

## Identification of the vigour test to predict planting value of chickpea seeds

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**ABSTRACT** The studies were conducted on various seed vigour parameters, including accelerated ageing test and electrical conductivity of seed leachate in relation to field emergence and storability of chickpea (*Cicer arietinum*). The materials for study constituted of two seed lots i.e. freshly harvested and four years old. Each lot consisted of two genotypes each from desi (Pusa 256 and Pusa 2028) and kabuli (Pusa 1053 and Pusa 1108) types. Inter-variety and inter-ageing differences were observed for the vigour parameters amongst the eight different chickpea seed lots. The results on each variety for various seed parameters and vigour tests revealed significant differences for test weight, seed coat weight, first count, germination per cent, abnormal seedling, vigour index I and vigour index II. The electrical conductivity values were found significantly high in old seed lot. Accelerated ageing test conducted at 40°C and 100% RH for period of 36 hrs gave significant differences between kabuli varieties. Electrical conductivity was significantly correlated with most of vigour parameters studied, while the accelerated ageing test and the first count also gave significant correlation with the field emergence. Thus, the electrical conductivity test could be suggested as 'The Vigour Test' for chickpea.

**Key words:** Chickpea, electrical conductivity, accelerated ageing, vigour index, field emergence

Chickpea (*Cicer arietinum* L.) belongs to genus *Cicer*, tribe Cicereae, family Fabaceae, and sub family Papilionacea. It originated in South Eastern Turkey [1]. The name *Cicer* is of Latin origin, derived from the greek word 'Kikun' meaning force or strength. However, there are two major constraints *viz.*, biotic stresses like fusarium wilt, pod borer etc. and abiotic stresses like drought, salinity, heat and cold, which hamper chickpea production. Besides, these constraints the viability of seed is adversely influenced by the viability of seed id adversely influenced by prolonged storage and ageing. In tropical and subtropical conditions, under high ambient relative humidity the seeds uptakes moisture from the environment, thus resulting in changes in genetic integrity, leading to deterioration of seeds. The rate of deterioration rapidly increases with increase in either seed moisture content or storage temperature [2]. The physiological and biochemical changes during seed ageing have been extensively reviewed [3-4].

Desi and kabuli chickpea seeds show large variability among genotypes in seed germination and seedling vigour. The results of standard germination test, which most farmers use to determine seeding rates, is performed under ideal laboratory conditions (e.g., optimum moisture, temperature, light, no soil microbes, etc). These conditions seldom occur in the field. The standard germination test has been designed to determine the highest germination potential of a seed lot under ideal conditions, but not to differentiate between seed lots based on their capabilities to perform better under unfavourable field conditions. This may explain the puzzle when several seed lots with similar germination percentages result in totally different plant stand in the field. Thus, vigour testing is being perceived as an important management tool and routine practice in many crop production programs, nowadays. There are no universally accepted vigour tests for all plant species.

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While, selecting choosing a vigour test, one should take into account the biology, physiology and other specific features of plant species. Wang *et al.* [5] reported that the controlled deterioration and electrical conductivity tests are more sensitive and accurate for indicating storability of lucerne (*Medicago sativa* L.) seed lots than the germination index and standard germination. The present investigation was aimed to investigate the loss of quality, viability and vigour either due to ageing or effect of adverse environmental factors in both desi and kabuli chickpea.

## MATERIALS AND METHODS

This study was carried out on four lots each of fresh and aged seeds of two varieties of both *Desi*: Pusa 256 and Pusa 2028 and *Kabuli*: Pusa 1053 and Pusa 1108 type of chickpea. The following observations were recorded on all the eight lots.

### *Test weight (g)*

A random sample from pure seed fraction was used for estimating 1000 seed weight. Four replicate of 1000 seeds were weighed. The mean value was expressed in grams.

### *Seed coat weight (g)*

Seed coat weight of 100 seeds was taken after soaking the seeds in water for 24 hr, followed by the seed coats, which were dried at  $70 \pm 1^\circ\text{C}$  for 48hr. It was measured in grams.

### *First count (%)*

The number of normal seedlings recorded on 5<sup>th</sup> day from the days to putting for germination. It was expressed in percentage [6].

