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Seed viability and vigour in naturally aged seeds of coriander (*Coriandrum sativum*)

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ABSTRACT

In the present investigation, three seed lots of eight varieties/genotypes, viz. DH 224, DH 226, DH 242, DH 259, DH 294, DH 296 (advance genotypes) and DH 228 (Hisar Bhoomit) and Hisar Anand (released varieties) of coriander were subjected to study the effect of natural ageing on different seed quality parameters. All the seed lots were analyzed for standard germination test (%), root length (cm), shoot length (cm), seedling dry weight (mg), seedling vigour index-I, seedling vigour index-II, accelerated ageing test (%), electrical conductivity (mS/cm/seed) of seed leachates, seed density (g/cc), dehydrogenase activity test (OD/g/mL), field emergence index and field establishment (%). Results revealed that all the varieties/genotypes showed the germination percentage above the Minimum Seed Certification Standards (60%) in Lot-A (½ year old seed) and Lot-B (1½ years old seed). Standard germination (%), seedling length (cm), seedling dry weight (mg), seedling vigour index-I &II, accelerated ageing test (%), dehydrogenase activity test (OD/g/mL), field emergence index and field establishment (%) decreased significantly and progressively as the ageing period increased. The electrical conductivity was negatively and significantly correlated with all seed viability and vigour parameters. Results also revealed that viability and vigour of seeds declined with faster rate in Lot-C (2½ years old seed). Among all the varieties/genotypes, genotypes DH 224 and DH 228 were found most promising for various parameters of viability and vigour.

Key words: Ageing, Coriander, Dehydrogenase activity, Electrical conductivity, Viability, Vigour

Sidhawani (1991) reported that quality seed is prerequisite to enhance the production and productivity in coriander (*Coriandrum sativum* L.). It has been demonstrated to realize that use of quality seeds increased productivity of crop by 15-20%. Rapid deterioration of stored seed is a serious problem in tropical and sub tropical countries like India where high temperature and high relative humidity greatly accelerate the seed ageing phenomenon. Among seed spices, the quality of seed is more important because of its high value. Accelerated ageing of seeds over several days of exposure to high temperature and high humidity has been recognized as good predictor of seed storability (Chiu *et al*. 1995). The performance capabilities of many seeds deteriorate during prolonged storage, but the rate of deterioration varies greatly among species (Priestly 1986, Roberts 1989). This ageing or loss of vigor is evidenced by delayed germination and emergence, slower growth, increased susceptibility to stress, and ultimately a decline in germinability (Douglas 1975, Woodstock 1973). Accelerated seed ageing technique is a widely used tool to test the seed quality. The ageing test of seed vigor can give better indications of probable field emergence for vegetable crop seeds than germination and growth tests (Pandey *et al*. 1990). High temperature, ambient relative humidity, and seed moisture content are the main factors influencing seed storage capability (Abdul-Baki 1980). However, the rate at which seeds age depends upon their physiological status, their genetic constitution and the storage conditions. The availability of an adequate supply of crop seeds of a uniform high quality is essential for a successful seed industry and the maintenance of a viable and productive agriculture (Barens 1986). It was found that plants from high quality seed produced 18% higher yield than those obtained from low quality seed (Khadi 2006). Availability of viable and vigorous seeds at planting time is important for achieving targets of agricultural production because quality seeds act as a catalyst for realizing the potential of other inputs.

Keeping in view the importance of this crop, it is very important to determine the quality of seed of this crop as seed deteriorates with passage of time or during the period of prolonged storage. Higher the moisture content of the seeds and higher the temperature at which they are stored, sooner the viability is lost (Abba and Lovato 1999). As seeds aged, they come to germinate more slowly than fresh seeds, respire slower and become more susceptible to disease,

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chromosomal abnormalities and increased proportion of abnormal seedlings. Coriander being one of the most important spices crop at International level, the information on the physiological and biochemical basis of seed viability and vigour is fragmentary and incomplete and still has contradictions, errors and difference of opinion. Hence, the present investigation was carried out to study the various seed lots of coriander for different seed quality parameters.

