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Evaluation of Seed Vigour in Onion

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ABSTRACT Vigour assessment of twelve seed lots of onion was made through standard germination (first and final count), seedling dry weight, seed vigour index, accelerated ageing, controlled deterioration, dual stress, electrical conductivity, and volatile aldehyde tests. The results of these vigour tests were correlated with field emergence. The highest positive correlation with field emergence at 1% significance was recorded with standard germination final count, (r = 0.937**), followed by seed vigour index (r = 0.916**), germination first count and dual stress test (r = 0.899**). To know the storage potential, seed lots were stored under ambient conditions and correlation was determined, between standard germination, accelerated ageing, dual stress test and field emergence with germination recorded at three months' interval during storage upto 12 months. The highest correlation was recorded between initial germination per cent and germination taken after every three months of storage upto twelve months. Hence, indicating the actual germination (%), rather than the minimum prescribed standards was found to be a better indicator of onion seed field emergence and storability.

Key words: Onion, vigour, field emergence, storage potential.

Onion is the second most important commercial vegetable crop in the world, after tomato. India, with a production of 0.82 million tonns and an area of 0.06 million hectare, occupies the second position after China. Onion seeds are relatively small sized, with small amounts of stored reserves and prone to rapid deterioration after physiological maturity. It contains high amounts of oil (20.8%), fiber (22.4%), and crude protein (24.8%) [1].

Loss of seed vigour and viability are more rapid in subtropical countries as compared to temperate environment. The conditions of high temperature and humidity in subtropical countries such as India, make it difficult to produce quality seeds and maintain their viability during storage. In terms of planting value, poor seed vigour results in faster loss of germination during storage, poor field emergence and inadequate stand establishment.

Innumerable vigour tests have been attempted and found useful in different crops within certain sets of conditions, but not accepted universally. Even in cases, where vigour tests have been proposed for a given crop, it is difficult to determine appropriate standards to indicate the true vigour status of a seed lot. In a poor storer crop like onion the loss of vigour is expected to have more pronounced effect on their plantability. Hence, developing reliable vigour tests is desirable for both the seedsmen as well as the farmers [2-7].

MATERIALS AND METHODS

Twelve seed lots of onion were collected from various research stations and seed markets for the present studies.

Evaluation of seed vigour

Germination (first and final count)

Germination was tested in three replications of 100 seeds each, following top of the paper

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method at 20°C [8]. First count was taken on 6^{th} day and final count was taken on 12^{th} day. The germination was calculated on the basis of normal seedlings, abnormal seedlings and ungerminated seeds counted on the final day. Germination percentage was recorded on the basis of normal seedlings only.

Seedling dry weight

Seedling dry weight was estimated in three replications, following the standard method [9]. Ten normal seedlings were picked up randomly from the germinated seeds from each replication and dried in oven at 80°C for overnight. It is expressed as mg/10 seedlings.

Electrical conductivity (EC) of seed leachate

The electrical conductivity was measured following the standard procedure given by Agrawal and Dadlani [10]. One gram of seeds, in two replications, was imbibed in 25 ml of deionised water at 20 ± 1°C for 24h. The electrical conductance of the leachate were measured at room temperature and calculated as μ si/cm/g.

Accelerated ageing test

The accelerated ageing test was conducted as the method described. Two gram seeds were kept in nylon net bag at 100 per cent RH in a sealed desiccator maintained at 41°C in an incubator. The lower chamber of the desiccator was filled with 500 ml distilled water and was equilibrated at the given temperature 24h before keeping the sample. The samples were drawn after 72h of accelerated ageing and the germination was tested on three replications of 100 seeds for each variety as described before.

Controlled deterioration test

The seed moisture content was determined according to ISTA, 2004 [8]. Two grams of seed was taken in a sealable foil pouch and to raise the moisture content of seed to 24 per cent, required amount of water was added and the pouch was sealed and kept in a refrigerator for 24h to equilibrate the seed moisture. The pouch containing high moisture seed was then transferred to an incubator at 45°C for 24h. Germination test was conducted as mentioned above.

