

# A New Cheap and Efficient Single-Grain Shatter Tester for Use in Rice Breeding

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## ABSTRACT

Grain shattering, also called grain shedding, is the relative ease with which rice (*Oryza sativa* L.) seed is dislodged from the pedicel at maturity. Several qualitative methods have been used to assess grain shattering and they tend to underestimate yield loss. Equipments available for quantitative assessment of grain shattering are mostly laboratory based. However, a recently developed prototype in Japan for fieldwork can be used, but the cost is prohibitive. An improved grain-shattering tester that uses a hand-held device has been developed and reported here. It is an improvement over the stationary laboratory device. It has the following modifications: a crocodile clip instead of a bench clamp and a helical spring for testing tensile material instead of tension gauge. The new device is affordable, handy for fieldwork, and effective for large experimental trials. This equipment was designed and developed at the University of KwaZulu-Natal (UKZN) mechanical workshop (Pietmeritzburg, South Africa). Its efficiency was tested at the National Crops Resources Research Institute (NaCRRI) at Namulonge, Uganda. The efficiency was tested by first estimating yield loss in selected test plants using a method that involves shaking panicles. This finding was compared with the minimum force required to dislodge the grains using this new device and a high correspondence of  $R^2 = 99.9\%$  was found. This new equipment has therefore been recommended for field assessment of grain shattering.

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**Abbreviations:** ARC, Africa Rice Center; PVC, polyvinyl chloride; UKZN, University of KwaZulu-Natal.

**G**RAIN SHATTERING is a serious problem that occurs at the end of the growth cycle of rice (*Oryza sativa* L.), but it has received little attention in comparison with other constraints that emerge early in the crop's growth cycle. Part of the reason for this is a lack of quality data on yield loss caused by shattering. Yield losses exceeding 40% have been reported when susceptible cultivars are grown (Jacobi et al., 1974; Sahu and Sahu, 1981; Agarwal et al., 1989). Current advances in rice breeding that involve crossing of *O. sativa* with its relatives that have high shattering ability have caused the grain-shattering problem to reemerge (Fukuta, 1995a).

Precise estimation of grain shattering in cultivated rice is important because it helps in ranking grain shattering among other constraints, estimating genetic parameters for the trait, and selecting cultivars with acceptable shattering ability. The trait is controlled by genetic, morphological, and physiological factors. The genetic component is quantitative in nature (Fukuta, 1995b). Better understanding of its transmission would require quantitative assessment of shattering ability in a population segregating for the trait.

To date, methods of estimating grain shattering have been subjective in nature. The methods that are less influenced by

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environmental conditions are laboratory based, but the equipment is not portable and therefore not suitable for field evaluation. Currently, seven methods are available to assess grain shattering ability. In the first method, panicles are shaken and grains that fall off are recovered, counted, and grouped (Ramiah and Rao, 1953). However, because of variation in the density of the panicles, the impact of shaking is liable to be variable. The second method involves harvesting panicles and placing them on a smooth wooden board with an incline toward a horizontal board. The tip of the board is fixed facing downward to allow grain recovery (Rao, 1935). A glass roller is released and allowed to roll over the panicles. Shattering is computed from the ratio of the number of grains detached to total number of grains on the panicle. The third method involves counting grains that fall off freely 30 d after anthesis. In this method, the number of scar marks and non-grain-bearing pedicels are assumed to have shattered. This method is commonly used in screening grain shattering in cereals. The fourth method involves dropping panicles from some height on to a hard surface, and the grains that get dislodged from inflorescence are counted (Ramiah and Rao, 1953; Sahu and Sahu, 1981). This method has been improved in that panicles were placed on a wooden frame, which is released from a spring so that it strikes an inclined board (Jacobi et al., 1974). The effect of varying panicle densities is reduced. Both the third and fourth methods are still used in laboratory work.