MATERIALS AND METHODS

In the present investigation, three seed lots, viz. Lot-A (½ year old seed), Lot-B (1½ years old seed) and Lot-C (2½ years old seed) of eight varieties/genotypes of coriander, viz. DH 224, DH 226, DH 242, DH 259, DH 294, DH 296 (advance genotypes) and DH 228 (Hisar Bhoomit) and Hisar Anand (released varieties) were collected and present study was conducted at the Department of Seed Science and Technology, College of Agriculture, CCS Haryana Agriculture University, Hisar, India during 2010 and the following observations were recorded.

Hundred seeds of all the three lots of eight varieties/genotypes (6 genotypes namely, DH 224, DH 226, DH 242, DH 259, DH 294, DH 296 and two varieties, i.e. DH 228 (Hisar Bhoomit) and Hisar Anand were placed in between sufficient moistened rolled towel papers in three replicates and kept at 25^0C in seed germinator. The final count was taken on 21st day and only normal seedlings were considered for percent germination as per rules of International Seed Testing Association (ISTA 2001).

Root and shoot length was measured on five randomly selected normal seedlings taken from three replications of standard germination test and recorded in centimeter. At last average of five seedlings was taken for final calculation. All the five normal seedlings selected for measuring seedling length were further kept in hot air oven for taking dry weight. These are dried at 80°C for 48 hr and then seedling dry weight was recorded in milligram. At last average weight of five seedlings was taken for further calculations.

Seedling vigor indices were calculated according to the formulae suggested by Abdul-Baki and Anderson (1973).

Seedling Vigor Index-I = Standard germination (%)×Seedling length (cm)

Seedling Vigor Index-II = Standard germination (%)×Seedling dry weight (mg)

To observe the optimum time for accelerated ageing test (i.e. period of reduction of initial germination up to 50%), fresh seeds (½ year old) of all the varieties/genotypes were placed in single layer on the wire mess trays fitted in the plastic boxes. Each plastic box had 40 ml of distilled water. The boxes were placed in accelerated ageing chamber after closing of lids. The seeds were aged at 40±1°C temperature and 100% relative humidity for 72, 96 and 120 hr followed by standard germination in three replications of 100 seeds each. The treatment combination, i.e. 40±1°C temperature and 100 % relative humidity for 120 hr was found better for accelerated ageing of seed for all the varieties/genotypes.

One hundred seeds of each varieties/genotypes of all the lots in three replicates placed in between sufficient moistened rolled towel papers and kept at 25°C in seed germinator. Final count was taken on 21st day and only normal seedlings were considered for percent germination according to the rules of International Seed Testing Association (ISTA 2001).

To measure the electrical conductivity, 50 normal and uninjured seeds were soaked in 75 ml deionized water in 100 ml beakers in three replications. Seeds were immersed completely in water and beakers were covered with foil. Thereafter, these samples were kept at 25°C for 24 hr. The electrical conductivity of the seed leachates was measured using a direct reading conductivity meter. The conductivity was expressed in µS/cm/seed.

One hundred seeds from each sample were taken and weighed on electrical balance. These seeds were dipped in hexane solution; density of hexane is 0.655. Volume of hexane displaced by the seeds was recorded and seed density was calculated by using the following formula:

$$\text{Seed density} = \frac{\text{Weight of 100 seeds (g)}}{\text{Vol. of hexane displaced by seeds (cme}^3\text{)}}$$

The density of seeds was expressed as g/cc.

In DHA test, reduction of 2, 3, 5-Triphenyl tetrazolium chloride to red formazan by dehydrogenase enzyme in seed embryo is the basic principle for topographical tetrazolium test for seed viability. It is a quantitative method which may be used to determine varying dehydrogenase activity between seeds of similar viability and therefore, it is measure of seed vigour. Sample of 2 gram seed of each seed lot of all the varieties/genotypes replicated thrice were grinded to pass through a 20 mesh screen. 200 mg flour was soaked in 5 ml of 1% tetrazolium solution at 30°C for 3-4 hr. Then it was centrifuged at 10000 rpm for 3 minutes and the supernatant was poured off. The formazan was extracted with 10 ml acetone for 16 hr followed by centrifugation and absorbance of the solution was determined by spectrophotometer at 520 nm. These observations were expressed as change in OD/g/ml.

