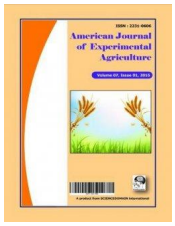

American Journal of Experimental Agriculture
9(2): 1-6, 2015, Article no.AJEA.19553
ISSN: 2231-0606

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Use of Vigor Tests in Cucurbit Rootstock Cultivars

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SE and EO conducted the work and managed the analyses of the study. Author ID designed the study, wrote the protocol and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEA/2015/19553
Editor(s):
(1) Mirza Hasanuzzaman, Department of Agronomy, Sher-e-Bangla Agricultural University, Bangladesh.
Reviewers:
(1) Anonymous, Universidad Autonoma De Bacrelona, Spain.
(2) Anonymous, Universiti Malaya, Malaysia.
Complete Peer review History: http://sciencedomain.org/review-history/10332

Original Research Article

Received 16th June 2015
Accepted 7th July 2015
Published 26th July 2015

ABSTRACT

Late and non-uniform emergence of rootstock seeds affect grafting efficiency in cucurbit species. As seeds age, (vigor decreases) they emerge later and the time to get graftable stage becomes longer. Therefore determination of emergence potential of a seed lot before sowing is crucially important.
This study was conducted to test accelerated ageing (AA), controlled deterioration (CD), cold tests (CT), electrical conductivity (EC) and mean germination (MGT) tests for the discriminating vigor of cucurbit rootstock seeds. AA (41°C, 72 h, 100% RH), CD (45°C, 24% seed m.c, 24, 48,72 h) tests were found to be successful for the ranking of 13 different cucurbit rootstock cultivar seeds, 12 of which were interspecific (*Cucurbita maxima X Cucurbita moschata*) hybrids. Cold test (CT), mean germination time (MGT) and electrical conductivity (EC) were found to be unsuccessful for the ranking of the lots. The most vigorous lots were Neffiac, Nun 9075 and Shintosa; while 90-44 and Zorba were the lowest vigorous cultivars according to the ageing tests.

Keywords: Accelerated ageing; controlled deterioration tests; electrical conductivity; cold test; rootstock seeds.

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1. INTRODUCTION

Grafting in vegetables has been carried out since the 1920s [1]. There are various benefits to be obtained from grafting, such as robust growth, resistance to disease, higher yield and plant development [2]. Watermelon is one of the main crops for which grafting is carried out intensively in Turkey, and the estimated grafted watermelon transplant production was about 100 million in 2012 in Turkey, and this value is rising gradually based on the benefits of grafting, which include higher yield and higher tolerance against disease (Dr. Hasan Ünal, Grow Seedling, Antalya, Turkey).

One of the prerequisites for successful grafting is fast seedling emergence, as fast emergence results in higher seedling size, better uniformity and more efficient grafting [3]. There are a number of different seed vigor tests, including accelerated ageing, controlled deterioration, mean germination time to estimate seedling emergence potential of any seed lot. These tests found highly correlated with seedling emerge potential (8,9,10,11). One reason for late emergence and small and uniform sized seedlings is seed deterioration, which may occur during production and storage, and as a result of genetic factors. Seed vigor is the component of seed quality that indicates the ageing level of any seed lot [4]. Various seed vigor tests have been developed and adopted for predicting the emergence and storage potential of different species [5,6], however, to date there have been no studies detailing rootstock seeds in this regard. This study reports on the application of vigor tests in cucurbit rootstock cultivars, which are widely used in Turkey for watermelon grafted transplant production.

The vigor tests used in the study are those that are recognized internationally, being accelerated ageing (AA), controlled deterioration (CD), cold test (CT), electrical conductivity (EC) and mean germination time (MGT), [5].

2. MATERIALS AND METHODS

A total of 13 different cucurbit rootstocks were obtained from different seed companies in Turkey in 2013. All were *C. maxima* X *C. moschata* interspecific hybrids, except one (Table 1).

