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Prediction of seedling emergence in onion (*Allium cepa*) seed lots through saturated salt accelerated ageing test

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ABSTRACT

The emergence of six seed lots of two onion (*Allium cepa* L.) cultivars Pusa Red and Pusa Madhvi was compared by standard germination, vigour index, germination at non-standard temperatures, accelerated ageing and saturated salt accelerated ageing tests. Germination tests were conducted at standard (20°C) and non-standard temperatures of 15°C, 25°C and 30°C. The accelerated ageing (AA) and saturated salt accelerated ageing (SSAA) tests were performed using four relative humidity levels, viz. standard AA (RH 100%), NaCl (RH 75%), Mg $(NO_3)_2$ (RH 53%) and $CaCl_2$ (RH 29%). These vigour tests were able to assess vigour differences among different seed lots. The field emergence percentage ranged from 32% to 55.5% in onion seed lots. Field emergence had non-significant correlation with germination at non-standard temperatures of 15°C, 25°C and 30°C. However, accelerated ageing (RH 100%) and saturated salt accelerated ageing using NaCl (RH 75%) showed positive and significant correlation value of 0.943 and 0.957, respectively. The regression analysis results for onion seed lots revealed that standard germination, accelerated ageing (AA) and saturated salt accelerated ageing (SSAA) test using NaCl (RH 75%) explain 88%, 88% and 91% variation in field emergence, respectively. The results suggest that saturated salt accelerated ageing (SSAA) test using NaCl (RH 75%) is useful vigour tests to predict seedling emergence in onion seed lots.

Key words: *Allium cepa*, Non-standard germination temperatures, Onion, Saturated salt accelerated ageing, Seedling emergence, Vigour tests

Most of the vegetables produced commercially depend primarily on seed for regeneration. As seed is the most important basic input, it should be of good quality and the most vital attribute of that quality is its emergence potential under field conditions. Therefore, seed vigour seems to be an important seed quality trait in addition to germination, purity and health especially in context of field emergence. Furthermore, there is an interest among vegetable growers to predict emergence, due to an increasing need to schedule their crop in precision to meet market demands for continuity of high quality products. To ensure the success of any planting programme a regular and continuous supply of high quality seed is vital. High quality seed is required for rapid and synchronous seedling emergence which is a prerequisite for successful stand establishment. Poor quality seed results in low stand establishment and sometimes crop failures.

The laboratory germination test provides information about the seedling emergence potential of seed lots under favourable conditions. The importance of seed vigour as a quality attribute has gained ground only recently as the germination potential did not reflect satisfactorily under varied environmental conditions. Since standard germination test usually overestimates field emergence under sub-optimal field conditions, there is a need for development of dependable seed vigour tests for different crops. The accelerated aging test is one of the most acceptable vigour test and is employed with high degree of consistency to predict emergence of large seeded agronomical crops. However, this test has limitation when used for small seeded vegetables due to rapid absorption of water during ageing. To overcome this problem in small seeded crops, saturated salt accelerated aging test (SSAA) has been used to detect the differences in physiological quality of seed lots. Therefore, in the present study, attempts have been made to identify dependable vigour test for predicting seedling emergence potential of onion (*Allium cepa* L.) seed lots.

MATERIALS AND METHODS

Six seed lots (A-F) of two onion cultivars Pusa Red and Pusa Madhvi produced during winter 2009-10 were collected from different public and private agencies. The seed lots were hermetically sealed in aluminium foil and

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stored at room temperature till the start of experiment. Standard seed germination was determined as per ISTA (2004). Seeds were placed in petridish on moist blotting paper and incubated in walk-in-germinator maintained at, 15°C, 25°C and 30°C. First and final count was taken on 6th and 12st day for onion and 7th and 14th day for carrot. Germination percentage was calculated based on number of normal seedlings on final count. Mean germination time was calculated according to Ellis and Roberts (1980).

High speed of germination is an indication of vigorous seed lot. Numbers of germinated seeds were counted daily from the first day and the cumulative index was made by the formula suggested by Maguire (1962).

$$\text{Speed of germination} = \Sigma\ (n/t)$$

where, n, number of seeds newly germinating at time t and t, days from sowing.