Three hundred seeds of all the three lots of all eight varieties/genotypes were sown in a Randomized Block Design (RBD) replicated thrice and the number of seedlings emerged daily were counted up to 30 days after sowing. The field emergence index was estimated as follow:

$$\text{FEI} = \frac{\text{No. seedlings emerged}}{\text{Days of first count}} + \text{..........} + \frac{\text{No. of seedlings emerged}}{\text{Days of final count}}$$

The field establishment was determined by counting the total number of seedlings when the emergence was completed.

RESULTS AND DISCUSSION

Results revealed that standard germination decreased as the ageing period increased in all the eight varieties/genotypes (Fig 1). In Lot-A and Lot-B, all the varieties/

genotypes showed germination percentage above Minimum Seed Certification Standards (60.00%). Among varieties/ genotypes, DH 259 recorded high germination (86.33) followed by DH 226 (84.00), Hisar Anand (83.67) in Lot-A. Standard germination declined with a fast rate in Lot-C as compared to Lot-B. The maximum standard germination was recorded in DH 224 (32.33) followed by DH 294 (30.67) and DH 296 (30.67) in Lot-C. It is interesting to note that germination decreased in DH 224 at lower rate as compared to other varieties/genotypes in Lot-C which showed the better storability. Varietal differences might be due to variation in genetic make up in these varieties and thus genotypic variation in these varieties have been observed for response to seed deterioration due to storage. As seed quality declined with age, there was a concurrent increase in the levels of free fatty acids. Elevated levels of free fatty acids, which are toxic to more cells, are not found in healthy seed tissues. The present results are also in agreement with the findings of Saxena *et al.* (1987) where loss of seed viability and vigour increased with increase in period of storage. Above results are in agreement with various workers in different crops such as okra (Narwal 1995), onion (Kumari 1994), carrot (Maskri *et al*. 2003) and turnip (Khan *et al*. 2005) under natural ageing.

The root length, shoot length and seedling dry weight were also affected by different seed lots and decreased significantly with passage of time. Root length and shoot length ranged from 8.87 cm (Lot-C) to 16.87cm (Lot A) and 9.60 cm (Lot-C) to 18.16 cm (Lot A), respectively. The highest root length was recorded in DH 224 (10.54) and lowest in Hisar Anand (6.59) in Lot-C. Similarly, maximum shoot length was recorded in DH 296 (10.89) followed by DH 224 (10.84) and minimum in Hisar Anand (7.90) in Lot-C. The mean seedling dry weight was found maximum in Hisar Anand (3.89) followed by DH 294 (3.55) and minimum in DH 226 (2.59) in Lot-A. The results indicated that genotype DH 296 (3.10) showed significantly maximum seedling dry weight and minimum in DH 226 (2.30) in Lot-B. Seedling length ranged from 2.30 (DH 226) to 2.64 (DH 228) in Lot-C. Similar findings were also observed by Nagarajan *et al*. (2004) and Verma *et al*. (2003) in mustard and Kumar (2004) in onion. It is well known that seed is a living organism whose biological quality may deteriorate with passage of time and also lack of adequate storage facilities. Many biochemical investigations have proven that lipid peroxidation and fat acidity (free fatty acid percentage) are the major causes of seed deterioration with passage of time, including cellular membrane disruption.

The standard germination test fails to account for the progressive nature of seed deterioration and the seeds are merely classified as either germinable or non-germinable with no distinction between strong or weak seedlings. These weaknesses have encouraged the interest in vigour testing to provide information about the vigour and viability of seed, which has not been realized by standard germination test. Seedling vigour index-I and 11 declined significantly in all the varieties/genotypes with the passage of seed storage time. Among lots, seedling vigour index-I and 11 ranged from 563.01 (Lot-C) to 2875.20 (Lot-A) and 75.78 (Lot-C) to 270.38 (Lot-A), respectively. Seedling vigour index–I ranged from 1480.57 (DH 228) to 1971.36 (DH 226) in Lot-B, whereas it was recorded maximum in DH 224 (690.01) followed by DH 296 (621.81) and minimum in Hisar Anand (423.48) in Lot-C. Seedling vigour index–II ranged from to 146.57 (DH 226) to 207.49 (DH 296) in Lot-B. Seedling vigour index-II was recorded maximum in DH 259 (79.80) followed by DH 294 (77.43) and minimum in DH 226 (69.08) in Lot-C. The variety Hisar Anand showed maximum value (325.18) followed by DH 294 (297.54) and minimum in DH 226 (217.69) in Lot-A. The genotypes DH 294 showed high vigour index-I and Hisar Anand recorded maximum vigour index-II, which indicated that these two vigorous varieties/genotypes can also be used in further breeding programmes for seed traits.