The seeds were kept in air-tight aluminum foil packets at 5°C until use. The germination of three replicates of 25 seeds from each cultivar was tested using the between paper method [7], at 20°C in the dark. The percentage of normal seedlings was determined after 8 days, while seed moisture content was determined using the high temperature oven method (130°C, 1h), [7].

The accelerated ageing test was conducted according to procedure described by [5], according to which 100 seeds of each cultivar were placed on a wire mesh tray (10x10x3 cm) and put in an outer ageing plastic box (11x11x4 cm) containing 40 ml of distilled water. The seeds were aged at 40°C for 72 h in a water-jacketed incubator. The germination test was conducted as described above after three hours of drying at room temperature.

Table 1. Initial standard germination percentages (normal, SG), seed moisture content (m.c. %. fwb.) of cucurbit rootstock cultivars

Cultivar name	Botanical name	SG (%)		MG (%)	
Neffiac	*Cucumis melo* L.	99	a	6.08	b
Nun 9075	*C. maxima*x *C. moschata*	99	a	6.29	ab
Shintosa	*C. maxima*x *C. moschata*	95	ab	7.05	a
P 360	*C. maxima* x *C. moschata*	94	ab	6.92	a
Gürdal	*C. maxima* x *C. moschata*	93	ab	6.34	ab
Domingo	*C. maxima* x *C. moschata*	93	ab	6.68	ab
Ferro	*C. maxima* x *C. moschata*	92	ab	6.66	ab
Strongtosa	*C. maxima* x *C. moschata*	92	ab	6.43	ab
Carnivor	*C. maxima* x *C. moschata*	91	abc	6.58	ab
Polifemo	*C. maxima* x *C. moschata*	88	bc	6.05	b
64-19	*C. maxima* x *C. moschata*	88	bc	7.01	a
90-44	*C. maxima* x *C. moschata*	87	bc	6.55	ab
Zorba	*C. maxima* x *C. moschata*	83	c	4.11	c

Means with different letters are significantly different at 5 % level in each column

The controlled deterioration (CD) test was conducted at 20 percent seed moisture (m.c) for 24, 48 and 72 hours at 45°C. For each cultivar, 225 seeds of known initial moisture were placed on moist paper and allowed to imbibe to the weight calculated to achieve a 20 percent m.c. This was achieved by frequent weighing. The seeds were then kept at 5°C in laminated foil bags for 24 hours to allow moisture equilibration among the seeds. The 39 sub-samples sealed in air-proof laminated foil bags and incubated at 45°C. Next, one sample of each cultivar was withdrawn at each ageing period and germination was tested as described above.

For the cold test, the seeds were kept at 10°C for 4 days in wet germination papers in plastic bags. The germination papers were transferred to 25°C and normal seedling counts were determined after 8 days.

For the electrical conductivity test, two replicates of 25 seeds were weighed (0.0001 g) and soaked in 40 ml distilled water for 24 h at 20°C in the dark. The conductivity of the seed-soak water was measured using a conductivity meter (Shott-Gerate, GmbH Hofheim) and expressed as per gram of seeds ($\mu Scm^{-1}g^{-1}$).

A statistical analysis was carried out using SPSS for the ANOVA, and the correlation coefficient values between vigor tests and emergence percentages were transformed prior to analysis.

3. RESULTS

The vigorous ranking of seeds lots was considered to be high when germination was above 80 percent, medium at 60–80 percent and low at less than 60 percent after ageing and cold test. The controlled deterioration and accelerated ageing tests successfully ranked seed lots, in which Neffiac, Nun and Shintosa recorded the highest values after ageing in both tests. These three cultivars had germination percentages of 82 percent and above when the mean of the two AA and CD results were taken. At the other end of the scale, 90–44 and Zorba were found to have the lowest vigorous cultivars, at 44.2 and 18.5 percent, respectively. The other eight cultivars were ranked as medium, with germination ranged between 65.7 and 77.7 percent (Table 2).

