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**Using Vigor Tests for Predicting
Seedling Emergence of Vegetable Seeds
under Greenhouse Conditions**

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Abstract

Copper citrate is a complex compound of copper, which is characterized by Standard germination test is used in seed industry to evaluate seed quality; however, since is conducted under laboratory conditions, sometimes the estimated results are quite different from those observed at field conditions. This study was developed with the objective to identify the vigor test highly correlated to seedling emergence in field conditions and in polystyrene trays under greenhouse conditions. Five vegetable crops were established in standard germination test, five vigor test, and emergence test at greenhouse conditions. A completely randomized experimental design with four repetitions of 100 seeds was used for each crop. The variables evaluated were: germination and emergence percentage, speed of germination and emergence, shoot and root length, and seedling dry weight. The cold test was associated to tomato and cucumber seedling emergence in greenhouse, meanwhile the accelerated aging test predicted onion and lettuce emergence. Summer squash seedling emergence was predicted by chloride ammonium, sodium hydroxide and germination standard tests.

Keywords: Vigor, germination, cold test, accelerated aging, ammonium chloride test.

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Introduction

Certified seed is mainly based on the standard germination test, which is developed in laboratory under favorable conditions of temperature, humidity and healthiness; so that results of the seeds in seedbed are sporadically lower to those percentages reported by this evaluation (Hyatt & Tekrony, 2008). This disagreement may be to environmental conditions in the field or greenhouse are less favorable than those at laboratory (Kavak *et al.*, 2008).

In the search for protocols that allow to predict what might happen in the seedbed trays and field conditions, the evaluation of vigor has been presented as an alternative for different vegetable species, particularly by using those tests trying to simulate the stress to which seeds might be exposed to, before or during germination (Hyatt & Tekrony, 2008; Ilbi *et al.*, 2009).

Vigor tests causing artificial deterioration of seeds had been useful on predicting seedling emergence in seedbeds for some crops, such as chili pepper (*Capsicum annuum* L.) (Kavak *et al.*, 2008), sugar beet (Silva *et al.*, 2006) and summer squash (Dutra and Vieira, 2006); while the cold test had been useful for eggplant (*Solanum melongena* L.) (Demir *et al.*, 2005) or maize (*Zea mays* L.) (Ilbi *et al.*, 2009); whereas the salt soaking test has shown good results for tomatoes (*Solanum lycopersicum* L.) and onions (Hayatt & Tekrony, 2008).

The aim of this study was to evaluate different vigor tests for their ability to predict seedling establishment in seedbed in several vegetables seeds, and to identify the best test that can predict such event.

Materials and Methods

From 2008 to 2011, five vigor tests (standard germination, cold test, accelerated aging, hot water immersion, sodium hydroxide and ammonium chloride immersion) were conducted in order to evaluate physiological seed quality using five vegetable commercial varieties. Previously, seeds were sorted by weight using a Dakota® seed blower machine. All tests were conducted under a completely randomized experimental design with four replications of 25 seeds. Each test consisted of a laboratory, greenhouse and field experiments, conducted in Universidad Autónoma Chapingo, Chapingo, México. The tests were the following: standard germination according to ISTA (1976) for each crop; cold test at 10 °C during a seven days period (Molina, 1992); accelerated aging at 40 °C and 100 % humidity for 72 h (Rincón & Molina 1990); hot water immersion at 96 °C over five seconds (Heydecker, 1972), immersion in a 2 % sodium hydroxide solution, during 2 minutes at 23 °C; and immersion in a 5% ammonium chloride solution at 40 °C for a 90 min period. Seedling emergence tests were carried out under greenhouse conditions using polystyrene trays with 200 cavities filled with Peat Moss Grow Mix 2 as substrate. To evaluate seedling emergence in field conditions, seedbeds were filled with clay loam soil with neutral pH; with average temperatures of 25.5 and 19.3 °C, respectively. Light irrigation was

provided daily. Seed germination and seedling emergence (expressed in percentages), speed of germination and speed of emergence (Maguire, 1962), shoot and root length and seedling dry weight, were evaluated. To identify the most suitable vigor test that predicts the behavior in seedbed, the main appreciation criteria was the relationship between the percentage of germination in vigor tests and the percentage of seedling emergence obtained in seed trays and seedbed, since this is what farmers expect to observe. Additionally, other traits related to seedling vigor were measured in order to identify the existence of a good relationship among any of them and the observed seedling emergence on seedbeds. In order to carry out the variance analysis, percentages data were transformed using the arcsine function; afterwards, Tukey's means comparison test was performed using the SAS 9.2 (SAS, 2009) software.

Results and Discussion

The analysis of variance showed significant differences for all sources of variation: vigor tests and crops. For all cases, variation coefficients were less than 16% (results not showed).