### *Germination (%)*

Seeds from pure seed fraction were used for estimation of germination (%). The

germination test was conducted by between paper (BP) method. Eight replicates of 50 seeds each were tested by following ISTA method [6]. In this method, seeds were placed between two layers of wet germination paper which were then rolled and wrapped in a sheet of wax paper so as to keep the surface evaporation to minimum and placed in germinator in an upright position at  $20 \pm 1^\circ\text{C}$  and 95% RH for 8 days. finally, seeds were evaluated into normal seedlings, abnormal seedlings, hard and dead seeds and percentage of normal seedlings only were used to calculate standard germination (%).

### *Abnormal seedling (%)*

All the damaged, decayed and deformed seedlings, which were not able to produced normal seedlings were reported as abnormal seedlings. It was reported in percentage.

### *Total seedling length (cm)*

Ten normal seedlings were taken at random from each replication and root and shoot length of each seedling was measured in cm.

### *Seedling Dry weight (mg)*

Ten normal seedlings per replication which were selected at random for recording total seedling length were dried in a hot air oven maintained at  $70 \pm 1^\circ\text{C}$  for 48 hr and then cooled in desiccators. Seedling dry weight was then measured in mg/10 seedlings.

### *Vigour index*

The vigour indices were computed by using following formula [7]:

Vigour index I = Germination (%)  $\times$  Total seedling length (cm)

Vigour index II = Germination (%)  $\times$  Seedling dry weight (mg)

*Field Emergence (%)* Field emergence was estimated by sowing one hundred seeds in four replications in the field. Observations were recorded on alternate day till 30<sup>th</sup> day of sowing. The emergence was expressed as percentage of seedling emergence.

#### *Electrical conductivity*

Four replicates of 50 seeds, each were soaked in 250 ml of deionized water for 24 hr. Seed leachate was collected and conductivity was measured using an Elico conductivity bridge as  $\mu\text{mhoscm}^{-1}\text{g}^{-1}$  of seeds. EC of distilled water was taken as control. The conductivity per gram of seed weight for each replicate was calculated after accounting for the background conductivity of original water and average of the four replicates using the following formula:

$$\text{Conductivity } (\mu\text{mhoscm}^{-1}\text{g}^{-1}) = \frac{\text{Conductivity reading} - \text{Back ground reading}}{\text{Weight of Replicate (g)}}$$

#### *Accelerated ageing*

A total of 400 seeds from each lot were subjected to high humidity (RH 100%) and high temperature ( $40^{\circ}\text{C} \pm 0.3$ ) for 24, 48 and 72 hr. After the treatment, the seeds were air dried and put for germination test as per ISTA rules [6] and expressed as germination (%) after accelerated ageing.

#### *Statistical analysis*

The seeds were sown in IARI experimental farm, New Delhi and data collected from the field experiments were analysed in Randomized Block Design (RBD), while data from laboratory experiments were analysed in complete randomized design (CRD) as described by Panse and Sukhatme [8].

## RESULTS AND DISCUSSION

The data of varietal profiles for seed quality

and vigour tests has been given in table 1. The test weight of chickpea varieties of desi types *viz.*, Pusa 256 (21.80g), Pusa 2028 (19.89g), were statistically different from kabuli type *viz.*, Pusa 1053 (26.86g) and Pusa 1108 (28.34). However, there was no significant difference within Desi + Kabuli types. The seed coat weight of 100 seeds ranged from 0.997g (Pusa 1053; kabuli type) to 2.393g (Pusa 256; desi type). All the varieties included in the study were differing statistically for seed coat weight from each other. Seed coat weight of 100 seeds was invariably higher in desi types than kabuli types. First count ranged from 69 to 85 per cent over the varieties. Though kabuli and desi groups were statistically different with higher mean values in desi type (84.00%) than kabuli types (70.00%), there was no difference within kabuli types. Among all the varieties, Pusa 256 had highest (85.00%) and Pusa 1108 lowest (69%) first count value. The germination test data revealed that all the varieties had more than 90% germination. There were significant differences in germination percentage between desi and kabuli types. However, within desi and kabuli types, no significant differences in germination was observed. Maximum germination percentage (95%) was recorded in Pusa 256 (desi type) where as minimum (91%) was observed in Pusa 1108 (kabuli type). Kabuli types suffer more loss of vigour due to faster imbibition of water and pathogenic incidence due to cracking in large seeded varieties [9]. There was no significant difference observed for seedling length within desi and kabuli groups, but significant difference were recorded between desi types (20.9 cm) and kabuli types (17.1 cm). Seedling dry weight varied from 528 g in Pusa 256 to 669 g in Pusa 1108.

