ACTA AGROBOTANICA
Vol. 54, z. 2 – 2001
s. 5–17

Use of tetrazolium (TTC), Germ's and greenhouse plant emergences methods for testing seed vigour of selected ornamental plant species

ROMAN HOŁUBOWICZ, XIAN WANG[1], WIESŁAW SIUTAJ

Department of Seed Science and Technology, Faculty of Horticulture, August Cieszkowski Poznań Agricultural University, Baranowo, 62-081 Przeźmierowo, Poland
1) Graduate student, present address: Beijing Academy of Agriculture and Forest Sciences, Beijing Vegetable Research Centre, Beijing Cun, West Suburb, 100081 Beijing, P.R.China

(Received: 6.04.2001)

Summary

In the years 1996-1997 the experiments were carried out on methods to investigate seed vigour of tassel flower (*Amaranthus caudatus* L.), sand pink (*Dianthus chinensis* L.), babies' breath (*Gypsophila elegans* M.B.), sweet pea (*Lathyrus odorathus* L.), African marigold (*Tagetes erecta* L.) and zinnia (*Zinnia elegans* Jasq.). The main goals of this research were to specify conditions for accelerated ageing (AA) of the seeds of a few selected ornamental plant species and to choose the most appropriate methods for their seed vigour evaluation in the laboratory and greenhouse conditions. All used in the experiments seeds came from the commercial seed lots from Polish seed company. Evaluation was carried out on the seed samples with high and low vigour. The latter ones were received through subjecting the seed samples to AA, i.e. by placing them in 100% relative humidity (RH) at 44°C, except African marigold – at 42°C, in the darkness and keeping them for 144, 88, 100, 48, 72 and 72 hours, respectively. The tested seed vigour estimated methods included the Germ' s method, the 2,3,5-triphenyl tetrazoilum chloride (TTC) method and the test of plant emergences in the greenhouse. The high vigour seeds samples were used as a check. The Germ' s method was found to be useful to evaluate sand pink, babies' breath and African marigold seed vigour, whereas the TTC method was found to be suitable for vigour evaluation of sand pink, babies' breath and zinnia. At present stage of our knowledge about seed vigour, the plant emergences in the greenhouse method was found to be the

best for evaluation of seed vigour of tassel flower, sand pink, babies' breath, sweet pea and zinnia. It is reasonable to combine a few methods of seed vigour evaluation for ornamental plant species.

Key words: seed vigour, tassel flower, sand pink, babies' breath, sweet pea African marigold, zinnia, *Amaranthus caudatus, Dianthus chinensis, Gypsophila elegans, Lathyrus odorathus, Tagetes erecta, Zinnia elegans,* tetrazolium method, Germ's method, accelerated ageing test

Abbreviations:
AA -accelerated ageing, RH -relative humidity, TTC - 2,3,5-triphenyl tetrazolium chloride, ISTA - International Seed Testing Association

INTRODUCTION

In the routine seed testing there has been constant search for additional, more precise parameters for evaluating seed quality. One of them could be seed vigour. The percentage of American seed laboratories conducting vigour tests has increased from 52 in 1978 to 75 in 1990 (C o p e l a n d, M c D o n a l d, 1995). Out of many tested seed vigour estimation methods, the conductivity and accelerated ageing (AA) methods seem to get the biggest attention from the seed analysts. Despite the already known methods, there has been also a continuous search for new methods based on biological markers, low molecular weight heat-shock proteins and ethylene production (H a l m e r, 2000; G r o o t et al., 2000).

Fay et al. (1993) reported that bulk conductivity and AA tests can be used to evaluate the seed quality of *Rudbeckia fulgida* and other herbaceous perennial seeds. K u o et al. (1996) successfully used the TTC test to determine the viability of *Salvia splendens* and *Salvia farinacea* seeds. Previous research by H o ł u b o w i c z et al. (1997) has shown that gold poppy, China aster, garden balsam and stock gillyflower seed vigour can be measured using the Germ' s, TTC or emergences in the greenhouse methods.