The fifth and sixth methods were developed by Ichikawa et al. (1990). The fifth method involves modification of a conventional rice thresher. Panicle stalks are cut to a length of 500 mm from the top. The stalks are divided into standard size units, which are then manually fed into the testing device. It is suitable when large sample sizes can be used for assessment but it is cumbersome for field experiments. The sixth device measures the force required to separate grains from panicles directly. This is a static test of shattering ability, but it is also too cumbersome for field experiments. The seventh equipment is a digital breaking strength device described by Li et al. (2006). It uses a digital force gauge (FGC-1B; NIDEC-SHIMPO Co., Kyoto, Japan) and is operated by attaching it upside down to a stand (FGC-50L) (Li et al., 2006). It is an improved version of the equipment developed by Ichikawa et al. (1990). Rice panicles from the main stem or primary tiller are harvested and fixed upside down to the force gauge using a flat chuck tensile grip and each grain is pulled down by forceps, and the maximum tensile strength is measured at the moment of pedicel breakage. This is the most accurate method but it is not practical for field screening of large numbers of genotypes. It is not **commercially produced**.

The new grain shattering equipment reported here is a modification of the device developed by Ichikawa et al. (1990). It was designed, constructed, and tested at the University of KwaZulu-Natal (UKZN; Pietmeritzburg,

South Africa). The modifications are (i) a single clip for holding the grain instead of two holding clips for the grain and the detached panicle, (ii) the panicle is not detached from the plant, and (iii) a helical spring for testing tensile material instead of a tension gauge is used.

## CONSTRUCTION AND OPERATION OF THE GRAIN SHATTERING TESTER

The assembled equipment is shown in Fig. 1. Its construction involves the following steps:

- Make a helical spring from a 0.5-mm diameter spring wire and estimate the pulling force for minimum and maximum tensile force it can estimate using a standard laboratory force gauge. The force required to pull the string is adjusted by altering spring diameter until it measures the pulling force in range of 0 to 40 m kg s<sup>-2</sup>.
- Fix the helical spring of spring constant 10-newton force (10 m kg s<sup>-2</sup>) on to a polyvinyl chloride (PVC) block machined into a groove to accommodate the spring. The other end of the spring is fixed onto a crocodile clip type K260.
- Attach an adjustable cork onto one end of the spring to allow estimation of force required to pull the spring.
- To calibrate the equipment, different weights in units of 5 m kg s<sup>-2</sup> are attached to the suspended end of the spring and the distance the cork moves to is carefully marked on an aluminum pocket that covers the spring. The corresponding value is engraved on the aluminum cover. This operation is conducted under minimum air resistance in the laboratory.

In operating this device, the adjustable cork is set to zero mark and the clip is allowed to clamp a single grain. The pedicel of the clamped grain is held firmly with one hand and the other hand used to pull the grain off the pedicel. The whole equipment is held parallel to the longest axis of the seed before pulling. The grain is then gently pulled until it snaps and the minimum force read off. The general posture for grain testing is shown in Fig. 2.

## TECHNICAL DETAILS OF THE GRAIN SHATTER TESTER

The technical detail of the single grain rice-shattering device is in Fig. 3. The first part of this detail is about tensile-adjustment gauge (front view) (Detail A) and side view (Detail C). It is made up of a PVC engineering block. The cork slides on a smooth aluminum surface with graded marks. A crocodile clip, type K260, is attached at the end of the pulling rod (Detail B).

## APPRAISAL OF THE EQUIPMENT

To test the performance of the UKZN-designed equipment, 10 rice genotypes with known shattering ability based on hand squeeze and shaking method were tested.

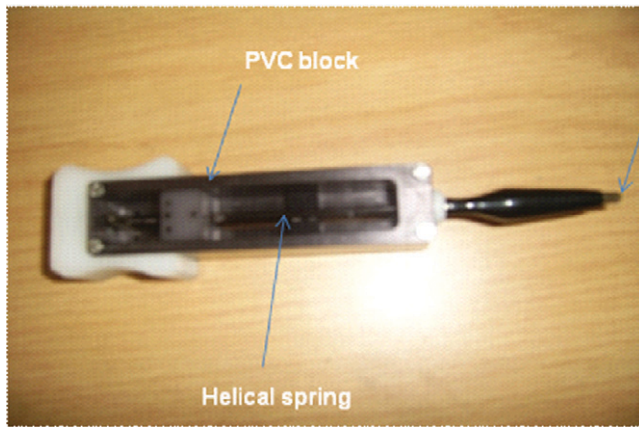


Figure 1. Photo of assembled grain shattering equipment.