**Table 1: Mean values of various vigour parameters of chickpea genotypes.**

Types	Varieties	TW	SCW	FC%	Ger %	SL	SDW	VI-I	VI-II	FE%
Desi	Pusa 256	21.80	2.939	85.00 (67.26)	95.00 (77.18)	20.4	528	1937	50203	91.00
	Pusa 2028	19.89	2.601	82.00 (64.96)	93.00 (74.74)	21.5	570	1999	52965	91.00
	Mean	20.85	2.770	84.00 (66.46)	94.00 (75.86)	20.9	549	1968	51584	91.00
Kabuli	Pusa 1053	26.86	0.997	71.00 (57.45)	92.00 (73.21)	16.9	645	1554	59340	87.00
	Pusa 1108	28.34	1.199	69.00 (56.20)	91.00 (72.60)	17.3	669	1569	60890	89.00
	Mean	27.60	1.098	70.00 (56.82)	91.05 (73.09)	17.1	657	1562	60115	88.00
	Grand Mean	24.22	1.934	77.00 (61.37)	92.75 (74.53)	19.3	614	1780	56540	89.50
CD (p=0.05%) for Varieties		3.60	0.079	2.26	2.18	1.33	10.2	146	1571	NS

TW: Test weight, SCW: Seed coat weight, FC%: First count percentage, Ger %: Germination percentage, SL: Seedling length, SDW: Seedling dry weight, VI-I: Vigour index I, VI-II: Vigour index II and FE% = Field emergence percentage.

There was significant difference in dry weight of the seedlings not only between the desi and kabuli types but also within each types. The mean data observed for vigour index indicated significant differences between desi (1968) and kabuli (1562) types; desi type being more vigorous than kabuli type. However, within each group, the varieties were at par for vigour index I. The score for vigour index II was maximum (60890) in Pusa 1108 (kabuli type) and the group differed significantly from each other. However, within each group, no statistical differences were observed for vigour index II. All the varieties were at par for field emergence data, though the value varied from 87 % in Pusa 1053 to 91% in Pusa 256 and Pusa 2028. Seed quality evaluation can be conducted by physical and physiological vigour tests [10] that provide information on the potential behavior of a seed lot under field conditions.

Data for germination percentage after accelerated ageing in the four varieties studied are presented in table 2. Accelerated

ageing led to the rapid loss of germination in kabuli than in desi type. In desi type, 66 % germination was observed even after ageing for 48 hours at 40°C and 100 % relative humidity. The differences between two types, however, narrowed down after 72 hours with desi and kabuli groups, but significant value for germination being 48.6 % in desi and 31.88 % in kabuli type. Statistically, no difference in germination was observed under the conditions of accelerated ageing within each group. The accelerated ageing led to the decline in all varieties but it was highest in variety Pusa 1053 of kabuli type. This decline was much higher in kabuli types as a whole and Pusa 1053 in particular (from 92.00% to 31.25%) after 72 hr of accelerated ageing. More reduction in germination after accelerated aging (AA) in kabuli type was again may be due to cracking of thin seed coat and rapid water absorption of moisture. Traditional vigour tests, such as the AA test, were developed especially to determine storage potential [11].

**Table 2: Standard germination (%) of chickpea genotypes after accelerated ageing tests.**

Treatments	GAA1	GAA2	GAA3	Mean	
Group	Variety				
Desi	Pusa 256	75.75 (60.53)	66.50 (54.66)	49.00 (44.45)	63.75 (53.21)
	Pusa 2028	75.25 (60.20)	65.75 (54.21)	48.25 (44.02)	63.08 (52.81)
	Mean	75.50 (60.36)	66.13 (54.44)	48.62 (44.23)	63.42 (53.01)
Kabuli	Pusa 1053	55.75 (48.33)	43.00 (41.00)	31.25 (34.01)	43.33 (41.11)
	Pusa 1108	58.75 (50.06)	45.00 (42.15)	32.55 (34.80)	45.43 (42.34)
	Mean	57.25 (49.19)	44.00 (41.58)	31.88 (34.39)	44.38 (41.72)
Grand Mean		66.34 (54.56)	55.06 (47.93)	40.25 (39.40)	53.88 (47.30)
CD (p=0.05%)	Treatment :	1.76	Variety :	1.19	
	Group :	1.09	Interaction(TXV) :	2.89	

GAA1: Standard Germination (%) after accelerated ageing (36hrs), GAA2: Standard Germination (%) after accelerated ageing (48 hrs) and GAA3: Standard Germination (%) after accelerated ageing (72 hrs).

