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INTERNAL MECHANICAL INJURIES DIAGNOSIS AND SUBSEQUENT EFFECTS ON SEED QUALITY OF SOME LEGUME SPECIES

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ABSTRACT

Seeds of faba bean (*Vicia faba* , L) cv. Giza $_{40}$, soybean (*Glycine max* , L) cv. Giza$_{22}$ and chickpea (*Cicer areitinum* , L) cv. Giza$_1$ were separated manually just after physiological maturity . Collected seeds contained 6.2 , 5.5 and 5.9 % moisture content for faba bean , soybean and chick pea respectively prior to expose them to mechanical damage by a device constructed and used to induce various levels of internal injuries. Different impact stresses were selected to induce invisible mechanical damage, whereas , six forces which is the combination between three different impact stresses and two seed positions were used . Seed placed on the device either seed side face the falling weight (SFL) or embryonic axis face the falling weight (EAFL). Increasing impact stress caused seed to lose its vigourisity when faba bean, soybean and chick pea seeds stressed by F_1 , F_2 and F_3. Seed position as well showed significant differences in all characters studied, whereas, seeds received falling load upon embryonic axis (EAFL) were more dramatically affected than seeds received falling load upon cotyledon area (SFL) of all studied legume species. Sensitivity of (EA) is not the only reason for vigourisity dramatic reduction occurred at (EAFL) position, but also the increment of figured loads from F_1, F_2 and F_3 in (N/mm^2) for (SFL) position to F_1, F_2 and F_3 in (N / mm^2) for (EAFL) position. Increasing contact area due to the seed shape caused figured impact stresses to be lower in faba bean seed, therefore, these lower forces enable the seed to survive the impact stress more than soybean and chick pea seeds . Not only the load used to induce internal damage affect the level of injury , but also part of seed to receive that load as well as the area that receive the load are factors to be paid more consideration when actual stresses to be figured . performance of (Fg) in diagnosing seed cracks in all studied species was the lowest , whereas , this method showed equal capability with other diagnosing method in diagnosing broken faba bean and soybean seeds. Visual, X-ray and tetrazolium had equal capability diagnosing seed cracks and broken areas of the three studied legume species. Highly negative correlation was found between broken seeds with vigour % as well as germination % of all studied legume species . Correlation analysis showed positive correlation between dead seeds % and all broken seeds of all studied legume species. Differences in diagnosing methods (visual , X-ray and Tz) capability were narrowed showing close capability in predicting seed quality as well as in diagnosing seeds internal damage.

Keywords: Faba bean, soybean, chick pea, seed-quality, internal-damage, tetrazolium, X-ray, fast green.

INTRODUCTION

Seed deterioration is a major problem in agriculture production. In the United States alone, seed sales amounted to over $ 2 billion. It has been estimated that about 25% of that value or approximately $ 500 million in revenue, was lost due to poor seed quality. Worldwide, these losses are even greater, particularly in lesser - developed countries where there are poor facilities of post - harvest processes. Seed production practices such as harvesting, storage, cleaning and shipping and handling inevitably lead to mechanical damage (Mc-Donald, 1985). Seed deterioration is inexorable and progressive, thus, small mechanically damaged areas that initially have little impact on seed performance may latter increase in size and cause deterioration of vital embryonic tissues , resulting in poor seed quality (Moore, 1972). Moreover, mechanical injuries promotes invasion by storage fungi, which can enter the seed through cracks in seed coat (Mamiepic and Caldwell, 1963) .

It is widely Known that mechanical injury is an important factor unfavourly influencing the quality of seed. The principle effect of the negative influence is a reduction of germination and subsequent yields of crops (Pollock and Roos, 1972). The general conclusion is that not only visible macro-damage but also invisible micro-cracks might significantly reduced the viability of seed. In early work , the internal micro-cracks were successfully detected by X-ray radiography (Milner and Shellenberger, 1953). However continuos focusing in using X-ray as a helpful tool assist seed analyst to detect seed micro-cracks become remarkable, whereas, recently investigators worked on wheat seed quality by inducing internal cracks. These cracks were resulted of quasi-static compression in a cylindrical press, damaged seeds were exposed to X-ray producing roentgenograms that would exhibit any internal cracks. Then, seeds were divided into three groups based on the level of internal damage diagnosed by X-ray which are (seeds with no cracks, seeds with small cracks and seeds with rough cracks), It was found that the germination of seeds increased as the level and size of cracks decreased (Tryka *et al.,* 1997). X-ray analysis enabled visualisation of stress cracks that are invisible to the human eye and, therefore, gave a better estimate of the percentage of cracks (Maria *et al*., 1999), meanwhile, they went far to use scanning electron microscopy which revealed small cracks inside cotyledons not noticed by visual or X-ray inspection. They noticed that cracks are often located near middle of the seed along the embryo axes, but they were also located in other positions, their conclusion came to that if cracks is located inside or perpendicular to the embryo axis, it may affect the quality of the seed. Not only decline in germination percentage as a result of loosing quality, but also other seedlings characters such as abnormalities were observed due to mechanically damaged seed (Grass and Tourkmani, 1999). Over the past two decade, the tetrazolium test (Tz) has gained wide acceptance not only as rapid technique for estimation of viability but also as a powerful rapid tool for assessing vigour , detecting weathering damage and mechanical damage (Mason *et al*., 1982; Carbonell and Krzyzanowsky, 1995; Deswal and Chand, 1997 ; Carbonel *et al*., 1998 ; Abdelghany and El-Sahar, 1998). Also, it is previously documented that fast green test reveals physical fractures in seeds. Therefore, seed quality was assessed by the fast green staining test (Fg), whereas, the relationship between fast green evaluation of mechanical damage and germination percentage was also evaluated in an attempt to address the extent to which fast green may be useful in predicting the effect of damage on seed quality (Peterson *et al.*, 1995).