Figure 2. Demonstrating use of grain shattering tester in the field.

Three sets of experiments were conducted at Namulonge in Uganda 2009 season 2, 2009 season 2, and 2010 season 1 to estimate the effect of the environment on grain shattering. Each experiment was laid out as randomized complete block design with two replications. The same set of experiment was used to test grain shattering ability by (i)

estimating minimum force required to dislodge the grains using the UKZN new equipment, (ii) shaking the panicles, and (iii) estimating the grain loss. In this method, 10 ripened primary panicles at 30 d after anthesis were carefully

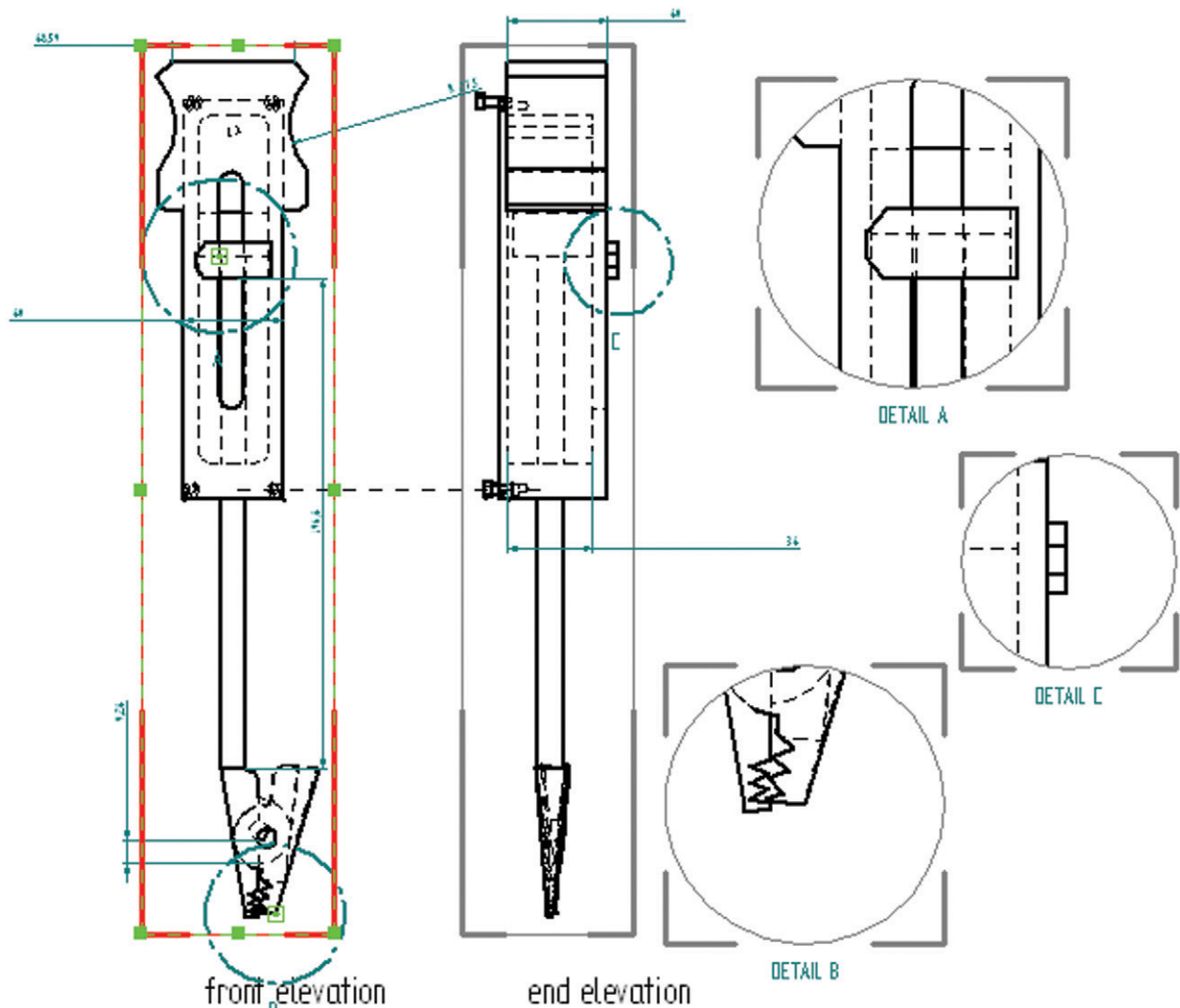


Figure 3. Detailed sketch of the single grain shattering tester.