The seed quality of both fresh and old seed lots as measured by EC of seed leachate (Fig. 1, 2 and 3) revealed that EC values for fresh seed lot were significantly lower before and after storage than old seed lots. Mean EC value in fresh seed lot before storage was 11.148  $\mu\text{mhos/cm/g}$  in comparison to 17.145  $\mu\text{mhos/cm/g}$  of old seed lot (Table 3). After 6 months of storage, no significant increase in EC value was observed in fresh seed lot, but in old seed lot it increased significantly and reached at 16.188  $\mu\text{mhos/cm/g}$ . Pusa 1053 and Pusa 1108 showed higher value of EC than Pusa 256 and Pusa 2028 and grouping was as per the type of gram. Conductivity test measure leakage of electrolytes from seed and can be used as a vigour test to predict field emergence. This test becomes an important approach for monitoring large seeded legume seed quality [12]. Degree of seed leakage during imbibition is influenced by stage of seed maturation, degree of seed ageing and incidence of damage [13]. Kabuli types are more prone to deterioration in loss of semi permeability of membrane resulting in higher leakage of ions in aged seed. Increased membrane permeability resulting in an increased EC of seed leachate has been reported in different crops with ageing [14-17].

The values of correlation coefficient between the different parameters are presented in table 4. The perusal of data indicates that field emergence was significantly correlated with the germination percentage after all the three stages of accelerated ageing, first count and electrical conductivity.

However, no significant correlation was found between field emergence and seedling length, seedling dry weight and both vigour indices. The maximum value of correlation coefficient ( $r=0.562$ ) of field emergence was observed with germination per cent after accelerated ageing for 48 hr at 40°C and 100% RH. Electrical conductivity showed significant negative correlation with all the parameters studied, except speed of emergence and germination per cent. Dahiya [18] also reported that speed of germination, 1000 seed weight and AA test gave good indication about plant establishment. The above results therefore, confirm the utility of AA, first count, standard germination and electrical conductivity as a indicators for assessing the quality of seed. Germination test alone does not reflect the potential of the seed lot in field conditions. Thus, use of these as vigour tests helps in predicting the

**Table 3: Variation in electrical conductance ( $\mu\text{mhos/cm/g}$ ) of chickpea genotype lots before and after storage**

Storage Period		EC( $\mu\text{mhos/cm/g}$ )				
Initial	Group	Varieties	Fresh lot	Old lot	Mean	
6 Month	Desi	Pusa-256	9.274	11.350	10.312	
		Pusa2028	7.156	11.273	9.215	
		Mean	8.215	11.312		
	Kabuli	Pusa1053	16.622	19.498	18.060	
		Pusa-1108	11.540	14.792	13.166	
		Mean	14.081	17.145		
		Mean over varieties	11.148	14.228		
	Desi	Pusa-256	Pusa-256	9.347	17.252	13.299
			Pusa2028	7.2230	12.089	9.656
			Mean	8.288	14.671	
		Kabuli	Pusa1053	17.252	19.768	18.510
			Pusa-1108	12.089	15.639	13.864
			Mean	14.671	17.704	
			Mean over varieties	11.479	16.188	
Mean over storage period			11.314	15.208		
CD (p=0.05%)						
Storage Period with in lot :			2.231	Group :		0.456
Varieties :			0.473	Lot :		1.462
Interaction :			2.674			

performance of seeds under field conditions and can be efficiently used as alternative to field emergence. Similar results were reported by Verma *et al.* [19]; Singhabumrung and Juntakool [20]. Hence, it is suggested that the electrical conductivity test is recommended as the vigour test for chickpea to carry the benefits of the crop. Chickpea is an environment friendly crop and sustains soil productivity. The results have shown there is clearly faster deterioration of quality in kabuli gram than in desi gram. Moreover, desi type has shown very good germination and other vigour parameter, the same may be introgressed in the kabuli type through intensive and selective breeding efforts.