Results indicated that seed density was ranged from 0.66 (Lot-C) to 0.72 (Lot-A). The maximum seed density was shown by DH 259 and DH 294 (0.72), whereas minimum seed density was expressed by DH 228 and Hisar Anand (0.68) in Lot-A. The seed density ranged from 0.67 to 0.70 and 0.66 to 0.68 in Lot-B and Lot-C respectively. The genotypes and genotypes× lots interaction found significant.

Seed ageing has come to be recognized as major cause of reduced germination, vigour and viability in many species. Maximum seed quality occurred at physiological maturity after which seed vigour and viability declined both during aging on the plants as well as during storage. In the present study, accelerated ageing period was first standardized by ageing the seeds for the period of 72, 96 and 120 hr at 40±1ºC with 100 percent relative humidity. Fresh seeds of all the varieties/genotypes were placed in accelerated ageing chamber for 72, 96 and 120 hr at 40±1ºC with 100 percent relative humidity. After accelerated ageing, the seeds of all the varieties/genotypes were placed for germination test (ISTA 2001). Results showed that all the varieties/genotypes recorded germination less than 50% after the accelerated ageing period of 120 hr, hence, the accelerated ageing period of 120 hr was standardized and all the varieties/ genotypes were placed for 120 hr at 40±1ºC with 100% relative humidity. The results revealed that DH 228 (50.33) showed maximum number of normal seedlings followed by DH 259 (48.67), whereas DH 224 and Hisar Anand (41.33) recorded minimum number of normal seedlings in Lot-A. Results revealed that maximum germination was recorded in DH 259 (40.00) followed by DH 228 (37.33), whereas minimum in DH 294 and Hisar Anand (28.33) in Lot-B. Maximum germination was recorded in DH 228 (17.67), whereas Hisar Anand (9.33) showed minimum germination in Lot-C. DH 228 recorded maximum germination in all the lots after accelerated ageing test. It revealed that this variety was found more vigorous than others and this genotype may be rated as good storer. The decline in seed germination and vigour during accelerated ageing as well as storage treatments were influenced by chronological age of seed rather than initial germination percentage. The EC of the seed leachates was significantly more with aging. Low EC is an indication of improvement in seed vigor. Increased electrolyte leakage with aging confirmed the inferior quality of aged seeds. The increased seed leakage is believed to be associated with aging induced changes in cellular membranes of imbibed seeds. Decrease in percentage germination and root and shoot lengths were accompanied with AA. Sinha and Agarwal (1980) also reported decrease in seed germination and vigour during accelerating ageing as well as during storage.

The measurement of electrical conductivity is very important to determine the vigourness of seeds. The electrical conductivity test of solution reflected the amount of leachates extruded from the seeds. Mean electrical conductivity increased as the ageing period increased which was found 0.14, 0.37, 0.69 in Lot-A, Lot-B and Lot-C, respectively. The range of electrical conductivity of seeds varied from 0.12 (DH 226, DH 294) to 0.16 (DH 242, Hisar Anand) in Lot- A whereas it ranged from 0.31 (DH 224) to 0.44 (DH 296) in Lot-B. The data on electrical conductivity of leachates indicated that the highest membrane integrity was present in the DH 226 and DH 294 (0.12) in Lot-A. In comparison with other seed quality parameters altogether a reverse trend was observed for electrical conductivity test. The genotype DH 296 (0.44, 0.81) showed highest value of electrical conductivity in Lot-B and Lot-C, respectively and thus it was rated as poor storer. All the varieties/genotypes lost their membrane integrity significantly and gradually with advancement of storage period. Many researchers have used electrical conductivity test to indicate seed vigour. The electrical conductivity is related to the deterioration processes of seeds as degradation of cell membranes and leackage out of the cell. In aged seeds or partially deteriorated seed, the electrical conductivity will be higher, owing to decrease in membrane integrity on account of detrimental changes in biomembranes occurring in stored seeds Higher leachates which leach out with increase in storage period due to loss of membrane integrity and due to peroxidation of unsaturated fatty acids. Similar results were also reported by Basra *et al.* (2003) in cotton. The seed leachates increased with increased in storage period of sunflower Pallavi *et al.* (2003).