The main objective of this study was to seek, if these three methods can be also used to evaluate seed vigour of other ornamental plant species.

MATERIALS AND METHODS

Seeds of six ornamental plants species were used in the experiments. All of them came from commercial seed lots from the Polish seed company „Torseed" S.A. located in Toruń (Northern Poland). The 6 tested species were: tassel flower (*Amaranthus caudatus* L.), sand pink (*Dianthus chinensis* L.), babies' breath (*Gypsophila elegans* M.B.), sweet pea (*Lathyrus odorathus* L.), African marigold (*Tagetes erecta* L.) and zinnia (*Zinnia elegans* Jasq.). Their seed quality met Polish standard criteria for ornamental plants (A n o n., 1990).

The (AA) test. A single layer of seeds was placed on a gauze screen tied up with a small basket and put into a glass container over 40 ml of distilled water. Then, the containers with the seeds were put into a thermostat at 42°C or 44°C ± 1°C and 100% relative humidity (RH) in the darkness for 48, 72, 88, 100 and 144 hours. Neither temperature of the AA test nor the time needed for lowering the seeds' germination capacity were known before starting the experiments. After exposing seeds to AA, they were put on the routine germination test following ISTA rules (A n o n., 1979). The used in the experiments seeds with low vigour were received by placing the commercial seeds at 100% RH at 44°C, except African marigold – at 42°C, in the darkness and keeping them for 144, 88, 100, 48, 72 and 72 hours, respectively.

Vigour estimation. Seeds of the investigated plant species of low vigour were received by subjecting them to the AA. The high vigour seeds were the check. Seed vigour in the laboratory was investigated by two methods: measuring seed viability (TTC) and by the Germ's method, whereas in the greenhouse – by evaluating the plant emergences. In the Germ's method, seeds were treated as described by P e r r y (1987) and H o ł u b o w i c z et al. (1997). Both high and low vigour seeds of each species were run in 4 replications and each of them consisted of 25 seeds. All the paper blotter tubes with the seeds were placed vertically in a bucket with water and were put in the darkness at 20°C. After 14 days, the tubes were unrolled and germs were counted. Ten seedlings of each species, which had the largest root length in each replication, were evaluated and their selected characters - measured. These were: the length of hypocotyl, root and whole seedling as well as fresh and dry weights of 1 plant. The dry weights were described after drying the plants for 2 hours at 102 ± 1°C.

The TTC test. For each of the tested species, 3 replications, 50 seeds each, were used for both high and low vigour seeds evaluation. The used method followed the TTC ISTA instructions (Anon., 1985). Seeds were first soaked in water of the room temperature for 18 hours, then prepared for staining. They were then processed either by cutting or puncturing them (Fig. 1). Then, the seeds were soaked in 1.0% solution of TTC at 30°C for 24 hours, except tassel flower seeds, which were soaked at 34°C. After that, seeds were evaluated to distinguish the number of alive and dead seeds.

Plant emergences in the greenhouse. All seed samples of high and low vigour were sown in the greenhouse at 15/20°C. They were tested in 4 replications, each of 50 seeds. Plastic trays of 39.5 cm x 19.5 cm were filled with peat and sand, mixed at the volume ratio of 3: 1 and then the seeds were sown. Since first emerged plant, seedlings were counted every day at the same time until no new seedling emerged.

Statistical data analysis. The statistical calculations were done separately for each of the tested species. All received data were analysed calculating variance and significant differences by using the Duncan' s range test at 5% level of significance.

RESULTS

The AA test. Results received from the standard germination tests had indicated that the seed germination capacities of 6 tested species, which were used as high vigour seeds, ranged from 77.7% to 94.6% (Table 1). After the AA, in different conditions, depended upon the tested species, seeds' vigour was generally lowered. This

affected the seeds germination capacities, which in all of the species decreased significantly. These seeds, which were effectively processed through the AA, had lower vigour than the check seeds (Table 1).