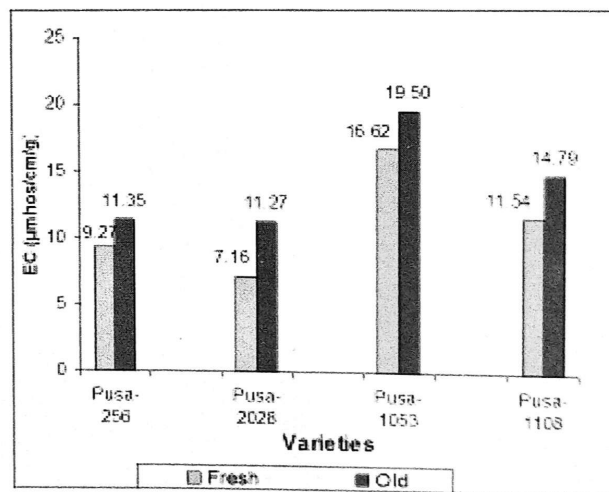


Fig. 1. Frequency distribution of electrical conductivity ( $\mu\text{mhoscm}^{-1}\text{g}^{-1}$ ) of fresh and 4yr old lot of chickpea before storage

Table 4: Correlation matrix of vigour parameters in chickpea

Characters	1	2	3	4	5	6	7	8	9	10	11	12
1 Germination (%) after A.1	1.000											
2 Germination (%) after A.2	0.984**	1.000										
3 Germination (%) after A.3	0.994**	0.979**	1.000									
4 First count	0.760**	0.775**	0.754**	1.000								
5 Germination (%) seedling dry weight	0.573*	0.588*	0.567*	0.631**	1.000							
6	0.916**	0.924**	0.931**	0.852**	0.687**	1.000						
7 Seedling length	0.904**	0.924**	0.909**	0.744**	0.566*	0.901**	1.000					
8 VI-I	0.902**	0.928**	0.907**	0.806**	0.594*	0.925**	0.990**	1.000				
9 VI-II	0.902**	0.894**	0.920**	0.774**	0.679**	0.967**	0.867**	0.863**	1.000			
10 Field Emergence	0.559*	0.562*	0.533*	0.500*	0.480*	0.485	0.383	0.405	0.438	1.000		
11 Speed of emergence	-0.196	-0.174	-0.234	0.034	0.241	0.140	0.276	-0.243	-0.115	-0.185	1.000	
12 Electrical conductivity	-0.870**	-0.840**	-0.839**	-0.551*	-0.371	-0.636**	-0.771**	-0.740**	-0.633**	-0.501*	-0.122	1.000

AA1-Accelerated ageing (36hr), AA2-Accelerated ageing (48hr), AA3-Accelerated ageing (72hr)  
 \*\*Correlation is significant at 1%  
 \*Correlation is significant at 5%

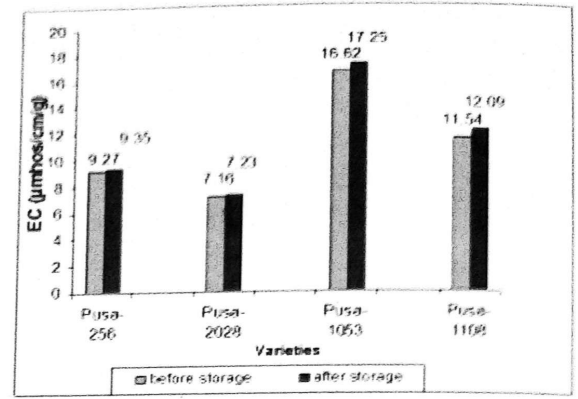


Fig. 2. Frequency distribution of electrical conductivity ( $\mu\text{mhos cm}^{-1} \text{g}^{-1}$ ) of Fresh lot of chickpea before and after storage

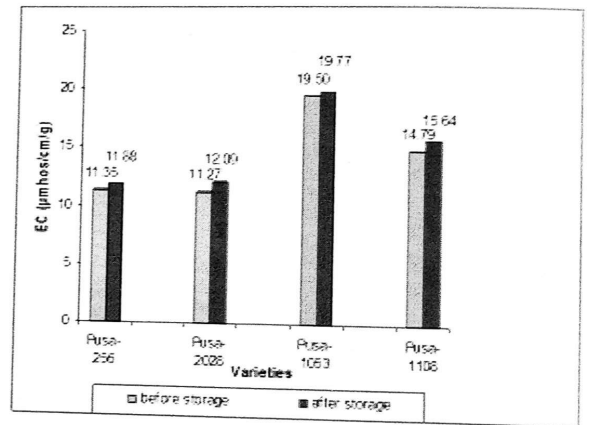


Fig. 3. Frequency distribution of electrical conductivity ( $\mu\text{mhos cm}^{-1} \text{g}^{-1}$ ) of Fresh lot of chickpea before and after storage

