, 41 oC / 48h of AA, 45 oC /72h of SSAA, can be recommended in geranium and salvia. MGT in salvia and impatiens, 2nd day germination in pelargonium and gazania are also successful vigor tests. Standard laboratory germination percentages also have value as vigor discrimination for seed lots.

**Acknowledgment**

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REPLY TO REFEREES PAPER NO: JPOP618 (Guloksuz, T and Demir, I.)

Our reply is bold,

Referee 2

**Vigour tests in geranium, salvia, gazania and impatiens seeds**

**The objective of the study was to apply a range of stress tests / ageing treatments in order that seed lots could be ranked according to their vigour. The approaches taken are comprehensive - working on a range of species and seed lots - the results clearly presented and the discussion valuable. This is an interesting and well-executed study on comparative seed longevity.**

Thank you for complementary sentences.

Abstract

**Numbers below 10 should be spelled out in full; so, ‘nine lots of geranium (*Pelargonium* sp.),’ etc. This applies throughout the manuscript.**

We have done that**.**

**On line 20, it is stated that ‘accelerated ageing was more successful in geranium.’ But this statement is rather vague. I presume what is meant is that the geranium seeds aged quicker in this test compared to the others. There are other examples in the Abstract where greater precision in the description of the findings would help the reader. For example, I am not sure (without looking elsewhere in the paper) as to what the following means: ‘SSAA gave higher correlations in salvia.’ Higher than what, I wonder.**

**The Abstract should convey the main findings in plain language. Overall, there are too many statistics in here that are not needed.**

We tried to shorten abstract. Also made more clear through removing some sentences. Some statistical values are necessary to emphasize the conclusion.

Introduction:

**The rationale for conducting this study is clearly presented.**

Materials and Methods:

**Pages 114-121: For accelerated ageing (AA) seeds were placed above water and held at 41 and 43°C for 72 h. Is it known how long the seeds took to equilibrate above the water, as this would ultimately affect the ageing rate. As the seeds increased in humidity towards about 85% RH, ageing would speed up; but above this level, hydration may have been sufficient for the repair of damage, which could slow ageing. Please see the review ‘Water and Seed Survival’ by Roberts and Ellis (1989) in *Annals of Botany*. The dynamic nature of the ageing tests (NB the same seems to apply to the SSAA?) should be dealt with briefly in the Discussion.**

Good point. We have earlier on observed occurrence of repair mechanism during AA test. Such as pepper seeds. We have not measured how long moisture equilibration happened during ageing. But obviously the relative humidity occurs well above 85 % ( about 95-98 %) in the box which is in the range of occurrence of repair systems. Some lots showed that repair system in flowers as well. But we have not presented individual seed lot results. Because 4 species and 9 lots 9 vigor tests would be huge tables which are not easy to comprehend for readers. Therefore we gave the means of ageing results (table 3). However, we mentioned about occurrence of repair mechanism during ageing test (Lines 212-216)

Discussion.

Results:

**Lines 165-7: It is stated that ‘salvia is a slower-germinating species than the others.’ However, Table 4 indicates that impatiens is the slowest for seedling emergence. It will be important in revising the manuscript to be clear as to which small differences between species are statistically significant that those that are not.**

* This deals with slow germination after ageing in Table 3. Table 4 mentions emergence times in seedling stages.

**Lines 174-175 (and Table 2): This is one of the main findings that should be clearly stated in the Abstract, in my opinion. Another main finding appears to be stated in Lines 183-4.**

* We add that the higher emergence percentages were obtained from hybrid seed lots compare to open-pollinated ones (Abstract line 16).

**There may be a little more that could be gleaned from the data. For example, I plotted Table 4 data by hand for the dependency of percentage of normal seedlings on mean time to emergence (MTE); the relationship appears to be linear for the species and variable between species. So,**

**I agree that ‘vigor information regarding seedling emergence potential of any seed lot along with laboratory germination has great value for transplant production purposes’ (Lines 209-11). Presenting such co-plots could form the basis of a type of standard ‘performance’ curve for each species.**

* We have done similar analyses in pepper, cucurbit seeds. But in this paper we just concentrate on to find out the ideal vigor tests for species. MET and emergence relations can be meaningful but I presume higher number of seed lots may be needed to get more solid results on that matter.

**Finally, I also wonder if the precise ranking of the seed lots under one ageing test are the same under another stress test. That is, a comparison could be made between the relative decreases in seed quality by the AA and the SSAA tests on a seed lot by seed lot basis. Are the effects proportionally the same under both conditions, or do some seed lots survive relatively better under one set of conditions than the other. This could be assessed by Spearman’s Rank (or similar). This addresses a wider issue of the predictability of longevity under all conditions being assumed from tests conducted under one condition. I would be happy to discuss this point further if the authors so wished.**

We took mean of germination of the aged seed lots. Giving results of individual seed lots would give excessive number of value. So we compare species as a global ageing level rather than concentrating ageing level of individual lots.