Volatile aldehyde Estimation

The passive collection of volatile aldehydes was conducted on two replications according to the procedures described by Wilson and McDonald [11].

To perform this test, 250 ml conical flasks were lined with 3 discs of Whatman No. 1 filter paper and moistened with distilled water. One gram of seed was placed over the moist blotter in three replications in separate flasks. A small test tube containing 7ml 0.2 per cent (w/v) MBTH (n 3-methyl-2-benzothiazolone hydrazone) solution was kept inside each flask with seeds. The flasks were sealed with rubber stopper and incubated at 25°C in the dark for 48h. At the end of 48h period, the flasks were opened and the reagent tubes were taken out.

Colorimetric analysis: An aliquot of 1ml of MBTH trapping solution from each flask was pipetted into test tubes containing 2.5 ml of 0.23 % (w/v) $FeCl_3$ and incubated for 5 minutes at room temperature (25 ± 2°C). To this 6.5 ml acetone was added and absorbance was read at 635 nm in a spectrophotometer (Beckman DU-640, USA).

Dual stress test

A new vigour test, based on dual stress, was developed by subjecting the seeds to two types of stress i.e., (i) low O_2 concentration and (ii) high concentration of volatile aldehydes. To create the above conditions, seeds were incubated in sealed, small volume containers (to restrict O_2 availability and to build up the levels of VAC released by the hydrated seeds). To perform this test, small vials lined with Whatman No. 1 filter paper or foam layer, moistened with distilled water were used. Twenty five seeds were placed over the moist substratum in two replications. Immediately after placing the seeds, vials were capped tightly. The capped vials sealed with masking tape and were incubated at 25°C for 7 days. After 7 days, the germination per cent was calculated on the basis of number of seeds sprouted or germinated.

Field emergence

Hundred seeds of each lot were sown (dibbled) on two raised beds in four replications (2 x 50 x 4 = 400) in the experimental fields of the Division of Seed Science and Technology, IARI in July, 2006 under optimum conditions. Observations were recorded after 6 and 15 days of sowing on the number of seedling emerged in each row. The seedling emergence was expressed as per cent.

Seed storage

About 100g seeds of each lot were packed in paper bags and stored under ambient condition from June, 2006 to 2007. Samples were drawn at every three months interval and germination per cent was recorded.

Statistical analysis

Statistical analysis was carried out separately for each test using a completely randomized design for the variance analysis using MSTAT and correlation was also worked out.

RESULTS AND DISCUSSIONS

Field emergence

Thirteen parameters of seed vigour were determined to assess the planting values of twelve seed lots of onion with respect to their field emergence and storability. The analysis of variance showed that lots significantly differed for all vigour parameters studied (Table 1). In twelve seed lots, the first count of the standard germination ranged from 13 per cent to 91 per cent, with a mean value of 62 per cent. The final count of the standard germination ranged from 29 per cent to 92 per cent, with a mean value of 68 per cent. Seedling dry weight taken after standard germination test varied from 17.75 mg to 20.90 mg and the vigour index i.e. germination (%) x seedling dry wt. (mg) ranged from 515 to 1900.

The first count of germination after accelerated ageing test ranged from 6 per cent to 81 per cent, with a mean value of 49 per cent. The final count of germination after accelerated ageing ranged from 6 per cent to 87 per cent, with a mean value 53 per cent. Seedling dry weight after accelerated ageing test varied from 16.13 mg to 19.00 mg. The first count of the germination after controlled deterioration ranged from 1 per cent to 53 per cent, with a mean of 35 per cent. Germination final count after controlled deterioration ranged from 3 per cent to 59 per cent, with mean value of 38 per cent. Seedling dry weight after controlled deterioration varies from 16.25 mg to 20.50 mg. Thus, reduction in germination (%) was more after AAT than after CD, whereas the seedling dry weight declined after AAT but remained comparable to non-aged seed in case of standard germination test. A new vigour test, based on dual stress response, was developed in the present study. Germination (based on radicle emergence and/or elongation) after the test ranged from 6 per cent to 90 per cent, with a mean value of 70 per cent. Release of volatile aldehydes ranged from 0.250 to 0.500 HCHO mg/g seed, with mean value of 0.308 HCHO mg/g seed. Electrical conductance of seed leachate ranged from 6 to 9.5 mmhos/cm/g with mean value of 7.52 mmhos/cm/g.