REFERENCES

- LADIZINSKY G (1975). A new *Cicer* from Turkey. Notes from the Royal Botanic Garden, Edinburgh, **34**: 201 -2.
- ELLIS RH, HONG TD AND ROBERTS EH (1985). Handbook of Seed Technologies for Gene Banks: Principle and Methodology, Vol. 1. IBPGR, Rome, pp: 518-37.
- MCDONALD MB (1999). Seed deterioration: Physiology, repair and assessment. *Seed Sci Technol*, **27**: 177-237.

4. JATOI SA, AFZAL M, NASIM S AND ANWAR R (2001). Seed deterioration study in pea, using accelerated ageing techniques. *Pak. J Biol Sci*, **4**: 1490-4.
5. WANG YR, YU L, CHEN JH (1992). The relationship between standard germination, vigour, moisture content and storability in *Medicago sativa* seed lots (Chinese with English Abstract). *Pratacul-tural Sci*, **6**: 34-8.
6. ISTA, 2004. *International Rules for Seed Testing*. Published by International Seed Testing Association, Zurichstr. 50, CH-8303 Bassersdorf, Switzerland
7. ABDUL-BAKI BS AND ANDERSON JD (1973). Vigour determination in soybean seed by multiple criteria. *Crop Sci*, **13**: 630-3.
8. PANSE VG AND SUKHATME PV (1985). *Statistical Methods for Agricultural Workers*, Indian Council of Agricultural Research, New Delhi.
9. YADAV SP AND SHARMA SP (2001). Seed coat cracking and its effect on seed quality characteristics in Kabuli gram (*Cicer arietinum* L.). *Seed Res*, **29**(1): 7-12.
10. MCDONALD MB (1995). Standardization of seed vigour tests, in *Seed vigour Testing*. (Ed. H.A. Van De Venter, Published by International Seed Testing Association, Zurich, 88-97.
11. HAMPTON J (1993). The ISTA perspective of seed vigour testing. *J Seed Technol*, **17**: 105-9.
12. HAMPTON J AND TEKRONY DM (1995). *Handbook of vigour test methods*, (Ed.) Published by International Seed Testing Association, Zurich, 117.
13. POWELL AA (1995). The controlled deterioration test, in *Seed Vigour Testing*. (Ed. H.A. Van De Venter, Published by International Seed Testing Association, Zurich, 73-87.
14. DADLANI M AND AGRAWAL PK (1983). Mechanism of soybean seed deterioration. *Pl Physiol Biochem*, **10**: 23-30.
15. FERGUSON JM (1993). AOSA perspective of seed vigour testing. *J Seed Technol*, **17**: 101-4.
16. YAKLICH RW, KULIK MM AND ANDERSON JD (1979). Evaluation of vigor tests in soybean seeds: relationship of ATP, conductivity, and radioactive tracer multiple criteria tests to field performance. *Crop Sci*, **19**, 806-10.
17. SINGH KK AND DADLANI M (2003). Effect of packaging on vigour and viability of soybean [*Glycine max* (L.) Merrill] seed during ambient storage. *Seed Res*, **31**: 27-32.
18. DAHIYA OS (1992). Seed viability, vigour and genetics of speed of germination in chickpea (*Cicer arietinum* L.) Ph.D. thesis, CCS, HAU, Hisar India, 86 p.
19. Verma SS, Verma U, Dahiya OS and Punia RC (1998). Comparative seed vigour studies in wheat. *Crop Res*, **16**(2): 235-8.
20. SINGHABUMRUNG V AND JUNTAKOOL S (2004). Vigour test results for prediction of field emergence for sweet corn. pp. 291-299. In *Proceeding in the 42nd Kasetsat University Annual Conference*, Available source: [www.lib.ku.ac.th/KUCONF/KC4201036.pdf](http://www.lib.ku.ac.th/KUCONF/KC4201036.pdf).