**Table 1. Mean squares for gram force required to dislodge rice grains from panicles and yield loss of ten rice varieties evaluated in the field at Namulonge in Uganda.**

Source of variation	df	Minimum gram force to dislodge		Percent yield loss	
		Mean square	p-value	Mean square	p-value
Variety	9	149.98	0.001	151.24	0.001
Season	2	54.51	0.001	243.83	0.001
Variety × season	18	2.66	0.001	2.30	0.002
Error	31	40.1		79.2	

harvested and placed on a clean sheet. Each panicle was gently squeezed and the panicle pulled out slowly. The grains that were dislodged were counted. All the remaining grains were also harvested and counted separately. The grain-shattering degree was the ratio of grains recovered to total number of grains in each panicle. Percentage grain-shattering ability for each genotype was calculated.

To record shattering using the new grain shattering tester, five plants from each of the 10 were sampled. The panicles of the primary plant were tagged on the date when 50 to 60% of the inflorescences had reached anthesis. Tagging was done between 1000 and 1500 h, the time when most inflorescences flowered at Namulonge in Uganda. All the florets that flowered earlier along with those that had not flowered on the date tagged were carefully excised off the peduncle immediately after tagging. The anthesis date was considered to be the day when the middle portion of the panicle length had flowered. Shattering was recorded on 10 seeds per panicle on the 30th day after anthesis. Overall, 50 records were taken per plot.

Results indicated significant, at  $p = 0.05$ , differences between varieties for both minimum forces required to dislodge grains from the panicles and yield loss for season, cultivar and season × varieties interaction (Table 1). The minimum force required for each cultivar, the corresponding mean yield loss for 2010 season 1, and the standard grouping of the cultivars according to African Rice Center (ARC) seed import notes are shown in Table 2. In this table, three lines considered to be high shattering according to ARC notes required less than 3 m kg s<sup>-2</sup> for dislodging grain using the single-grain tester and yield loss of >50% was recorded. Genotypes that were nonshattering required more than 10 m kg s<sup>-2</sup> for dislodging their grain and less than 20% yield loss was recorded. When the percent yield loss was regressed on minimum force required to dislodge the grains ( $R^2 = 0.99.9\%$ ), there was high correspondence in the way the two methods estimated the importance of grain shattering (Fig. 4). This new equipment was, therefore, able to discriminate among the populations for grain-threshing ability and strongly corresponded with the standard grouping conducted by African Rice Center.

Generally, the minimum force required to dislodge grains varies with rice populations and methods used to dislodge the grains. According to Ichikawa et al. (1990), the minimum force required to dislodge nonshattering grains was 18 m kg s<sup>-2</sup> in the populations they tested using their laboratory grain shattering tester. In another study, mean force required to dislodge nonshattering in rice populations from Japan was 20 and 15 m kg s<sup>-2</sup> for lowland and upland rice types, respectively (Fukuta, 1995b). A study involving a collection of rice from India

**Table 2. Mean shattering degree (m kg s<sup>-2</sup>) and yield loss (%) for 10 reference rice types for 2010 Season 1.**