The first and final count of field emergence ranged from 3 per cent to 61 per cent and 5 per cent to 61 per cent with a mean value of 40 and 41 per cent, respectively.

The highest positive correlation of field emergence at 1 per cent significance was recorded (Table 2) with standard germination final count (r = 0.937**). It was followed by germination first count (r = 0.924**), seedling vigour index (r = 0.916**), germination after dual stress test (r = 0.899**), germination final count after accelerated ageing (r = 0.896**) and germination first count after accelerated ageing (r = 0.853**), germination final count after controlled deterioration test (r = 0.842**) and germination first count after controlled deterioration test (r = 0.833**). Volatile aldehyde production (r = -0.690*) and electrical conductivity (r = -0.684*) were negatively correlated with field emergence at 5% significance. Thus, actual germination value of a seed lot was the most reliable indicator of its emergence ability. The prediction of performance, based on standard germination, was more reliable in case of onion, where eight out of twelve lots were having germination well above the minimum standard of 70 per cent, one was

Table 1. Evaluation of vigour in twelve seed lots of onion

Lot No.	SG FC (%)	SG Fin C (C)	SDW (mg)	SV	AAFC (%)	AA Fin C (%)	AA SDW (mg)	CD FC (%)	CD Fin C	CD SDW (%)	DST (%)	VA µg HCHO /g	EC (µ si/ /g)	FE (%) 6th day	FE (%) 15th day
LI	74(59)	79(63)	18.75	1482	47(43)	57(49)	17.25	52(46)	52(46)	16.75	90(72)	0.225	7.50	61(51)	61(51)
L2	69(56)	71(57)	19.20	1364	64(53)	67(55)	17.50	49(44)	54(47)	17.00	80(63)	0.250	7.50	45(42)	48(44)
L3	75(60)	81(64)	19.00	1540	60(51)	64(53)	17.75	38(38)	43(41)	17.00	85(67)	0.300	8.35	51(46)	52(46)
L4	89(71)	92(74)	20.65	1900	81(64)	87(69)	18.75	53(47)	55(48)	20.50	90(72)	0.250	6.75	54(47)	57(49)
L5	85(67)	89(71)	18.85	1678	71(57)	81(64)	16.75	45(42)	50(45)	17.25	90(72)	0.300	6.00	59(50)	59(50)
L6	91(73)	92(74)	20.00	1840	64(53)	71(57)	19.00	50(45)	59(50)	18.50	85(67)	0.250	6.00	52(46)	53(47)
L7	65(54)	72(58)	18.95	1364	55(48)	57(49)	18.00	31(34)	35(36)	18.25	80(63)	0.350	7.15	47(43)	51(46)
L8	65(54)	71(57)	18.80	1335	43(41)	47(43)	16.75	29(33)	32(34)	17.25	75(60)	0.250	7.65	40(39)	43(41)
L9	64(53)	69(56)	19.10	1318	59(50)	59(50)	16.50	35(36)	35(36)	16.25	68(56)	0.350	7.50	38(38)	38(38)
L10	20(27)	30(33)	20.90	624	9(18)	9(18)	17.75	1(4)	3(10)	17.50	36(37)	0.375	8.85	18(25)	18(25)
L11	13(21)	29(33)	17.75	515	4(12)	6(14)	16.13	2(8)	5(13)	16.25	6(14)	0.500	9.50	3(7)	5(12)
L12	39(39)	44(42)	19.85	873	27(31)	29(33)	17.25	29(33)	3(33)	16.50	60(51)	0.300	7.50	8(16)	8(16)
Mean	62(53)	68(57)	19.32	1319	49(43)	53(46)	17.45	35(34)	38(37)	17.42	70(58)	0.308	7.52	40(38)	41(39)
CD(P=0.05)	3.67	4.79	1.55	146	1.65	1.55	0.87	4.05	2.5	0.86	3.00	0.032	1.19	9.00	7.00