Table 1. Mean germination capacities (%) of the 6 ornamental plant species with high (H) and low (L) seed vigour. The low vigour seeds were first subjected to the AA at 100% RH, at 44°C (African marigold – 42°C) for 144, 88, 100, 48, 72 and 72 hours respectively

Seed vigour	Species					
	tassel flower****	sand pink	babies' breath	sweet pea	African marigold	zinnia
H*	94.6 b ***	87.3 b	90.3 b	92.0 b	77.7 b	80.6 b
L**	88.6 a	48.0 a	44.2 a	76.3 a	35.1 a	34.7 a

* H – high vigour seeds i.e. the check seeds.

** L – low vigour seed, i.e. seeds subjected to the AA.

*** means followed by same letters are not significantly different at $\alpha = 0.05$ level by Duncan' s range test.

**** the statistical calculations were done separately for each of the tested species.

Vigour estimation. Out of three tested seed vigour estimation methods, none of them was found to be definitely better than others.

The Germ's method. The carried out experiments showed that in 5 species, out of 6 tested, there were significant differences in their seedlings characters between the plants received from the seeds of various vigour (Phot. 1). The low vigour seeds of African marigold and sand pink gave seedlings with shorter hypocotyls than high vigour seeds (Table 2). The seedlings received from low vigour seeds of babies' breath, sweet pea and zinnia developed shorter roots than the seedlings received from high vigour seeds, whereas seedlings grown out from low vigour seeds of sand pink, babies' breath, African marigold and zinnia were shorter than those received from high vigour seeds. Seedlings grown out from low vigour seeds of sand pink, babies' breath, sweet pea and African marigold had lower mean fresh weight of one plant than the seedlings from the high vigour seeds. The seedlings grown out from low vigour seeds of sand pink and African marigold had lower mean dry weight of one plant than those grown out from high vigour seeds (Table 2). The received results showed that these differences were in varying and large degree dependent on the tested species. For tassel flower, no differences in characters of the seedlings received from high and low vigour seeds were found. The biggest differences in characters of seedlings received from the seeds of high and low vigour were found for sand pink and African marigold (Table 2).

The TTC method. The carried out experiments showed a relationship between the viability of seeds of 4 (out of 6 tested) species and their seed vigour (Table 3). For sand pink, babies' breath, African marigold and zinnia seeds, the lowering of seed vigour was accompanied by significant decrease in the number of alive seeds and at the same time – the significant increase in the number of dead seeds. The percentage of alive seeds, in the high vigour seeds, varied from 65.5 to 94.1, whereas the percentage of dead seeds – from 5.9 to 34.5. The percentage of alive seeds, in the low vigour seeds, varied from 0.2 to 91.1, whereas the percentage of dead seeds – from 8.9 to 99.8

Table 2. Effect of high (H) and low (L) seed vigour of 6 ornamental plant species on their selected plant characters in the Germ's test

Characters	seed vigour	Species					
		tassel flower****	sand pink	babies' breath	sweet pea	African marigold	zinnia
Mean length of hypocotyls (cm)	H *	4.73 a***	2.30 b	4.52 a	19.15 a	5.87 b	7.29 a
	L **	4.49 a	1.81 a	3.68 a	19.46 a	4.70 a	6.98 a
Mean length of root (cm)	H	2.94 a	3.37 a	6.25 b	15.86 b	6.29 a	7.08 b
	L	2.93 a	2.80 a	5.17 a	12.58 a	5.81 a	5.20 a
Mean length of whole seedling (cm)	H	7.67 a	5.67 b	10.76 b	38.84 a	12.20 b	14.37 b
	L	7.35 a	3.88 a	9.03 a	32.38 a	10.51 a	12.18 a
Mean fresh weight of 1 plant (g)	H	0.0075 a	0.0098 b	0.0088 b	0.4708 b	0.0347 b	0.0802 a
	L	0.0071 a	0.0051 a	0.0045 a	0.3585 a	0.0229 a	0.0740 a
Mean dry weight of 1 plant (g)	H	0.0003 a	0.0006 b	0.0005 a	0.0514 a	0.0015 b	0.0026 a
	L	0.0003 a	0.0003 a	0.0005 a	0.0539 a	0.0011 a	0.0091 a