The present study is an attempt to evaluate the capability of different mechanical injuries diagnosing tests as a helpful tool to estimate seed quality of different legume species and their correlations with vigour and germination characteristics .

MATERIALS AND METHODS

Plant material

Faba bean (*Vicia faba , L*) cv. $Giza_{40}$, soybean (*Glycine max , L*) cv. $Giza_{22}$ and chickpea (*Cicer areitinum , L*) cv. $Giza_1$ were submitted from Field Crop Insti. , Agricultural Research Center (ARC) , Giza . Seeds of harvested plants were separated and manually threshed just after physiological maturity. The collected seeds were used in the experiment immediately after threshing. The average moisture contents of seeds just before exposing them to mechanical damage were 6.2 , 5.5 and 5.9 % for faba bean, soybean and chick pea, respectively.

Mechanical damage

A mechanical device to induce damage to the seeds were constructed and used in this investigation. The device consists of three different solid metal cylinders, their weights are 0.26 , 0.08 and 0.17 kg to produce loads on seeds of faba bean, soybean and chick pea respectively, while falling of these loads was through three different distances representing three different forces F_1, F_2 and F_3 . A steel cylinder was used to adjust the fall height and permit the load to fall perpendicularly on seed . Detailed description of the device was documented by (El-Sahar *et al.*, 2000) . Impact stresses applied to induce seed mechanical damage used in the present investigation were demonstrated in Table (1), whereas, changing in load weight and falling distance were selected to produce impact stresses in a range that keep the invisible damage as low as possible , also seeds were placed in the device either in position that side facing the falling load (*SFL*) or in position that embryonic axis facing the falling load (*EAFL*) . The following formula was used to calculate the produced stresses , this formula was described by (El-Sahar *et al.*, 2000) :

$$\sigma = F/A\ [\ 1+ (\ 1+ 2AEh\ /\ FL\ )^{-1/2}]$$

whereas ;

σ = Impact stress (N /mm^2)

F = Load applied with impact (N)

A = Contact area exposed to load (mm^2)

E = Modules of elasticity (N / mm^2)

h = Falling height (cm)

L = Seed thickness (mm)

Germination test

Germination tests were carried out on rolled paper towels , according to ISTA procedure (ISTA , 1996) on four replicates of 100 seeds for each treatment. Seedlings morphology was evaluated as normal or abnormal seedlings and non-germinable seeds were classified into dead seeds and solid seeds according to ISTA guidelines .

Vigour test

Vigour tests were carried out on sand , according to ISTA procedure (ISTA , 1996) on four replicates of 100 seeds for each treatment . Seedlings emergence percentage was calculated according to ISTA guidelines .

Table (1): Impact stresses used for inducing seed mechanical damage for different seeds species .

Crop	Position	F (N)	A (mm^2)	E (N/mm^2)	h (mm)	L (mm)	σ (N / mm^2)
Faba bean (Giza 40)	SFL	2.6	22.50	65.8	90 110 130	8.0	13.20 14.60 15.80
	EAFL	2.6	5.00	240.4	90 110 130	10.0	48.20 53.10 57.60
Soybean (Giza 22)	SFL	0.8	1.54	325.0	60 70 80	6.0	58.70 64.80 67.50
	EAFL	0.8	1.70	1200.0	60 70 8.0	7.5	94.80 102.30 109.20
Chick pea (Giza 1)	SFL	1.7	4.15	200.0	60 75 90	6.0	41.00 45.70 50.00
	EAFL	1.7	2.30	2250.0	60 75 90	6.3	177.10 197.70 216.30

SFL : Seed side facing falling load ; EAFL : Seed embryonic axis facing falling load

Mechanical damage diagnosis

A- Visual diagnosis

Faba bean , soybean and chick pea seeds (400 seeds each treatment in four replicates) were placed in moistured rolled paper towel to allow seeds to imbibe water for 24 , 4 and 8 hours , respectively . Seed coat was removed carefully using the edge of sharp razor blade . Seeds were examined for mechanical damage , whereas , cracks and broken areas of cotyledons externally and internally were diagnosed by the help of magnifier lens . Percentages of cracked and broken seeds were calculated .