Table 1. Means comparison of physiological seed quality of tomato (*Solanum lycopersicum L.*) evaluated by vigor tests and standard germination

TEST	GER (%)	SE	SL (cm)	RL (cm)	DW (mg)
Accelerated aging	64.57 c ^t	4.92 d	3.59 bc	4.63 c	13.84 de
Hot water immersion	35.31 d	1.22 g	3.75 b	6.13 a	11.55 e
Cold test	76.91 ab	4.52 e	3.51 c	5.50 b	13.52 de
Ammonium chloride	78.14 ab	5.86 b	3.56 c	5.50 b	14.57 d
Sodium hydroxide	79.95 a	6.19 a	3.30 d	5.43 b	14.98 cd
Emergence in trays	73.95 b	2.56 f	3.27 d	3.70 d	76.70 a
Emergence in seedbed	65.76 c	2.48 f	1.85 e	2.89 e	47.07 b
Standard germination	79.77 a	5.37 c	4.18 a	5.90 ab	17.14 c
HSD	5.03	0.27	0.18	0.49	2.48

^tMeans with the same letters within columns are not significantly different according to Tukey's test at $P \leq 0.05$; GER: percentage of germination; SE: speed of emergence; SL: shoot length; RL: root length; DW: seedling dry weight; HSD: Honestly Significant Difference.

Seedling emergence of tomato. The lower values for both, speed of germination and germination, were obtained at the hot water immersion test (Table 1); therefore indicating a strong sensitivity of tomato seeds to extreme temperatures. Germination percentages from the cold vigor test and ammonium chloride immersion test, obtained in the laboratory, showed a similar behavior to the emergence in seed trays (Table 1), therefore the stress generated by those tests may be equivalent to that occurred under greenhouse conditions (Kavak *et al.*, 2008). Except for the result of immersion in hot water, the speed of germination of the remaining vigor tests was higher than sowing in trays and in

seedbeds in field conditions, because when seeds had to face the physical resistance of the substrate, seed germination and emergence are prolonged (Dutra and Vieira, 2006); thereby, this test was useless to predict the behavior in seedbeds.

Furthermore, high temperatures also reduced seedling dry weight (DW), perhaps due to heat stress produced by both, accelerated aging test and immersion in hot water test, causing a rapid consumption of plant reserves and consequently reducing vigor expression (Hyatt and Tekrony, 2008). An opposite behavior was observed in the emergence in trays and seedbeds in open field, probably due to the advantage provided by the presence of natural light and nutrients in substrates compared to the sterile conditions maintained under laboratory conditions, where dry matter accumulation was due to cotyledon reserves (Rincon and Molina, 1990).

Seedling emergence of onion. Accelerated aging test predicts better the onion emergence in polystyrene trays since both showed the highest values of germination, similar to that found by Palanisamy *et al.* (1994) who argued that seeds subjected to accelerated aging were favored by temperature and humidity (Table 2). The lower values for both, speed of germination and germination, were obtained at the hot water immersion test; therefore indicating a strong sensitivity of onion seeds to extreme temperatures.

Table 2. Means comparison of physiological seed quality of onion (*Allium cepa* L.) evaluated by vigor tests and standard germination

TEST	GER (%)	SE	SL (cm)	RL (cm)	DW (mg)
Accelerated aging	66.76 a ^f	12.81 a	6.95 d	2.02 de	14.84 b
Hot water immersion	12.90 e	5.12 g	4.16 g	1.30 f	8.22 e
Cold test	58.84 b	11.34 b	8.48 c	2.73 c	14.98 b
Ammonium chloride	50.55 c	8.86 c	6.10 ef	1.93 de	9.57 d
Sodium hydroxide	44.17 d	7.32 de	6.49 de	2.12 d	9.73 d
Emergence in trays	66.78 a	6.68 ef	13.31 a	3.93 b	38.69 a
Emergence in seedbed	62.57 b	6.51 f	9.20 b	4.49 a	38.03 a
Standard germination	58.98 b	8.02 d	5.76 f	1.74 de	11.36 c
HSD	0.74	0.74	0.60	0.33	1.95

^fMeans with the same letters within columns are not significantly different according to Tukey's test at $P \leq 0.05$; GER: percentage of germination; SE: speed of emergence; SL: shoot length; RL: root length; DW: seedling dry weight; HSD: Honestly Significant Difference.

Seedlings produced in polystyrene trays and seed bed showed higher seedling length as well as dry matter to those obtained after a vigor test in laboratory conditions, since they were exposed to sunlight, absorption of minerals by means of irrigation and soil water (Molina, 1992), what did not happen in the laboratory.

Seedling emergence of cucumber. The cold test could estimate germination and emergence in trays and seedbed of cucumber since low temperature during the test was less than the optimum required by the specie

(Table 3), resulting in a stress that produced results comparable to field stress (Tekrony, 2003). At the same time, lowest values of germination (GER) were observed in polystyrene trays and seedbed, difference that was due to a mechanical stress of the substrate (Tekrony, 2003). However, it not reduced the possibility of the seedling to increase the length of the root and the shoot as well as dry matter, because they were exposed to light, absorption of minerals by means of irrigation and soil water (Molina, 1992).

Table 3. Means comparison of physiological seed quality of cucumber (*Cucumis sativus* L.) evaluated by vigor tests and standard germination.