Seedling vigour indices were calculated following formula suggested by Abdul Baki and Anderson (1973). Seedling length was recorded by taking 10 normal seedlings at random from each replication and root and shoot length of each seedling was measured. The mean value was measured in cm. For seedling dry weight 10 normal seedlings per replication were selected at random and were dried in a hot air oven maintained at 80 ± 1°C for 24 hr. Seedling dry weight was expressed as g/10 seedlings.

Vigour Index I = Germination % × Seedling length (cm)

Vigour Index II= Germination % × Seedling dry weight (g)

Four different saturated salt solutions, producing different relative humidities (Water RH 100%, NaCl RH 75%, Mg $(NO_3)_2$ RH 53% and $CaCl_2$ RH 29%) were used for accelerated aging. The saturated salt solutions were prepared by dissolving individual salt in distilled water and further adding the same salt till saturation point was achieved. The accelerated ageing test was conducted according to the procedure given by Delouche and Baskin (1973). A small quantity of seed was drawn from each seed lot and spread out in thin layer in nylon net bag. These were then placed in ageing boxes and were kept in an incubator at 41°C for 72 hr. For accelerated ageing test, distilled water was placed in ageing boxes where as for SSAA test respective saturated salt solutions were used. After the completion of ageing duration, seeds were tested for moisture content and standard germination. A seed which produces an identifiable seedling regardless of their size were counted as germinated and the per cent germination after accelerated ageing was calculated.

Seeds of six lots of onion were sown under Delhi conditions at Division of Seed Science and Technology, IARI fields in November, 2010 and the emerged seedlings were counted daily until emergence is ceased. From the daily counts data final emergence percentage was recorded. Mean emergence time (MET) was calculated according to Ellis and Roberts (1980). Field emergence index (FEI) was calculated based on the procedure used by Egli and TeKrony (1985).

The data from laboratory experiments were analyzed by adopting complete randomized design (CRD). Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS). Data were subjected to analysis of variance and means were compared. All the replicated data were subjected to Duncan's multiple range test. Correlation coefficients (r) of various vigour tests with field emergence were calculated. The significance of the fitted model was assessed by R^2 (Co-efficient of determination).

RESULTS AND DISCUSSION

The results of standard germination test (Table 1) showed that all the seed lots of two different onion varieties had germination above minimum standard (70%). The germination percentage varied significantly among the different vigour level seed lots. Seed lot A (84.3%) of Pusa Red recorded maximum standard germination, whereas seed lot F (70.6%) of Pusa Madhavi showed significantly lower germination. Under favourable conditions, the germination test can predict the field emergence of garden peas with some degree of precision (Duczmal and Minicka 1989). Speed of germination varied significantly among the different vigour level seed lots. Seed lot C (22.28) of Pusa Red recorded maximum speed of germination, whereas seed lot B (18.70) of Pusa Madhavi showed significantly lower speed of germination.

Standard germination does not always indicate seed lot potential performance, especially if field conditions are less than optimal (Hampton and TeKrony 1995). Germination tests fail to account for the progressive nature of seed deterioration which is reflected by seed vigour (McDonald and Copeland 1997). Vigour index I calculated on the basis of seedling length showed no significant difference among different seed lots. However, vigour index II calculated on the basis of seedling dry weight showed that seed lot A (2.95) of Pusa Red had maximum vigour index II which was on par with seed lot C, E and F. Seed lot D (2.50) of Pusa Madhvi cultivar showed significantly lower vigour index II and was at par with seed lot B, C, E and F. Yadav and Dhankar (2001) reported that vigour indices were positively

Table 1 Germination, emergence and vigour of onion seed lots

Seed lot	Germination (%)	Speed of germination	Vigour index-I	Vigour index-II	Field emergence (%)	Mean emergence time
A	84.3a	21.10a	573.44a	2.95a	55.50a	2.44ab
B	80.6ab	18.70b	526.58a	2.68ab	50.50b	2.8 a
C	72.6c	22.28a	559.84a	2.71ab	32.00c	2.30bc
D	71.3c	20.83ab	533.58a	2.50b	41.00d	2.65ab
E	74.6bc	22.23a	587.18a	2.67ab	47.00e	2.36abc
F	70.6c	22.10a	544.66a	2.40b	32.50e	1.90c
CD (P=0.05)	6.42	2.37	N.S	0.37	3.31	0.48

Means followed by the same letters are not significantly different. Separation by Duncan's Multiple Range test at 5% level of significance.

and significantly correlated with standard germination, seedling length and seedling dry weight and negatively correlated with electrical conductivity in okra.