B- X-ray image analysis

All different injured seeds (400 seeds each treatment in four replicates) were glued to paper sheet (43 cm in length and 35 cm in width) and exposed to soft X-ray. The source of X-ray was supplied by voltage of 40 kV, 0.3 mA for 0.03 sec. The sheet was placed on top of the film on cassette-type holder at a distance of 110 cm from the X-ray source. Soft X-ray went through the seeds and made a roentgen image of a natural size. The roentgenograms obtained were used to detect the invisible damage, whereas, the seeds were divided into three groups, the first of which was without any visible damage while the second and the third had small cracks and broken areas, respectively. Percentages of the damaged groups were calculated.

C- Tetrazolium test (Tz)

Four hundred seeds of each treatment were soaked in 0.1 % 2,3,5 tri-phenyl tetrazolium chloride for two hours according to ISTA regulations (ISTA 1996). Prior to soaking, faba bean, soybean and chick pea seeds were pre-conditioned in wet paper towel for 24 , 4 and 8 hours , respectively . Seed coat were removed and the embryos were excised longitudinally with sharp razor blade and percentages of cracked and broken seeds were evaluated .

D- Fast green test (Fg)

Mechanical damage was assessed by fast green staining test (Fg) according to (AOSA , 1988) . Seeds (400 seeds each treatment in four replicates) are soaked in a 0.1% fast green solution for 24 , 4 and 8 hours for faba bean , soybean and chick pea , respectively . During this period , fast green penetrates any area of the seed coat which has been fractured and stains the endosperm green , after the soak period , the seed are washed and fractures then become apparent in the seed coat .

Statistical analysis

Collected data were arranged in a complete random design and were exposed to statistical analysis as described by (Snedecor and Cochran 1989). For comparisons between means, analysis of variance and LSD values were used. Correlation analysis between cracked and broken seeds diagnosed by visual, X-ray, tetrazolium and fast green methods and characters studied were carried out according to (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

Effect of mechanical damage on vigour and germination characteristics:
A- Faba bean seeds :

Faba bean seeds significantly affected by impact stress , whereas , vigour and germination characteristics (Table 2) showed significant differences except solid seed percentage . Increasing impact stress caused seed to lose its vigourisity, whereas, percentages of 82.50 , 60.25 and 41.88% were recorded when faba bean seeds stressed by F_1 , F_2 and F_3 respectively. Not only germination percentage reduced but also abnormal seedlings were also increased , whereas, germination percentages of 85.00 , 68.23 and 57.50 % included a percentage of 5.63 , 9.88 and 12.50 % of abnormal seedlings . As a result of the reduction occurred in germination percentage , dead seeds percentages of F_1 , F_2 and F_3 gradually increased . Therefore , various level of mechanical damage was successfully obtained by the selected forces which caused seed quality to be varied accordingly .

Seed position showed significant differences in all characters studied except solid seed percentage , meaning that the part of the seed which receive the impact stress strongly affect the seed behavior towards loosing germinability. Data presented in (Table 2) revealed that seeds received falling load upon embryonic axis (EAFL) were more dramatically affected than seeds received falling load upon cotyledon area (SFL). Accordingly, vigour, germination and normal seedlings percentages reduced and abnormal seedlings and dead seeds percentage increased of (SFL) position if compared with (EAFL) position. It could be concluded that the embryonic axis part is the most sensitive part in the seed causing seed to loss its vigourisity if exposed to damage. Sensitivity of (EA) is not the only reason for vigourisity dramatic reduction occurred at (EAFL) position, but also the increment of figured loads from 13.20, 14.60 and 15.80 N/mm^2 for (SFL) position to 48.20, 53.10 and 57.60 N/mm^2 for (EAFL) position, respectively , is a considerable reason as well (Table 1). Although both heavier load used to induce damage to faba bean seeds reached 2.6 N than those of 0.8 and 1.7 N used in soybean and chick pea seeds, and falling distances of 90, 110 and 130 mm higher than those used in soybean and chick pea seeds , the figured stress forces of faba bean in N / mm^2 were lowest than forces figured for soybean and chick pea seeds. Increasing contact area due to the seed shape caused figured impact stresses to be lower in faba bean seed, therefore, these lower forces enable the seed to survive the impact stress more than soybean and chick pea seeds. Although interaction between impact stress and seed position (Table 2) showed no significant differences at all studied characters, six different levels of seed quality were obtained. Accordingly, vigour and germination percentages varied from 86.25 and 89.50 % to 36.00 and 54.50 %, respectively.

Table (2): Various levels of mechanical damage effects on germination and vigour of faba bean seeds (Giza$_{40}$) .