Values in parenthesis indicate angular transformed values.

SGFC=Standard germination first count; SGFinC=Standard germination final count; SDW=Seedling dry weight; SV=Seed vigour; AAFC=Accelerated ageing first count; AA FinC=Accelerated ageing final count; CDFC=Controlled deterioration first count; CDFinC=Controlled deterioration final count; DST=Dual stress test; VAC=Volatile aldelydes; EC=Electrical conductance; FE=Field emergence.

Table 2. Correlation between different vigour tests with field emergence in onion

Lot No.	SG FC (%)	SG Fin C(%)	SDW (mg)	SV	AAFC (%)	AA Fin C (%)	AA SDW (mg)	CD FC (%)	CD Fin C (%)	CD SDW mg	DST (%)	VA µg/ HCHD /g	EC µsi/ g	FE (%) 6th day	FE (%) 15th day
SGFC		0.995**	0.114ns	0.993**	0.941**	0.972**	0.493ns	0.932**	0.947**	0.502ns	0.940**	-0.730**	-0.836**	0.924**	0.924**
SG Fin C			0.059ns	0.993**	0.935**	0.967**	0.469ns	0.911**	0.927**	0.506ns	0.921**	-0.688*	-0.809**	0.935**	0.937**
SDW				0.161ns	0.182ns	0.114ns	0.704*	0.098ns	0096ns	0.600*	0.183ns	-0.328ns	-0.253ns	0.031ns	0.020ns
SV					0.947**	0.968**	0.547ns	0.910**	0.927**	0.584*	0.913**	-0.690 *	-0.822**	0.914**	0.916**
AAFC						0.989**	0.479ns	0.890**	0893**	0.580*	0.874**	-0.600*	-0.780**	0.845**	0.853**
AA Fin C							0.445ns	0.923**	0 930**	0.512ns	0907**	-0.638*	-0.816**	0.893**	0.806**
AASDW								0.441ns	0487ns	0.806**	0.477ns	-0.419ns	-0.460ns	0.414ns	0.427ns
CD FC									0.991**	0.389ns	0.912**	-0.738**	-0.793**	0.839**	0.833**
CD Fin C										0.411ns	0.906**	-0.722**	-0.813**	0.845**	0.842**
CD SDW											0.419ns	-0.303ns	-0.483ns	0.431ns	0.462ns
DST												-0.863**	-0.794**	0.903**	0.899**
VA													0.620*	-0.699*	-0.690*
EC														-0.691*	-0.684*
FE 6th day															0.997**
FE 15th day															

*,**indicates significance at 5% and 1% respectively; Abbreviation same as in Table 1.

marginally below (69%) and only three lots were below the standard. Several previous studies indicated that onion seeds, being relatively small-sized with less amounts of stored reserves, high oil content and a precariously located embryo at the tip (which is prone to mechanical injury), required a sensitive vigour test to differentiate the actual planting value of the seed [12, 13, 14, 6]. The accelerated ageing and controlled deterioration tests were suggested, having better ability to discriminate between the seeds of different vigour levels, and hence predict the storability as well as emergence potential. A good correlation is generally obtained between the standard germination and field performance, particularly under near optimum soil conditions [15, 16]. On the other hand, reliability of the vigour tests were found to be more under sub-optimal/stressful field conditions in several crops *viz.*, water melon [7], soybean [5, 17] and maize [18].