The germination percentage at different non-standard temperatures varied significantly among the different vigour level seed lots. Seed lot A (84%) and C (84%) of Pusa Red recorded maximum standard germination at 15°C, whereas seed lot F (68%) of Pusa Madhavi showed significantly lower germination at 15°C. However, at 25°C seed lot E of Pusa Madhvi had maximum germination (82%) and seed lot B of Pusa Red and lot F of Pusa Madhvi had significantly lower germination. At 30°C seed lot C of Pusa Red cultivar showed maximum germination of 82%. The overall germination of all the seed lots increased at 30°C temperature as compared to other standard and non-standard temperatures. The range of temperatures at which the maximum germination percentage occurs, differs among crops and with seed quality. In general, temperature range becomes narrower as a seed lot deteriorates (Ellis and Roberts 1981). Germination percent after accelerated ageing of 6 different onion seed lots differed significantly. Seed lot B of Pusa Red maintained maximum germination after accelerating ageing at RH 100% and was at par with seed lot A, D and E. The germination in seed lot F of Pusa Madhvi was minimum following accelerating ageing test indicating higher deterioration and low vigour of this seed lot. Maximum overall reduction in germination of different onion seed lots was recorded following accelerated ageing as compared to saturated salt accelerated ageing. At high humidity fungal infection was also recorded in onion seed lots. SSAA using RH 75% showed significantly higher germination in seed lot A of Pusa Red (71.3%) while seed lot F of Pusa Madhvi recorded lowest germination (40.3%). At RH 29% maximum germination of 72.3% was observed in seed lot B of Pusa Red and minimum in seed lot F of Pusa Madhvi (56.0%). According to Rodo and Marcos Filho (2003), the use of saturated salt accelerated ageing using NaCl (RH 75%) was found successful for differentiating seed lots of onion that had similar germination percentage but different levels of vigour. Pandita *et al*. (2014) reported that controlled deterioration test was efficient in predicting seedling emergence in okra seed lots over accelerated aging test at RH 100%.

Table 2 Seed vigour of six onion seed lots assessed by germination at non standard temperatures, accelerated aging and saturated salt accelerated ageing tests

Seed lot	Germination at non-standard temperatures			AA test	Saturated salt accelerated ageing test		
	15°C	25°C	30°C	RH 100%	RH 75%	RH 53%	RH 29%
A	84.0a	80.0ab	79.3a	62.6a	71.3a	70.0ab	70.6a
B	73.3bc	71.3c	78.0a	63.3a	61.0b	70.3ab	72.3a
C	84.0a	79.3abc	82.6a	40.6b	44.3c	71.6a	70.0a
D	79.3ab	81.6a	72.6a	56.0a	59.0b	62.3ab	67.3a
E	77.3ab	82.0a	78.0a	62.0a	61.3b	64.6ab	68.6a
F	68.0c	72.0bc	76.0a	39.3b	40.3c	57.6b	56.0b
CD (P=0.05)	8.10	8.41	NS	7.94	7.15	11.74	10.81

Means followed by the same letters are not significantly different. Separation by Duncan's Multiple Range test at 5% level of significance

The maximum field emergence was recorded in seed lot A (55.5%) of Pusa Red followed by seed lot B (50.5%) of same variety. The minimum field emergence was recorded with seed lot C (32%) of Pusa Red followed by seed lot F (32.5%) of Pusa Madhavi. It is evident from the field emergence data that emergence percentage was far below the standard germination values in onion seed lots. The mean emergence time was highest in seed lot D (2.65) of Pusa Madhavi and it was lowest in seed lot F (1.95) of same variety. Although differences in physiological attributes of seed lots can be demonstrated in the laboratory, but it was recommended that the term should be used to describe the performance of seeds when sown in the field (Perry 1984).