There were some unstable results in the cold test. The highest vigor cultivars according to this test were found to be P360, Gurdal, Domingo, Polifemo and 64-19, all of which scored 80 percent and above germination. Ferro and Nun 9075 had the lowest germination values after CT test, at 41 and 47 percent, respectively (Table 3).

Mean germination time and electrical conductivity tests gave unstable rankings of the cultivars. Domingo and Neffiac and 90-44 gave the lowest EC values in all cultivars, which contradicted both the mean germination and ageing tests (Table 4).

4. DISCUSSION

This study indicated that the best seed vigor tests for rootstock seeds are ageing tests, controlled deterioration and accelerated ageing, all of which discriminated seed cultivars well (Table 2). CD and AA tests are the most common vigor tests, and are used for a wide range of various crop seeds [5], although we are not aware of any vigor studies detailing specifically rootstock seeds. The seed lots were classified as high (<80%), medium (60–80%) or low (>60%) vigor in germination after ageing, according to which, Neffiac, Nun 9075 and Shintosa were found to be high, while 90-44 and Zorba were deemed to be low.

Vigor studies are in general conducted on seed lots belong to the same cultivar and with the same genetic structure [5]. For the present study, we selected 13 widely used cucurbit rootstock cultivars in Turkey, 12 of which were interspecific crosses between *C. maxima* x *C. moschata,* excluding Neffiac (*Cucumis melo* L.), (Table 1). This was an obligation in the study, in that each cultivar belongs to one company with its own production rights, and has mother and father parent lines. In this regard, it is not possible to obtain more lots of the same cultivar, and this may be one of the reasons for the differences that were observed among the lots and the non-unanimity of the tests. That said, there are vigor studies that have been conducted for ranking cultivars [8,9]. [4] tested the CD of a large number of seed lots belong to various cultivars in small seeded species. For example they used 30 lots in 13 different cultivars of turnip, and found high significance between the CD test and emergence in the field (P<.01). The authors mentioned that there was not much effect varietal differences in vigor expressed either by field emergence or sensitivity CD. Vigor variations originate as a result of a combination of both genetic and environmental differences [5].

Table 2. Normal seedling percentages of cucurbit rootstock seed cultivars after accelerated ageing, AA (41°C, 72 h, 100% RH) and controlled deterioration, CD test after 24, 48, 72 h at 45°C with 24% seed m.c. Vigor level was considered as high above 80, medium between 60 and 80%, and low below 60%

Cultivar name	AA		CD								Vigor level
			24		48		72		Mean		
Neffiac	24	a	89	ab	97	a	92	a	94.0	a	High 80% above
Nun 9075	98	ab	88	abc	89	ab	68	bc	83.5	ab	
Shintosa	89	ab	81	abc	87	b	76	b	82.2	ab	
P 360	85	b	79	bcde	74	c	76	b	77.7	ab	
Gürdal	82	ab	85	abc	63	d	74	b	77.0	ab	
Domingo	86	e	87	abc	90	ab	80	b	76.5	ab	
Ferro	49	bc	92	a	73	c	69	bc	77.2	b	Medium 60-80%
Strongtosa	75	b	72	def	87	b	60	cd	75.5	b	
Carnivor	83	bc	81	abc	73	c	60	cd	72.7	b	
Polifemo	77	de	77	cde	64	d	67	bcd	67.0	b	
64-19	60	cd	69	ef	71	cd	55	d	65.7	b	
90-44	68	f	65	f	53	e	26	e	44.2	c	Low
Zorba	33	g	32	g	16	f	11	f	18.5	d	60% below

Means with different letters are significantly different at 5 % level in each column

Table 3. Normal seedling percentages of cucurbit rootstock seed cultivars after cold test 4 days at 10°C then 10 days at 25°C. Vigor level was considered as high above 80 medium between 60 and 80% and low below 60%

