**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 9 lots of 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. 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. The number of significant correlations obtained was 14 in geranium, 12 in gazania, 9 in salvia and impatiens. Accelerated ageing was more successful in geranium (r=0.75-0.98, p<0.05-0.001) and salvia (r=0.67-0.95, p<0.05-0.001). SSAA gave higher correlations in salvia (r=0.88-0.93, p<0.01-0.001). 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. Even though it was not very high, standard laboratory germination was successful in predicting both emergence and emergence time in all except MET in salvia (p<0.05). In conclusion, 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 and 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. Studies that was used the vigor tests as mentioned above are rather to rank seed lots, and their correlation with emergence is not considered. This study was designed to evaluate the use of AA, SSAA, mean germination time and 2nd day germination as vigor tests for estimating 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 2001). 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 2001) 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 6 and 7 of impatiens and the lowest in lots 1-4 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 (1-7) had higher emergence percentages in all four species than those of open-pollinated (8-9) 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 2 and 3).

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). 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 in geranium and salvia, can be recommended in order to discriminate vigor. MGT in salvia and impatiens and 2nd day germination in pelargonium and gazania are also successful vigor tests. Even though correlations are low, standard laboratory germination percentages also have value as vigor discrimination for seed lots.

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