Stress Position	Vigour %	Germination characteristics				
		Germ. %	Normal seedlings%	Abnorm. seedlings %	Dead seeds %	Solid seeds %
Control	90.25	94.50	94.25	0.25	2.00	3.50
F_1	82.50	85.00	79.13	5.63	12.50	2.50
F_2	60.25	68.13	58.25	9.88	29.25	1.88
F_3	41.88	57.50	45.00	12.50	40.75	1.75
L.S.D. 0.01	4.44	3.27	3.90	2.80	3.60	N.S.
L.S.D. 0.05	3.24	2.38	2.85	2.04	2.63	N.S.
SFL	65.42	73.75	66.25	7.42	23.42	2.33
EAFL	57.67	66.67	55.33	11.25	31.58	1.75
L.S.D. 0.01	3.63	2.67	3.18	2.28	2.94	N.S.
L.S.D. 0.05	2.65	1.95	2.32	1.67	2.14	N.S.
F_1						
SFL	86.25	89.50	85.50	3.75	7.75	2.75
EAFL	78.75	80.50	72.75	7.50	17.25	2.25
F2						
SFL	62.25	71.25	64.50	6.75	24.75	4.00
EAFL	58.25	65.00	52.00	13.00	33.75	1.25
F3						
SFL	47.75	60.50	48.75	11.75	37.75	1.75
EAFL	36.00	54.50	41.25	13.25	43.75	1.75
L.S.D. 0.01	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
L.S.D. 0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

B- Soybean seeds :

Increasing impact stress on soybean seed (Giza $_{22}$) significantly caused seed to lose its vigourisity as well as germinability . Data presented in (Table 3) revealed that using the lowest impact stress force (F_1) caused soybean seeds to lose about 60.87 % and 50.82% of its vigour and germination capacity when compared to control value . Meanwhile , this dramatic reduction reached 6.13 % and 7.25 % for vigour and germination percentages respectively , and accordingly high percentage of dead seed reached 92.75 % when the greatest force of stress (F_3) was applied.

Significant seed quality reduction occurred when soybean position was changed from (SFL) position to (EAFL) position , revealing that seeds were injured dramatically on (EA) area . Understanding the stress upon seed based on the force only is unfairly point of view since the seed position while, receiving the stress (Part of seed to receive stress) as well as seed contact area (area to receive the stress) are factors to be paid more consideration when actual stresses to be figured .Interaction between impact stress and position showed significantly differences only at 0.05 level except abnormal seedling % and dead seeds %. Although the applied stresses selected within an average to induce internal damage only, soybean seed quality greatly affected by such stresses . The reason that may cause that dramatic death to soybean seed is the great stress applied , whereas , F_1 figured (58.70 and 94.80 N/mm^2), F_2 figured (64.80 and 102.30 N/mm^2) and F_3 figured (67.50 and 109.20 N/mm^2) for (SFL) and (EAFL) positions , respectively (Table 1) .

Table (3) : Various levels of mechanical damage effects on germination and vigour of soybean seeds (Giza $_{22}$) .

Stress	Position	Vigour %	Germination characteristics				
			Germ. %	Normal seedlings%	Abnorm. seedlings %	Dead seeds %	Solid seeds %
Control		88.50	91.50	90.50	1.00	3.00	5.50
F_1		34.63	45.00	43.63	1.38	51.63	0.88
F_2		14.38	17.50	16.50	1.00	82.50	0.00
F_3		6.13	7.25	6.63	0.63	92.75	0.00
L.S.D. 0.01		2.04	2.80	2.61	N.S.	5.51	0.45
L.S.D. 0.05		1.49	2.04	1.90	0.53	4.02	0.33
	SFL	23.00	28.17	26.33	1.83	71.42	0.42
	EAFL	13.75	18.33	18.17	0.17	79.83	0.17
L.S.D. 0.01		1.66	2.28	2.13	0.59	4.50	N.S.
L.S.D. 0.05		1.22	1.67	1.55	0.43	3.29	N.S.
F_1	SFL	40.00	51.25	49.00	2.25	47.50	1.25
	EAFL	29.25	38.75	38.25	0.50	55.75	0.50
F_2	SFL	19.50	22.75	20.75	2.00	77.25	0.00
	EAFL	9.25	12.25	12.25	0.00	87.75	0.00
F_3	SFL	9.50	10.50	9.25	1.25	89.50	0.00
	EAFL	2.75	4.00	4.00	0.00	96.00	0.00
L.S.D. 0.01		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
L.S.D. 0.05		2.11	2.89	2.69	N.S.	N.S.	0.46

The estimated stresses force used with soybean were even greater due to seed structure and shape which allow small contact areas (1.54 and 1.70 mm^2) to receive the falling load . Seedlings abnormalities increased when lower stress (F_1) was used (1.38%) (Fig 2-O) , while increasing stress forces to (F_2) and (F_3) caused percentage of abnormal seedlings to be reduced to 1.00 and 0.63% , respectively .