	NERICA† 6	FKR 14	WITA 3	WITA 7	WITA 12	WAB 56-104	WAB 365-B-1H1-HB	IDSA 10	WAB 56-39	FKR 41
Shatter degree										
Mean	23.5	26.0	27.5	29.0	37.0	65.0	106.0	108.0	117.0	128.0
Standard error	1.7	0.7	1.1	1.3	2.8	7.1	6.6	6.0	5.6	6.7
Standard deviation	11.7	4.9	7.6	9.3	19.7	50.0	47.0	42.4	39.9	47.3
Range	50.0	25.0	25.0	25.0	75.0	175.0	175.0	175.0	175.0	175.0
Minimum	0.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	50.0	50.0
Maximum	50.0	50.0	50.0	50.0	100.0	200.0	200.0	200.0	225.0	225.0
CV %	3.3	1.4	2.2	2.6	5.6	8.9	13.4	12.0	11.3	13.4
Yield loss										
Mean	6.5	6.4	6.5	5.7	3.3	2.9	2.0	1.7	1.4	1.2
Standard error	3.5	3.1	4.3	5.2	2.3	2.5	2.5	1.9	1.1	0.6
Standard deviation	11.1	9.8	13.8	16.5	7.2	7.9	8.0	5.9	3.5	2.0
Range	3.2	2.9	5.1	5.3	2.6	2.6	2.9	1.6	1.0	0.6
Minimum	5.2	5.1	3.8	4.0	2.1	2.1	0.1	1.0	0.8	1.0
Maximum	8.4	8.0	8.9	9.3	4.7	4.7	3.0	2.5	1.8	1.6
CV %	7.9	7.0	9.8	11.8	5.2	5.7	5.7	4.2	2.5	1.4
Africa rice rating										
	Shatter	Shatter	Shatter	Shatter	Normal	Normal	Not shatter	Not shatter	Not shatter	Not shatter

†NERICA, New Rice for Africa (Cotonou, Benin); FKR, Farakouba Rice (Farakouba, Bukina Faso); WITA, West African Rice Development Association (WARDA) at IITA rice (Ibadan, Nigeria); WAB, WARDA-ADRAO (Association Pour le développement de la riziculture en Afrique de l'Ouest) Bouaké (Bouaké, Cote d'Ivoire); IDSA, Institut des Savannes (Bouaké, Cote d'Ivoire).

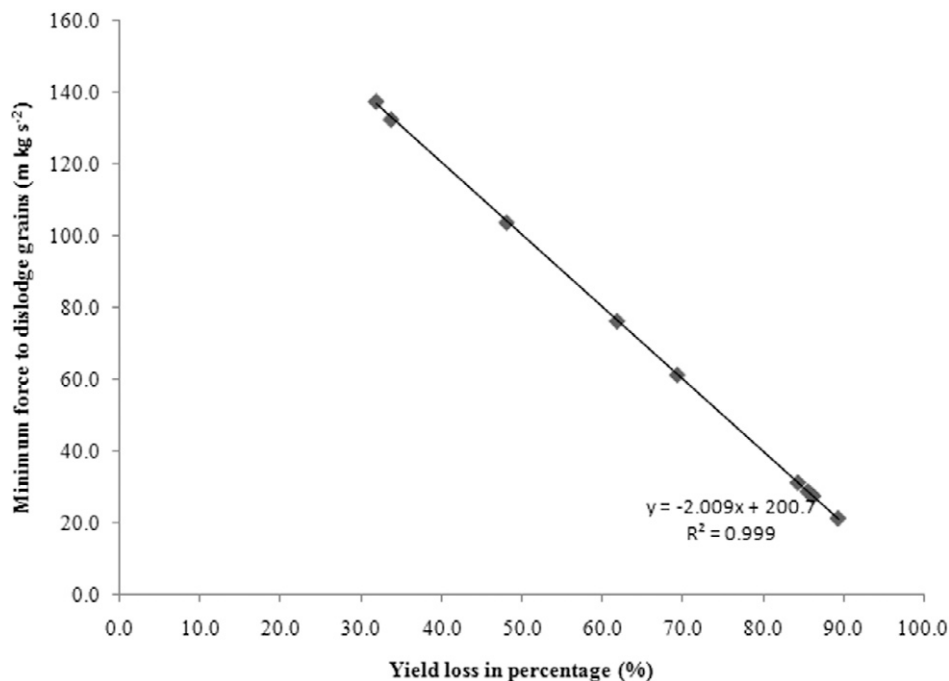


Figure 4. Regression of grain dislodged by hand to minimum force required to dislodge the grains.

and China showed that the maximum force required to dislodge shattering grains was  $5 \text{ m kg s}^{-2}$ , while the minimum force required to dislodge the nonshattering grains was  $7 \text{ m kg s}^{-2}$  (Fukuta, 1995b). This new equipment measures minimum force required to dislodge the grains in the range of 0 to  $40 \text{ m kg s}^{-2}$  which caters for all the ranges for shattering degree of rice in general and it could be used in grain-shattering studies under field conditions.

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