The relationship between various vigour tests results and field emergence of six seed lots of two onion varieties was studied by determining simple correlation co-efficient (Table 3). The correlation between standard germination

Table 3 Comparison of independent variables against field emergence in onion seed lots

Vigour tests		Correlation coefficients	Regression equation	R^2	P-value
Standard germination		0.938***	Y=1.202x–45.73	0.880	***
Germination at non-standard temperatures	15°C	0.245	Y=0.377x + 13.74	0.060	
	25°C	0.139	Y=0.279x + 21.38	0.019	
	30°C	0.017	Y=0.051x + 47.05	0.000	
Mean germination time		0.408	Y=11.17x–7.389	0.166	
Speed of germination		0.551	Y=3.866x + 125.0	0.304	
Vigour Index I		0.215	Y= 0.088x–5884	0.046	
Vigour Index II		0.691	Y=35.12x–50.06	0.478	**
Saturated salt accelerated ageing	100% RH	0.943***	Y=0.813x–0.844	0.889	***
	75% RH	0.957***	Y=0.791x–1.408	0.916	***
	53% RH	0.413	Y=0.724x–4.777	0.171	
	29% RH	0.613	Y=1.004x–24.71	0.376	

Significance level: ** P < 0.01, *** P < 0.001

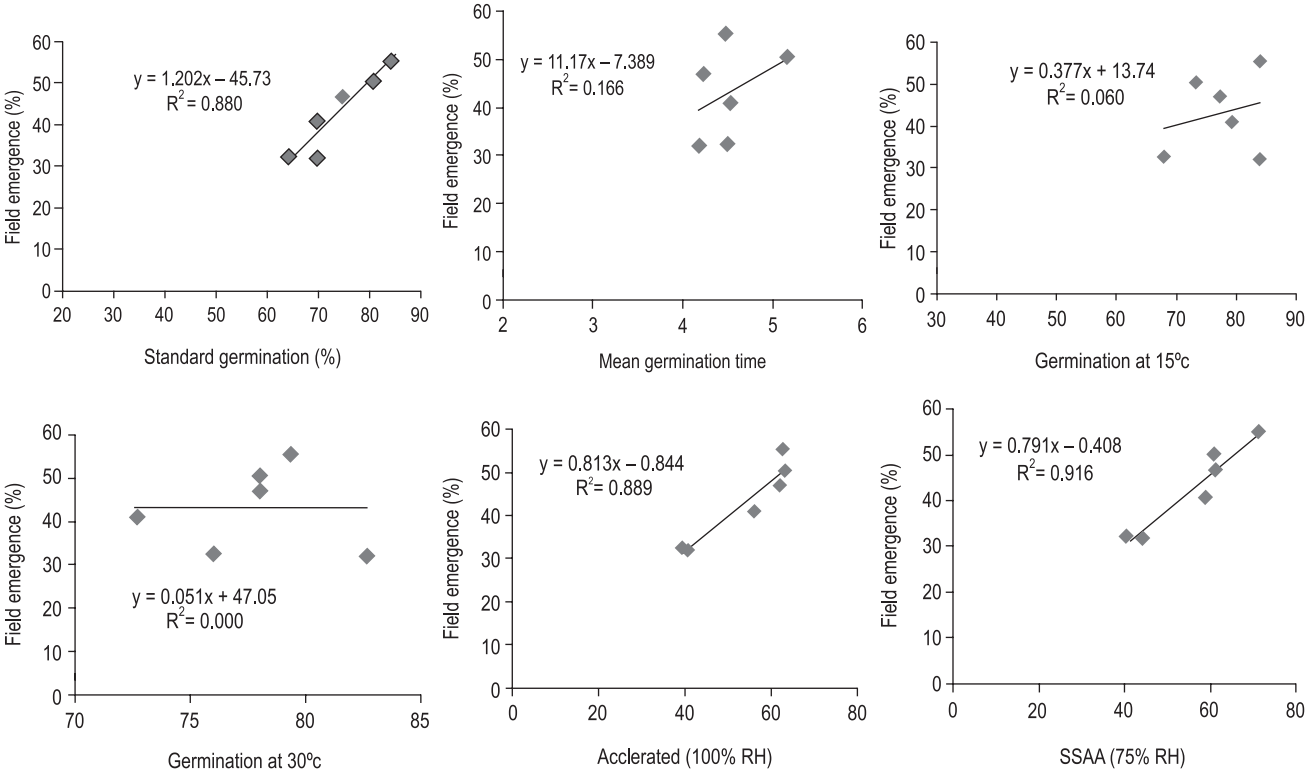

Fig 1 Relationship between field emergence and standard germination, mean germination time, germination at 15°C, germination at 30°C, accelerated ageing, saturated salt accelerated ageing of six onion seed lots.

and field emergence was significant (r=0.883**), whereas vigour indices, viz. vigour index I (r=0.215) and vigour index II (r=0.691) were not significant with field emergence. The correlation co-efficient (r) between field emergence and the results of germination at non-standard temperature were ranging from 0.017 (at 30°C) to 0.245 (at 15°C). These results indicate that germination at non-standard temperatures do not correlate with seedling emergence in onion and cannot be used to predict field emergence. The correlation between mean germination time(r=0.408) and speed of germination (r= 0.551) with field emergence was also non significant. Germination after accelerated ageing at 100% RH and 73% RH showed significant correlation with field emergence. It ranged from r=0.413 (53% RH) to r=0.957*** (73% RH). These results indicate that accelerated ageing (r=0.943***) and saturated salt accelerated ageing using NaCl 73% RH (r=0.957***) correlate better with field emergence in onion. Hyatt and Tekrony (2008) also reported that saturated salt accelerated ageing test using saturated NaCl for 72 hr at 45ºC provided better separation of vigour in onion seed lots.