References:

**All references cited in the text are listed at the end of the paper. Minor changes needed are:**

**1) The abbreviation ‘AOSA’ is used in the text, but not in the list.**

-We put AOSA abbreviation in reference list,

**2) The International Seed Testing Rules citation of 2001 should be updated.**

* ISTA (2009) was given in the list and it was changed in the text**.**

**3) Oakley et al 1994 on page 11, Line 252 is listed as 2004.**

It was corrected as 1994 in the list

Referee 3,

Reply is bold,

I feel that the authors have performed a significant amount of work to evaluate seed vigor tests in several flower crops. It extends our knowledge of the response of Geranium, Salvia and Gazania to vigor tests. I feel that the work was performed properly and the results were appropriately analyzed. I believe the conclusions reached in the manuscript are justified.

I have no concerns about the technical parts of the study, nor the way the data was collected and analyzed. My concern is with the objectives for the study, the performance of the seed lots and the discussion of the results.

Objectives: I am not sure that I am completely clear what the objectives are. I am not clear as to what “estimating the seedling emergence potential” is referring to. Is it to demonstrate that vigor tests can discriminate high and low emerging seed lots or is that vigor tests can predict emergence? I am not completely clear how correlations between vigor tests and emergence is estimating the seedling emergence potential”.

* **Our aim in this paper is relate the germination after AA, SSAA tests, and MGT values and 2nd day radical germination values with seedling emergence and mean emergence time. Higher correlation in any vigor test results mean that it gives us an idea that it discrimates the lots correctly. The final part of introduction was explained our aim.**

Seed lot performance: From Table 2, the standard germs indicate that most of the seed lots would be commercially acceptable. There are a few low germinating lots, but I believe there was a good range of seed lot performance in the Petri dish experiments. Table 4 does a good job of characterizing emergence in the plug tray. However, except for geranium, very few seed lots performed near a commercially accepted standard. In Geranium 6 of 9 were above 86%; in salvia 2 of 9 were above 81%; in gazania 1 of 9 were above 71%; and impatiens 0 of 9 were above 70% emergence. This suggests that most seed lots were medium to low vigor seed lots.

* **These are the commercial seed lots obtained from different companies. So the production history of seed lots are not known by us. Therefore seed lots appear to be medium range vigor group. Definitely, lots of factors affect seed vigor performance one of which is pre-storage growing environments.**

Discussion: Seed producers and growers would like a lab vigor test to be able to identify seed lots that will provide high, uniform emergence and those seed lots that will not meet commercial standards for emergence. I feel that in the geranium and salvia seed lots, the authors have very good data sets (with emergence % and rate) to make significant conclusions about the ability for single or a combination of vigor tests to discriminate between high, medium and low vigor seed lots in these species. I have not grown gazania before, so I am not sure what would be commercially acceptable emergence, but there is a range of emergence percentages that could provide valuable base-line vigor information for this species (since little information is currently available). I am not sure that there are significant conclusions that can be derived from the impatiens data. There is a good range of poor emergence in the seed lots, but there were no acceptable emerging seed lots in the group to use as a high vigor control. The authors may want to consider leaving out the impatiens data. I am not as concerned with this because there are already numerous vigor studies available for impatiens.

* **That is right. There are number of vigor studies in impatiens. However, they are rather done to rank seed vigor performance but not relate emergence percentages. We have done the correlations between vigor results and emergence percentages. In that sense we are not willing to exclude impatiens from the results. Our results add extra information what we know already in impatiens.**

Conclusions: I would like to see the authors make suggestions for which vigor tests and specific vigor test values to use with each of the species. For example, AA values above x% identified high vigor seeds in geranium, while those below y% were low vigor seed lots

* **It was mentioned at the end of discussion section. Lines 253-256.**

**Full post address, fax and e-mail must be indicated after the author’s name**

**Please do not abbreviate the name of the journal in the list of references.**

**The correct citation is:**

* **Copeland L. O., McDonald M. B. (1995) instead Copeland L.O., and McDonald MB (1995).**
* **Demir I., Celikkol T., Sarıkamıs G., Eksi C. (2011). instead Demir I., Celikkol T., Sarıkamıs G. and Eksi C. (2011).**

We did give full names of Journals and re-organised reference list according to journals publishing rules.

Apart from these comments, as referee suggested we added s.e. of means in the tables no: 2 and 4.

With my best regards,

Dr. Ibrahim Demir