TEST	GER (%)	SE	SL (cm)	RL (cm)	DW (mg)
Accelerated aging	77.35 a ^f	22.45 a	3.71 bc	8.54 a	178.02 c
Cold test	60.82 c	11.10 c	3.64 c	5.89 cd	194.35 c
Ammonium chloride	71.35 b	16.03 b	4.06 ab	7.17 b	182.28 c
Sodium hydroxide	67.67 b	9.45 d	4.06 ab	7.17 b	189.86 c
Emergence in trays	66.93 c	3.91 e	4.18 a	6.85 b	422.03 a
Emergence in seedbed	60.41 c	2.67 f	2.63 d	5.65 d	293.43 b
Standard germination	67.46 b	16.03 b	3.51 c	6.63 bc	171.98 c
HSD	4.71	0.68	0.36	0.80	24.02

^fMeans with the same letters within columns are not significantly different according to Tukey's test at $P \leq 0.05$; GER: percentage of germination; SE: speed of emergence; SL: shoot length; RL: root length; DW: seedling dry weight; HSD: Honestly Significant Difference.

Accelerated aging test was not efficient to predict behavior at the field, because high humidity and temperature during the test, promoted an easier germination and emergence, similar results were obtained in cucumber by Demir (2005).

Seedling emergence of lettuce. Accelerated aging test predicts better the lettuce seedling emergence in polystyrene trays since both showed the same values of germination (GER) and speed of emergence (SE) (Table 4). Meanwhile the highest values of GER and SE were observed in standard germination, maybe because environmental conditions in the field or greenhouse are less favorable than those at laboratory (Kavak *et al.*, 2008).

Hot water immersion test was not efficient to predict behavior at the field (Table 4), because high temperatures during the test, promoted embryo decease; therefore indicating a strong sensitivity of lettuce seeds to extreme temperatures.

The lowest values of shoot length were observed in polystyrene trays and seed bed as well as accelerated aging and hot water immersion tests, probably as a result of mechanical stress of the substrate (Tekrony, 2003) and strong sensitivity to high temperature, respectively.

Seedling emergence of summer squash. Summer squash seed germination (GER) data recorded after chloride ammonium, sodium hydroxide and germination standard tests were correlated to those obtained in polystyrene

trays, since they showed no significant difference between the results (Table 5), indicating that all tests could be used to predict the germination performance in polystyrene trays. The lowest values were obtained on hot water immersion and accelerated aging test, showing that germination process of summer squash is more sensitive to high temperature promoted under both tests (Dutra and Vieira, 2006). However, the seedling growth (SE) was affected by the stress generated by all vigor tests, including seedling emergence in polystyrene trays, where used substrate probably promoted a mechanical stress (Tekrony, 2003).

Table 4. Means comparison of physiological seed quality of lettuce (*Lactuca sativa L.*) evaluated by vigor tests and standard germination.

TEST	GER (%)	SE	SL (cm)
Accelerated aging	57.50 b ^f	7.39 ab	13.91 bc
Hot water immersion	0.50 c	0.12 d	3.50 d
Ammonium chloride	78.50 a	6.67 b	18.03 ab
Sodium hydroxide	81.00 a	4.43 c	19.91 ab
Emergence in polystyrene trays	56.50 b ^f	7.19 ab	13.90 bc
Emergence in seedbed	48.00 b	3.15 c	10.12 cd
Standard germination	79.50 a	8.32 a	23.41 a
HSD	11.54	1.59	7.29

^fMeans with the same letters within columns are not significantly different according to Tukey's test at $P \leq 0.05$; GER: percentage of germination; SE: speed of emergence; SL: shoot length; HSD: Honestly Significant Difference.

Table 5. Means comparison of physiological seed quality of summer squash (*Cucurbita pepo L.*) Var. Grey zucchini (ps), evaluated by vigor tests and standard germination

TEST	GER (%)	SE	SL (cm)	RL (cm)	DW (mg)
Accelerated aging	80.25 c ^f	3.47 b	2.7 b	6.79 ab	0.073 a
Hot water immersion	45.00 d	0.56 d	1.49 c	3.81 c	0.055 b
Ammonium chloride	87.25 abc	2.47 c	3.02 ab	6.68 ab	0.068 a
Sodium hydroxide	83.00 bc	2.32 c	2.75 b	6.6 b	0.071 a
Emergence in trays	90.50 ab	2.91 bc	2.93 ab	6.74 ab	0.070 a
Standard germination	93.25 a	10.51 a	3.26 a	7.18 a	0.058 b
HSD	9.6	0.726	0.407	0.577	0.007

^fMeans with the same letters within columns are not significantly different according to Tukey's test at $P \leq 0.05$; GER: percentage of germination; SE: speed of emergence; SL: shoot length; RL: root length; DW: seedling dry weight; HSD: Honestly Significant Difference.

Conclusions

Vigor tests could be used for predicting seedling emergence in polystyrene trays of vegetable seeds.

The cold test was associated to tomato and cucumber seedling emergence in polystyrene trays under greenhouse conditions, meanwhile the accelerated aging test predicted onion and lettuce emergence.

Summer squash seedling emergence in polystyrene trays was clearly predicted for chloride ammonium, sodium hydroxide and germination standard tests.

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