The regression analysis or Coefficient of determination (R^2) was analyzed for 6 seed lots of two onion varieties (Table 3). The R^2 values of various vigour tests with field emergence ranged from 0.003 to 0.916. The coefficient of determination for standard germination (R^2=0.88*) was found to be significant and it showed linear relationship with field emergence. It also showed that standard germination test contributed to 88% of the variation in the field emergence. The coefficient of determination for vigour index I (R^2=0.04) and vigour index II (R^2=0.47) were non-significant. Only 4% and 47% of the variation in field emergence could be explained by vigour index I and vigour index II respectively. The regression analysis for germination at non-standard temperatures was also done. The regression analysis for germination at 15°C temperature (R^2=0.06) was not significant. It also showed that non-standard germination test (15°C) contributed to 6% of the variation in the field emergence (Fig 1). The regression analysis for germination at 25°C temperature (R^2=0.01) was also non significant and contributed to 1% of the variation in the field emergence. The regression analysis for germination at 30°C temperature (R^2=0.003) was also non-significant. It showed that non-standard germination test (30°C) contributed to 0.3% of the variation in the field emergence. These results suggest that germination at non-standard temperatures cannot be used to predict seedling emergence in onion. The regression analysis for accelerated ageing test (R^2=0.88*) was also found to be significant. It explains that 88% of the variation in field emergence could be explained by AA test. The regression analysis for saturated salt accelerated ageing test at 73% RH (R^2=0.91**) was also found to be significant. It explains that 91% of the variation in field emergence could be explained by SSAA test at 73% RH. The regression analysis for saturated salt accelerated ageing test at 53% RH (R^2=017) and 29% RH (R^2=0.37) were non-significant. These observations (Fig 1) indicate that saturated salt accelerated

ageing test using saturated salt NaCl (RH 73%) can be successfully used to predict seedling emergence in onion.

Evaluation of vigour tests revealed vigour differences among the seed lots of onion. Vigour tests like standard germination, accelerated ageing (AA) and saturated salt accelerated ageing (SSAA) test using NaCl (RH 75%) were apparently able to identify seed lots with differing physiological potential. Correlation and regression analysis suggest that saturated salt accelerated ageing (SSAA) test using NaCl (RH 75%) is useful vigour tests to predict seedling emergence in onion seed lots.

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