C - Chick pea seeds :

Chick pea (Giza 1) seed respond similarly as those of faba bean and soybean seeds (Table 4) to impact stress used in this investigation . As the impact stress increased the vigour and germination percentages decreased . Abnormal seedlings decreased significantly from 5.50 to 5.03 and 1.75% when stress increased from F_1 (41.00 and 177.10 N/mm^2) to F_2 (45.70 and 197.70 N/mm^2) and F_3 (50.00 and 216.30 N/mm^2) for both (SEL) and (EAFL) respectively (Table 1). However, these values were higher than abnormal seedling seen in germination of control seeds. It could be concluded that exposing seed to stress caused abnormalities of seedlings to be increased due to injuries occurred to the seed by such stresses (Fig 2-P) . On the other hand, increasing stress up to F_2 and F_3 caused abnormalities to be reduced since the seed in this case showed less viability (53.63 and 71.38 % dead seeds, respectively) meaning that chances to survive even in abnormal manner was reduced since chances for seed death was greater .

Table (4): Various levels of mechanical damage effects on germination and vigour of chick pea seeds (Giza $_1$) .

Stress Position		Vigour %	Germination characteristics				
			Germ. %	Normal seedlings %	Abnorm. Seedlings %	Dead seeds %	Solid seeds %
Control		86.50	90.75	89.75	1.00	2.75	6.50
F_1		62.50	67.00	61.50	5.50	29.50	3.50
F_2		38.88	44.38	39.25	5.13	53.63	2.13
F_3		14.13	27.38	25.75	1.75	71.38	1.25
L.S.D. 0.01		3.25	3.12	3.75	1.79	3.31	1.53
L.S.D. 0.05		2.57	2.28	2.74	1.30	2.41	1.11
	SFL	43.75	52.17	47.25	4.92	44.33	3.58
	EAFL	33.25	40.33	37.08	3.33	58.67	1.00
L.S.D. 0.01		2.87	2.55	3.07	1.46	2.70	1.25
L.S.D. 0.05		2.10	1.86	2.24	1.06	1.97	0.91
F_1	SFL	67.50	71.50	67.00	4.50	23.25	5.25
	EAFL	57.50	62.50	56.00	6.50	35.75	1.75
F_2	SFL	45.75	53.25	46.00	7.25	43.50	3.50
	EAFL	32.00	35.50	32.50	3.00	63.75	0.75
F_3	SFL	18.00	31.75	28.75	3.00	66.25	2.00
	EAFL	10.25	23.00	22.75	0.50	76.50	0.50
L.S.D. 0.01		N.S.	4.41	N.S.	2.53	4.68	N.S.
L.S.D. 0.05		N.S.	3.22	3.88	1.84	3.41	N.S.

Seed position also successfully produce variation in seed quality since data obtained in (Table 4) revealed that (EAFL) position significantly affect seed quality more than (SFL) position. Data of impact stress and seed position interaction showed that chick pea seeds has been categorized to six varied quality groups based on stresses and seed positions used .

Evaluation of internal damage diagnosing methods

Data in (Table 5) presented the internal damage (cracks % & broken %) occurred in faba bean , soybean and chick pea seeds diagnosed by visual examination, X-ray, tetrazolium (Tz) and fast green (Fg), whereas , fast green diagnosing method significantly recorded the lowest cracks percentages (8.42, 1.88 and 4.75%) while the other three methods did not significantly vary, that was true for faba bean, soybean and chick pea seeds, respectively. Therefore, performance of (Fg) in diagnosing seed cracks in all studied species was the lowest, meaning that other diagnosing methods gave similar estimation for the cracks in legume species. Pattern used in diagnosing internal damage by X-ray , tetrazolium and fast green presented in (Figs 1 , 2 and 3) , respectively . Although fast green (Fg) diagnosing method did not show promising capability in diagnosing seed cracks of all different seeds used in this experiment, it showed equal capability with other diagnosing method in diagnosing broken faba bean and soybean seeds since no significant differences were detected among values of estimated faba bean and soybean broken seeds. While, chick pea broken seeds varied significantly showing less capability if diagnosed by (Fg) method compared to the other methods. Furthermore, visual, X-ray and tetrazolium methods showed no significant differences in diagnosing seed broken parts of all studied legume species. It is concluded that methods of visual , X-ray and tetrazolium had equal capability diagnosing seed cracks and broken areas of the three studied legume species , while fast green method showed only less capability except in diagnosing broken areas of faba bean and soybean seeds.

Table (5): Visual , X-ray , tetrazolium staining (Tz) and fast green staining (Fg) diagnosis methods estimating internal seed damage (cracks and broken percentages) occurred on faba bean , soybean and chick pea seeds .