Cultivar name	%		Vigor level	
Neffiac	73	bc	Medium	
Nun 9075	47	e		Low
Shintosa	68	cd	Medium	
P 360	88	a	High	
Gürdal	87	a	High	
Domingo	85	ab	High	
Ferro	41	e		Low
Strongtosa	68	cd		Low
Carnivor	59	d		Low
Polifemo	88	a	High	
64-19	85	ab	High	
90-44	77	abc	Medium	
Zorba	66	cd		Low

Means with different letters are significantly different at 5% level in each column

Cold and EC tests were found to be unsuccessful in ranking the lots (Tables 3 and 4). High and low vigorous seed lots were mixed in the cold test, in which P 360, Domingo and Polifemo were found to be high quality and vigorous, while the others were medium and low. The cold test is a common means of testing maize seed in North America and Europe [10], however it does not work well in cucurbit seeds, which may be attributed to the fact that maize seed is sown in cold soils (Northern Europe), while cucurbits are sown in rather warm soils.

The electrical conductivity test was first established in pea seeds as a vigor test [5], and was then widely adopted for legume seeds. The test is currently validated to be applied to large grain legume species, peas (*Pisum sativum* L.), soybean] *Glycine max* (L.) Merr.], and green bean (*Phaseolus vulgaris* L.). The increased leakage in low vigor seeds can be attributed to membrane deterioration during aging, and there is experimental evidence that leakage from dead tissue causes membrane deterioration [11]. In this study, no clear difference was identified among the cultivars. The high vigorous seed cultivars identified during ageing tests, like Neffiac and Nun 9075, had higher EC values than lower vigorous ones like Ferro and 90-44 (Table 4). This indicates that there is no relationship between vigor and EC levels in rootstock seeds, and supports the results of earlier studies in some cucurbit species. [12,13] found no relationship between seed quality parameters and EC level in melon and vegetable marrow seed lots, respectively. [14] found that differences in leakage among species may be attributed to the seed coat structure and permeability. Some species have a non-leakage barrier beneath the seed coat that prevents the

leakage of the material out of the seed, and cucurbit seeds may belong to that group. The seed lots used in this work were hybrids of *C. maxima* and *C. moschata,* both parents of which are from the *Cucurbitaceae* family.

Seed vigor tests reveal the potential of seed lots for seedling emergence or seed storage longevity [5]. We were unable to carry out emergence or storage measurements in this work due to the limited amount of seed available at this stage, and given the expense of purchasing hybrid seed. Further studies may consider the relationship between the vigor results and emergence and storability to justify seed vigor assessments in rootstock seeds, in that faster emerging seeds result in better developed seedlings, which means more available seedlings being available for grafting. In conclusion, AA and CD tests appeared to be promising for the assessment of rootstock seed vigor; however, more research needs to be done into these testing methods in further studies.

Table 4. Changes in MGT (h) and EC values (μmS $cm^{-1}g^{-1}$) of 13 cucurbit rootstock cultivar seeds

Cultivar name	MGT (h)		EC (μmS $cm^{-1}g^{-1}$)	
Neffiac	68	de	11.2	f
Nun 9075	70	de	20.5	e
Shintosa	68	d	22.7	de
P 360	58	b	20.4	e
Gürdal	68	d	23.7	de
Domingo	43	a	11.9	f
Ferro	84	f	23.8	de
Strongtosa	66	cd	77.4	a
Carnivor	73	e	73.5	b
Polifemo	67	cd	27.8	c
64-19	63	c	21.8	de
90-44	70	c	9.7	f
Zorba	66	cd	25.9	cd

Means with different letters are significantly different at 5% level in each column. Underlined values are the highest and lowest values in each test

5. CONCLUSION

Accelerated ageing and Controlled deterioration tests were found to be promising in discriminating seed vigour of cucurbit rootstock seeds. Variation in response to different vigour tests stemmed from different genetic background among the cultivars. We appreciate for the financial support of TUBITAK (The Scientific and Technological Research Council of Turkey).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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