The range of optical density of formazan was estimated with the help of spectrophotometer. The results also revealed that dehydrogenase activity decreased significantly as the age of the seeds increased. The range of optical density of the formazan in different varieties/genotypes varied from 0.05 to 0.08 in Lot-A, 0.02 to 0.06 in Lot-B and 0.01 to 0.02 in Lot-C. The genotypes DH 294 and DH 296 (0.08) showed highest value of dehydrogenase activity in Lot-A, whereas in Lot-B, DH 294 (0.06) showed the highest value. In Lot-C, DH 228, DH 259 and DH 294 (0.02) showed the maximum value of dehydrogenase activity. Ray and Gupta (1980) also reduced dehydrogenase activity in terms of formazan formation in rice seeds undergoing deterioration. Similar findings were also reported by Narwal (1995) in okra seeds.

Field emergence index and field establishment decreased inconsistently and significantly as the age of seeds increased, which were found maximum (10.02 and 71.37) in Lot-A and minimum (2.53 and 26.34) in Lot-C. In Lot-A, DH 226 (11.78) followed by DH 228 (11.60) showed the maximum field emergence, whereas minimum value was recorded in DH 296 (9.50). Field emergence index ranged from 5.11 (Hisar Anand) to 6.89 (DH 228) in Lot-B, whereas it ranged from 1.87 (DH 294) to 3.25 (DH 228) in Lot-C. Irrespective of the varieties/genotypes, the seeds of Lot-A germinated with faster speed as compared with Lot-B and Lot-C. All the varieties/genotypes showed field emergence reduction as the age of the seeds increased but the reduction was more rapid in Lot-C. Field establishment varied from 64.67 (DH 242) to 77.33 (DH 224) in Lot-A. The range of field establishment was found from 54.33 (DH 228 and DH 296) to 60.67 (DH 224) in Lot-B. The genotypes DH 224 (34.33) followed by DH 226 (31.67) showed maximum field establishment whereas minimum was found in Hisar Anand (18.67) in Lot-C. The genotype DH 224 showed maximum field establishment in all the lots under study.

The correlation between field and laboratory parameters of coriander seed lot is presented in Table 1. Field emergence index showed positive and significant correlations with standard germination (0.93**), shoot length (0.85**), root length (0.86**), seedling dry weight (0.72**), seedling vigour index-I (0.93**), seedling vigour index-II (0.92**), accelerating ageing test (0.93**) and dehydrogenase activity test (0.79**) where it was negatively and significantly correlated with electrical conductivity (–0.94**). The standard germination showed positive and significant correlation with root length (0.93**), shoot length (0.88**), seedling dry weight (0.71**), seedling vigour index-I (0.97**), seedling vigour index-II (0.96**), accelerating ageing test (0.98**), dehydrogenase activity test (0.84**) and field establishment (0.98**) and negatively and significantly correlated with electrical conductivity (–0.96**). The similar observations to present findings have also been reported by Tao (1980) in soybean, Yadav and Dhankhar (2001) in okra. The correlation studies revealed that standard germination, seedling length, seedling vigour index, accelerated ageing test were found positively and highly significant correlated with field emergence. Therefore, the standard germination test, seedling vigour index-I, accelerated ageing test can successfully be used as prediction criterion of field emergence.

From the present investigation, it was observed that the viability and vigour of coriander seeds decreased as the age of the seeds increased. It was concluded that the seeds more than two and half year old, should not be used for sowing purpose as the viability and vigour of the seeds of all the varieties/genotypes declined with fast rate in Lot-C. Among the varieties/genotypes, the genotype DH 224 was found most promising and this genotype may be used for further breeding programme.

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