Damage estimated	Diagnosis methods	Faba bean	Soybean	chick pea
Cracks%	Visual	12.29	3.83	7.46
	X-ray	12.13	3.79	7.42
	Tz	11.04	3.29	7.25
	Fg	8.42	1.88	4.75
L.S.D. 0.05		1.07	0.72	1.07
Broken%	Visual	29.21	77.00	55.25
	X-ray	30.54	76.38	57.42
	Tz	29.75	76.25	54.67
	Fg	29.33	77.88	46.45
L.S.D. 0.05		N.S.	N.S.	2.42

Fig (1): Radiograph of faba bean , soybean and chick pea damaged seeds showing ; A) Undamaged faba bean seed , B) Crack appear on cotyledon of faba bean seed , C) Broken embryonic axis (EA) of faba bean seed , D) Undamaged soybean seed , E) Crack appear perpendicular on EA of soybean seed , F) Broken EA of soybean seed , G) Undamaged chick pea seed , H) Cracks appear on both cotyledons of chick pea seed , I) Broken EA of chick pea seed .

Fig (2): Faba bean, soybean and chick pea seeds stained with 0.1% tetrazolium chloride showing ; A) Pattern of undamaged faba bean seed , B) Faba bean seed exposed to impact stress where bruises occurred on cotyledon , C) Faba bean seed exposed to impact stress where arrow pointed on dead tissue on cotyledon , D) Faba bean seed exposed to stress parallel EA where arrow pointed on the damaged occurred on EA , E) Pattern of undamaged soybean seed , G) Unbroken severely damaged soybean seed (EA stained dark red) , F) Crack appear incline to EA on soybean cotyledon , G) Dark red stained soybean (loss of vigourisity) , H) Unstained dead soybean seed , I) Minor bruises on EA of chick pea seed , K) Minor bruises appear on chick pea cotyledon ,L) Crack appear perpendicular on EA of chick pea seed , M) Broken cotyledon of chick pea seed , N) Off stained dead chick pea seed , O) Faba bean abnormal seedling, P) Chick pea abnormal seedling.

Fig (3): Faba bean , soybean and chick pea seeds stained with 0.1% fast green showing ; A) & B) Undamaged faba bean seed , C) Crack on cotyledon appear on whole seed parallel to EA of faba bean seed , D) Broken cotyledon perpendicular on EA , E) Crack appear perpendicular on EA , F) Multiple broken area appear on faba bean cotyledon , G) Undamaged soybean seed , H) & I) Crack appear perpendicular on EA of soybean seed , J) Broken on soybean cotyledon perpendicular on EA , K) Undamaged chick pea seed , L) Broken appear on chick pea cotyledon , M) Severe damage appear on EA of chick pea seed , N) Chick pea seeds showing 1- Crack through EA , 2- Crack perpendicular on EA , 3- Crack on the other side of EA, O) Cracks through and perpendicular EA of chick pea seed, P) Broken appear perpendicular on EA of chick pea seed. (Seed coat was removed to obtain clear photographs)

Diagnosing methods correlation with vigour and germination characteristics

Correlation analysis of faba bean cracked and broken diagnosed methods described in this investigation with vigour and germination characteristics presented in (Table 6) . Highly negative correlation was found between broken seeds with vigour % as well as germination % , meaning that increasing percentages of faba bean broken seed negatively correlated with vigour and germination percentages while cracked seed did not reach the negative effects of broken seeds . Furthermore , the diagnosing methods showed similar capability in diagnosing broken seed which related to seed quality . However , fast green showed the highest value (-0.97) in correlation with vigour % while visual , Tz and Fg all showed the highest values (-0.97 and -0.97) in correlated with germination % . Fast green method did not show significant correlation with both vigour and germination percentages meaning that these methods are less capability in diagnosing faba bean cracked seeds . High negative correlation between faba bean broken seeds percentage and normal seedlings percentage of all diagnosing methods was found , while fast green method showed low significant correlation value (0.43) with detected cracked faba bean seeds . Abnormal seedlings character showed high positive correlation with broken faba bean seeds , whereas , different levels of significance were detected in cracked faba bean seeds . It is concluded that the figured forces used to induce faba bean internal damage (Table 1) was low due to the structure and shape of faba bean seed , and that caused seedling abnormalities to be increased as cracked seed increased, whereas, visual methods showed the highest capability in detecting seed crack correlated with seedlings abnormalities (-0.71). Correlation analysis showed positive correlation between dead seeds % and faba bean broken seeds, whereas, tetrazolium diagnosing method showed the highest correlation value (0.98) followed by visual and fast green methods (0.97) and finally X-ray method (0.96). No correlation found between all diagnosing methods and faba bean solid seeds .

Table (6) : Correlation of vigour and germination characteristics with faba bean cracked and broken seeds diagnosed by visual , X-ray , tetrazolium and fast green tests .