*, **, ***, **** – for explanation see Table 1

Table 3. Effect of seed vigour of 6 species of ornamental plants on their viability (%) measured by the TTC method

Species	Time of soaking (h)	Time of staining (h)	Temperature of staining (°C)	Seed preparation method	Seed vigour	Seed viability	
						alive seeds	dead seeds
tassel flower****	18	24	34	puncturing	H *	80.2 a ***	19.8 a
					L **	85.6 a	14.4 a
sand pink	18	24	30	puncturing	H	83.7 b	16.3 a
					L	56.7 a	43.3 b
babies' breath	18	24	30	puncturing	H	88.7 b	11.3 a
					L	62.0 a	38.0 b
sweet pea	18	24	30	cutting	H	94.1 a	5.9 a
					L	91.1 a	8.9 a
African marigold	18	24	30	cutting	H	65.5 b	34.5 a
					L	0.2 a	99.8 b
zinnia	18	24	30	cutting	H	92.1 b	7.9 a
					L	47.3 a	52.7 b

*, **, ***, **** – for explanation see Table 1

Table 4. Effect of seed vigour of 6 ornamental plants species on their plant emergences in greenhouse

Species	Seed vigour	Following days of measurements													
		3	4	5	6	7	8	9	10	11	12	13	14	15	16
tassel	H *	0.0 a***	11.8 a	58.6 b	82.1 b	87.9 b	88.3 b	88.3 b	88.7 b						
flower****	L **	0.0 a	0.5 a	14.5 a	60.8 a	64.7 a	65.2 a	65.2 a	65.2 a						
sand	H	0.0 a	0.0 a	13.1 b	51.1 b	71.8 b	76.4 b	79.2 b	81.2 b	81.2 b	81.2 b	81.2 b	81.2 b	81.2 b	81.2 b
pink	L	0.0 a	0.0 a	0.5 a	18.7 a	24.6 a	27.7 a	28.2 a	30.3 a	31.9 a	31.9 a	32.4 a	32.4 a	33.0 a	34.4 a
babies'	H	0.0 a	0.5 a	4.8 b	34.4 b	55.6 b	67.1 b	71.6 a	74.0 b	75.6 b	75.6 b	75.6 b			
breath	L	0.0 a	0.0 a	0.1 a	5.2 a	29.0 a	47.4 a	53.0 b	54.0 a	55.1 a	55.1 a	55.6 a			
sweet	H	0.0 a	11.3 b	50.5 b	79.4 b	83.3 b	84.3 b	84.8 b	85.4 b	86.8 b	87.5 b	87.5 b	87.5 b		
pea	L	0.0 a	2.8 a	26.3 a	51.5 a	54.0 a	56.0 a	58.0 a	58.6 a	60.6 a	60.6 a	61.1 a	63.3 a		
African	H	0.0 a	0.6 a	20.8 a	51.7 a	62.7 a	66.4 a	70.3 a	71.3 a	72.5 a	72.5 a	73.0 a			
marigold	L	0.0 a	0.6 a	21.6 a	43.8 a	51.5 a	56.2 b	56.2 a	56.2 a	59.7 a	59.7 a	59.7 a			
zinnia	H	2.2 b	14.2 a	39.0 b	56.0 a	63.6 b	68.2 b	71.2 b	74.3 b	74.3 b	74.8 b	74.8 b	75.3 b	75.3 b	
	L	0.0 a	4.4 a	23.6 a	40.3 a	47.0 a	49.0 a	51.0 a	51.0 a	52.0 a	52.0 a	52.0 a	52.0 a	53.0 a	

*, **, *** , **** – for explanation see Table 1