Seed storage

The difference in germination was significant (Table 3) among different lots of onion during ambient storage. The initial mean germination per cent ranged from 29 per cent to 92 per cent. There was significant decline in germination final count per cent during storage. The mean germination final count ranged from 68 per cent to 59 per cent during twelve month of ambient storage, recording a reduction of 9 per cent in a period of 12 months storage.

An attempt was made to determine the potential storability of the seeds on the basis of several laboratory tests performed before storage (Table 4). Though very high and significant correlations were obtained between germination per cent after accelerated ageing (r = 0.949** to 0.967**), germination after dual stress test (r =

Table 3. Germination (%) in twelve seed lots of onion upon ambient storage

Lot No.	Initial	3 months	6 months	9 months	12 months	Mean
LI	79 (63)	76(61)	75(60)	70(57)	75(60)	75(60)
L2	71(57)	70(57)	68(55)	68(56)	68(56)	69(56)
L3	81(64)	77(61)	75(60)	74(59)	73(59)	76(61)
L4	92(74)	85(67)	84(66)	81(64)	80(63)	84(67)
L5	89(71)	85(67)	86(68)	76(61)	76(61)	82(65)
L6	92(74)	89(70)	87(68)	85(67)	85(67)	87(69)
L7	72(58)	77(61)	75(60)	70(57)	67(55)	72(58)
L8	71(57)	67(55)	63(52)	60(51)	60(51)	64(53)
L9	69(56)	66(54)	61(51)	60(51)	58(50)	63(52)
L10	30(33)	29(32)	29(32)	26(31)	20(27)	27(31)
L11	29(33)	26(30)	27(31)	20(27)	20(27)	24(30)
L12	44(42)	39(39)	39(38)	35(36)	30(33)	37(38)
Mean	68(57)	65(55)	64(54)	61(51)	59(51)	64(53)
CD (P=0.05)						
Storage period (SP)	0.63	Lots	0.98	SP x Lots	2.19	

Values in parenthesis indicate angular transformed values

Table 4. Correlation between germination after AA, DST and field emergence with germination per cent taken at three months interval in onion seed lots during storage

	Initial germination (%)	After AA germination (%)	DST germination (%)	Field emergence (%)
Initial germination(%)		0.967'**	0.921**	0.937**
After 3months germination (%)	0.991**	0.958**	0.923**	0.954**
After 6 months germination (%)	0.997**	0.958"*	0.918**	0.954**
After 9 months germination (%)	0.992**	0.963**	0.928**	0.949**
After 12 months germination (%)	0.994**	0.945**	0.915**	0.958**

*,**indicates significance at 5% and 1% respectively

0.915** to 0.928**) and germination taken at three months interval up to twelve months, the highest correlation was recorded with standard initial germination per cent (before storage) with germination after various periods of storage (r = 0.991** to 0.997**). Hence, indicating the actual germination (%), rather than the minimum prescribed standard, may be considered to indicate the vigour status.

Powell [19] reported that different seed lots of onion showed a marked variation in moisture content, ranging from 11.8 to 24.0 per cent after 24h of accelerated ageing (100% RH at 45°C), whereas, Matthews [20] proposed controlled deterioration for prediction of storage potential in commercial storage. In the present study also very high significant correlations were obtained between accelerated ageing (AA) and dual stress test (DST) with germination after 9 months and 12 months of storage as well as between DST, AA and controlled deterioration test (CDT) with field emergence (0.899**, 0.853** and 0.842**, respectively), though these were lower than the correlations with the standard germination test (0.937**).