Diagnosing Characters	Cracks				Broken			
	Visual	X-ray	Tz	Fg	Visual	X-ray	Tz	Fg
Vigour %	0.58 **	0.47 *	0.53 **	0.34 N.S.	-0.59 ***	-0.96 ***	-0.95 ***	-0.97 ***
Germination %	0.60 **	0.48 *	0.53 **	0.38 N.S.	-0.97 ***	-0.96 ***	-0.97 ***	-0.97 ***
Normal seedlings %	0.66 ***	0.53 **	0.57 **	0.43 *	-0.97 ***	-0.96 ***	-0.97 ***	-0.97 ***
Abnormal seedlings %	-0.71 ***	-0.59 **	-0.58 **	-0.48 *	0.81 ***	0.81 ***	0.82 ***	0.84 ***
Dead seeds %	-0.64 ***	-0.52 **	-0.57 **	-0.43 *	0.97 ***	0.96 ***	0.98 ***	0.97 ***
Solid seeds %	0.40 N.S.	0.27 N.S.	0.28 N.S.	0.33 N.S.	-0.40 N.S.	-0.33 N.S.	-0.64 *	-0.38 N.S.

*****= High significant, **= Medium significant, *= Low significant and N.S.= Not significant**

Correlation analysis of soybean cracked and broken seeds diagnosed by methods described in this investigation with vigour and germination characteristics presented in (Table 7) , whereas , high negative correlation was found between these two characters and broken seeds diagnosed by all methods used. Meaning that, all diagnosing methods used showed great eligibility in diagnosing broken soybean seeds which highly correlated with seed quality .Visual method gave the highest value (-0.98) when correlation of broken seeds with vigour % was considered, however , other methods gave close values. On the other hand, Tz diagnosing method gave the highest value (-0.97) when correlation of broken seed with germination % was considered. Faba bean cracked seed did reach negative correlation with seed quality, meaning that broken seed successfully correlated with soybean seed quality rather than cracked seeds. It could be concluded that methods detecting internal damage may use in predicting seed quality since the estimated internal damage correlated with vigour and germination percentages in negative manner. Normal seedlings percentage as well as abnormal seedlings % showed negative correlation with broken seed diagnosed by all methods used in this investigation . Soybean cracked seed did not reach the negative effects upon correlation with seed quality but seed contained broken parts detected by any method of diagnosing methods used showed negative correlation with seed quality, this finding may be due to great figured forces applied to the seed (Table 1) as well as the nature of seed structure and shape. Additionally , broken seeds diagnosed by any method showed high positive correlation with dead seeds percentage. Furthermore, both visual and Tz diagnosing methods gave correlation value of (0.94) showing superior capability of these method in diagnosing soybean broken seed.

Table (7): Correlation of vigour and germination characteristics with soybean cracked and broken seeds diagnosed by visual , X-ray , tetrazolium and fast green tests .

Diagnosing Characters	Cracks				Broken			
	Visual	X-ray	Tz	Fg	Visual	X-ray	Tz	Fg
Vigour %	0.76 ***	0.79 ***	0.71 ***	0.73 ***	-0.98 ***	-0.97 ***	-0.97 ***	-0.96 ***
Germination %	0.73 ***	0.76 ***	0.66 ***	0.70 ***	-0.96 ***	-0.95 ***	-0.97 ***	-0.95 ***
Normal seedlings %	0.71 ***	0.74 ***	0.64 ***	0.68 ***	-0.59 ***	-0.95 ***	-0.97 ***	-0.95 ***
Abnormal seedlings %	0.81 ***	0.75 ***	0.75 ***	0.82 ***	-0.67 ***	-0.63 **	-0.56 **	-0.57 ***
Dead seeds %	-0.66 ***	-0.70 ***	-0.60 **	-0.62 **	0.94 ***	0.92 ***	0.94 ***	0.92 ***
Solid seeds %	0.66 ***	0.70 ***	0.42 *	0.57 **	-0.74 ***	-0.72 ***	-0.71 ***	-0.75 ***

*****= High significant, **= Medium significant, *= Low significant and N.S. = Not significant**

Correlation analysis of chick pea cracked and broken seeds diagnosed by methods described in this investigation with vigour and germination characteristics presented in (Table 8) , whereas , similar results as those of soybean correlation results were obtained. High negative correlation was found between seeds diagnosed as broken seeds with vigour, germination and normal seedlings percentages. Moreover, dead seed percentage showed positive correlation with broken seeds diagnosed by all diagnosing methods used in this investigation. Results showed that highest correlation (-0.94) of chick pea broken seeds with vigour % was detected by X-ray diagnosing method, with germination % (-0.97) was detected by X-ray diagnosing method, with normal seedlings % (-0.97) was detected by X-ray diagnosing method, with abnormal seedlings % (-0.67) was detected by Tz diagnosing method, and with dead seeds % (0.97) was detected by Tz diagnosing method. Differences in diagnosing methods (visual , X-ray , Tz and Fg) capability were narrowed showing close capability in predicting seed quality as well as in diagnosing chick pea seeds internal damage .

Table (8) : Correlation of vigour and germination characteristics with chick pea cracked and broken seeds diagnosed by visual , X-ray , tetrazolium and fast green tests .

Diagnosing Characters	Cracks				Broken			
	Visual	X-ray	Tz	Fg	Visual	X-ray	Tz	Fg
Vigour %	0.83 ***	0.71 ***	0.79 ***	0.78 ***	-0.94 ***	-0.95 ***	-0.93 ***	-0.90 ***
Germination %	0.85 ***	0.76 ***	0.80 ***	0.78 ***	-0.97 ***	-0.97 ***	-0.96 ***	-0.92 ***
Normal seedlings %	0.87 ***	0.78 ***	0.82 ***	0.78 ***	-0.97 ***	-0.97 ***	-0.95 ***	-0.92 ***
Abnormal seedlings %	0.45 *	0.36 N.S.	0.39 N.S.	0.51 *	-0.62 **	-0.64 ***	-0.67 ***	-0.56 **
Dead seeds %	-0.87 ***	-0.79 ***	-0.81 ***	-0.81 ***	0.96 ***	0.67 ***	0.97 ***	0.92 ***
Solid seeds %	0.77 ***	0.81 ***	0.63 ***	0.84 ***	-0.63 ***	-0.67 ***	-0.74 ***	-0.66 ***

***** = High significant, **= Medium significant, *= Low significant and N.S.= Not significant**

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تشخيص الإصابة الداخلية وتبعية تأثيرها على جودة بذور بعض الأنواع البقولية

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فصلت بذور الفول البلدى صنف (جيزة ٤٠) وفول الصويا صنف (جيزة ٢٢) والحمص صنف (جيزة ١) يدوياً بعد وصولها لمرحلة النضج الفسيولوجى مباشرة . أحتوت البذور على نسبة رطوبة قدرها ٦,٢ ، ٥,٥ ، ٥,٩ % لبذور الفول البلدى وفول الصويا والحمص على الترتيب قبل تعريضهم للضرر الميكانيكى المستحدث بجهاز صمم وأستخدم لإحداث مستويات مختلفة من الضرر الداخلى . إختيرت إجهادات صدمة مختلفة لأحداث ضرر غير ظاهرى وأستخدم ٦ إجهادات هى توافيق بين ٣ مستويات من إجهادات الصدمة وأثنين من أوضاع البذرة . وضعت البذور فى الجهاز إما فى وضع يقابل فية جانب البذرة الثقل الساقط (SFL) أو فى وضع يقابل فية محور الجنين الثقل الساقط (EAFL) . أدت زيادة إجهادات الصدمة (F_1,F_2,F_3) إلى فقد بذور الفول البلدى وفول الصويا والحمص لقوة الأنبات . كما أظهر وضع البذرة تأثير معنوى فى كل الصفات المدروسة حيث أظهرت البذور المعرضة للإجهاد على المحور الجنينى تأثراً شديدا عن مثيلتها المعرضة للإجهاد على جانب البذرة . ليست حساسية المحور الجنينى السبب الوحيد للإنخفاض الشديد الحادث للبذور المعرضة للإجهاد على المحور الجنينى ولكن أيضاً زيادة قوى الإجهادات المحسوبة (F_1,F_2,F_3) والمقدرة للبذور فى وضع (EAFL) عن قوى الإجهادات المحسوبة (F_1,F_2,F_3) والمقدرة للبذور فى وضع (SFL) سبب آخر . زيادة مساحة تلامس البذور تبعا لشكل بذرة الفول البلدى أدى إلى نقص الإجهادات المحسوبة الأمر الذى ساعد بذور الفول البلدى على تحمل الإجهادات بدرجة أعلى من بذور فول الصويا والحمص . ليس فقط الإجهاد المستخدم والمحدث للضرر الداخلى هو العامل المؤثر فى مستوى الإصابة ولكن أيضا الجزء من البذرة المقابل للإجهاد وأيضا المساحة من البذرة التى يحدث لها الإجهاد هى عوامل مؤثرة يجب أن تأخذ كثير من الإهتمام عند قياس الإجهاد الفعلى الحادث . كان آداء طريقة الصبغ بصبغة الفاست جرين فى فحص البذور ذات الشروخ منخفضا بينما أرتفع آدائها ليتساوى مع الطرق الأخرى عند فحص البذور المكسورة لبذور الفول البلدى وفول الصويا .أظهرت طريقة الفحص الظاهرى والفحص بأشعة إكس والصبغ بصبغة التترازوليم قدرات متساوية فى فحص البذور ذات الشروخ وذات الكسر فى الأنواع البقولية الثلاثة . كما وجد إرتباط سالب وقوى بين البذور المكسورة والنسبة المئوية لقوة البادرة وكذلك النسبة المئوية للإنبات فى الأنواع البقولية الثلاثة ، بينما وجد إرتباط موجب من جهة أخرى بين النسبة المئوية للبذور الميتة ونسبة الكسر الحادث فى البذور فى الأنواع البقولية الثلاثة . كانت الإختلافات ضيقة بين طرق الفحص (الظاهرى - أشعة إكس - التترازوليم) الأمر الذى يوضح تقارب فعاليات التنبؤ بجودة البذور وكذلك فحص البذور للضرر الداخلى لهذة الطرق .