The dual stress test (DST) was developed in this laboratory (Dadlani and Usha, unpublished) to differentiate the high vigour seed lots, based on their ability to germinate under stressful conditions of low oxygen availability (by using a small and tightly sealed container for germination) and high levels of volatile aldehydes, produced by germinating seeds and accumulated within the sealed container (Fig. 1). An indirect analysis of the levels of VAC (data not presented here), measured by the method of Wilson and McDonald [11] showed that seeds, having low germination released higher amounts of VAC trapped in the MBTH solution and measured spectrophotomeirically, compared to the high germination lots (Dadlani and Usha, unpublished). The volatile aldehydes released during seed ageing and accumulated in sealed containers or applied to germinating seeds are reported to inhibitor of germination and growth of the seedling [21, 22].

CONCLUSIONS

In onion, standard germination test is the best indicator of seed vigour, as highest significant correlations (P = 0.01) were obtained between initial germination (%) to determine field emergence (r = 8.937**) and storage potential (r = 0.994*). Accelerated ageing (AA) and dual stress (DS) tests were also highly correlated.

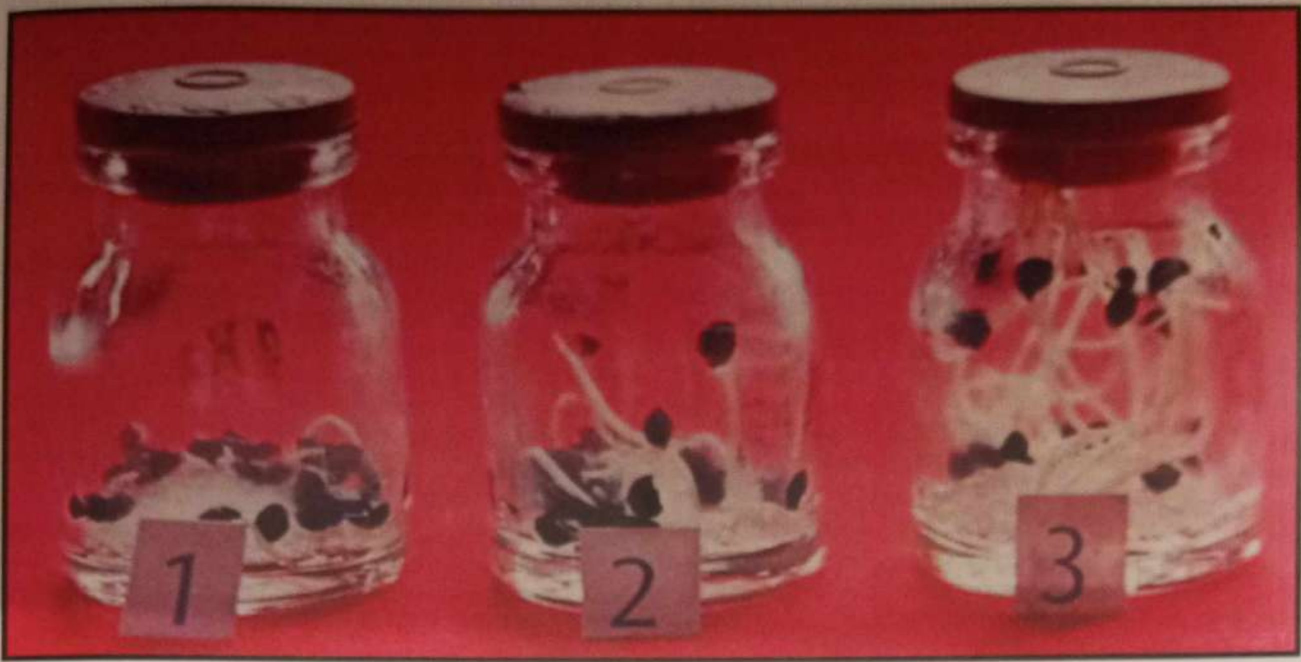

Fig. 1. Double stress test developed for assessing seed vigour in onion. Onion seeds put for germination in 5 ml bottles, 1= LV;2= MV & 3= HV seed lots

Fig. 2. Double stress test developed for assessing seed vigour in onion. Onion seedlings after 7 days of germination, 1,2 & 3 same as in Fig.1

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