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RESEARCH ARTICLE

CYTOPLASMIC INHERITANCE IN RICE GRAIN SHATTERING.

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Abstract

Rice production in Africa and the world in general has reached a stage where its need is felt like never before. Demand for the grain far exceeds production in recent times, especially in sub-Sahara Africa. Thus, there is the need to find a way to maximize the production of the crop. Ways to prevent pre-harvest and post-harvest losses of the crop must be taken seriously in order to increase the amount of the grains farmers produce. Grain shattering is one of the problems associated with pre-harvest losses of rice. Many genes have been discovered to play key roles in rice grain shattering. We performed an experiment to find the behaviour of the grain shattering gene by studying the expression of the shattering trait in F_1 plants generated from parental reciprocal crosses. The result of this work revealed a highly significant difference ($P<0.01$) between the F_1 plants in terms of percentage grain shattering. This is an indication that, apart from the nuclear genes responsible for major expression of the rice grain shattering trait, cytoplasmic genes, probably located in the mitochondria or chloroplast or both, could also influence the expression of the grain shattering trait in rice.

Introduction:-

Rice is the world's most important cereal crop (Kush, 2013). It represents the main food crop and calorie intake for one-third of the world's population (Courtois et al., 2009). In Africa, only 54% of total rice consumption is produced locally (Muthayya et al., 2014). This low production of rice in Africa is due to factors such as limited land area, climatic factors, water supply factors, farming practices and socio-economic factors (Degleh, 2013).

Grain shattering is one of the main causes of yield loss in rice (Kato, 2008). Such yield loss can cause drastic reduction in farmers' income. The gene controlling rice grain shattering is a QTL (Ji et al., 2006; Konishi et al., 2006; Lin et al., 2007; Zhou et al., 2012). However, different researchers have reported various categories of genes at various chromosomal positions that are responsible for grain shattering in rice. There are a number of loci that control grain shedding in rice with major and minor effects (Konishi et al., 2006). Kinoshita (1989) reported at least five genes that are involved in the control of grain shattering in rice. Four of these predicted genes have been mapped in the rice genome. They are, *sh1* on chromosome 11 (Nagao and Takahashi, 1963), *sh2* located on

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chromosome 1 (Oba et al., 1990), *Sh3* on chromosome 4 (Eiguchi and Sano, 1990; Nagai et al., 2002) and *Sh4* on chromosome 3 (Fukuta et al., 1994; Fukuta and Yagi, 1998). Nonetheless, only two of the possible rice shattering genes have been cloned (Thurber et al., 2010). They are, *qSH1* (Konishi et al., 2006), and sh4 or *SHA1* (Lin et al., 2007; Li et al., 2006). This indicates that a lot more work needs to be done in understanding the inheritance of the rice grain shattering trait.

To date, no research has been conducted to prove that some of the grain shattering genes that influence the expression of the grain shattering trait are found on either the chloroplast or mitochondrial DNA or both, all of which are found in the cytoplasm of the rice cell. However, Lamo (2010) detected maternal influence on rice grain shattering when the researcher crossed shattering genotypes with non-shattering ones, conferming the results obatained from this study.

The objective of this study was to find out whether there are other genes controlling the expression of the rice grain shattering trait apart from nuclear genes. The preliminary research we have conducted using a shattering and non-shattering rice lines has proven that cytoplasmic genes are involved in the inheritance of the rice grain shattering trait.

Further understanding of the genetic control of this trait is a key factor in managing the rate of rice grain shattering on the field. It will also aid in the breeding of moderate to low shattering rice varieties in order to reduce yield loss due to shattering.

Materials and Methods:-

Plant Material

A non-shattering rice cultivar, ARCCU12Fa1L6P7-3-14-1-1 developed by AfricaRice Research Centre (Cotonou, Benin) was used as the non-shattering parent. This line is an upland rice cultivar that is intermediate in height, has very high tillering ability and very difficult to thresh (IRRI, 2013).

CRI-138-13, the shatttering parent, is a stable rice accession that was obtained from a cross between ARCCU12Fa1L6P7-3-14-1-1 and ARCCU3Fa7L16P5-B-B-1. ARCCU3Fa7L16P5-B-B-1 is also an upland rice cultivar developed by AfricaRice Research Centre. This accession is similar to ARCCU12Fa1L6P7-3-14-1-1 in terms of morphology. However, ARCCU3Fa7L16P5-B-B-1 is moderately difficult to thresh (IRRI, 2013). At F_3, CRI-138-13 was noticed to exhibit higher degree of shattering, and was therefore subjected to further selfing to obtain a stable shattering line. Thus, a cross between the difficult to thresh line, ARCCU12Fa1L6P7-3-14-1-1 (from now on, shortened as ARCCU12) and the shattering line, CRI-138-13 was used for the study.

Generation of F_1 Reciprocal crosses

Fifty parents each of the shattering and non-shattering parental lines were planted in medium-sized plastic containers using clayey-loam soil. This was done after the rice seeds were pre-germinated on tissue papers and transferred into plastic containers when the seedlings were 5 days old. Ten seedlings were planted per container in clayey-loam soil. The seedlings were later thinned at one seedling per bucket when they were 21 days old. Irrigation was done when the water level in the buckets went low. N. P. K (15:15:15) was applied at the rate of 100 kg N ha^{-1}, 25 kg P ha^{-1} and 100 kg K ha^{-1} respectively in two split applications in each bucket (Ezui et al., 2010). The first application was done two weeks after planting and the second was done five days before panicle initiation. The systemic pesticide, Dimethoate was applied 7 days after translating to control stem borers. Weeding was done by the hand-picking method whenever weeds were seen growing in the buckets.

Before anthesis – which happened at 66 days after planting for ARCCU12Fa1L6P7-3-14-1-1 and 57 days after planting for CRI-138-13 – selected panicles from both parents were emasculated to produce only female flowers. These were later pollinated using pollen from the opposite lines. In all, 100 viable seeds were produced from each of the reciprocal crosses. The resulting seeds were then planted in buckets and arranged in randomized complete block design with three replications. The partial diallel mating design was used for all crosses (Acquaah, 2012). All cultural practices pertaining to the planting of the parental lines were repeated in generating the F_1 lines.

Grain shattering in both the parentals and the F_1s were measured in 8 periods. Thus, percentage shattering data were taken at 5 DAH, 10 DAH, 15 DAH, 20 DAH, 25 DAH, 30 DAH, 35 DAH and at harvest. Maturity date (harvest date) was determined using descriptions in the Standard Evaluation System for Rice. Similarly, percentage

shattering was obtained by grasping and pulling the hand over the selected panicle, and counting the number of rice grains that fell due to the applied pressure to the total number of grains present on the panicle (IRRI, 2013). To find out whether there was significant difference between the mean percentage shattering among the F_1 reciprocals, the student's t-test was performed using the mean percentage shattering obtained from the two crosses.

Results and Discussion:-

The results showed extreme shattering levels for the parental lines. The non-shattering line, ARCCU12 did not shatter at all from 5 DAH to 30 DAH. At 35 DAH, only 0.1% of the rice grains shattered. Finally, at harvest, it recorded an average shattering rate of 0.3%.

On the contrary, the shattering parent, CRI-138-13 shattered as early as 5 DAH (18.05%). At 35 DAH, an average of 87% of the rice grains shattered, and at harvest, 97.21% of the grains shattered (Fig 1).

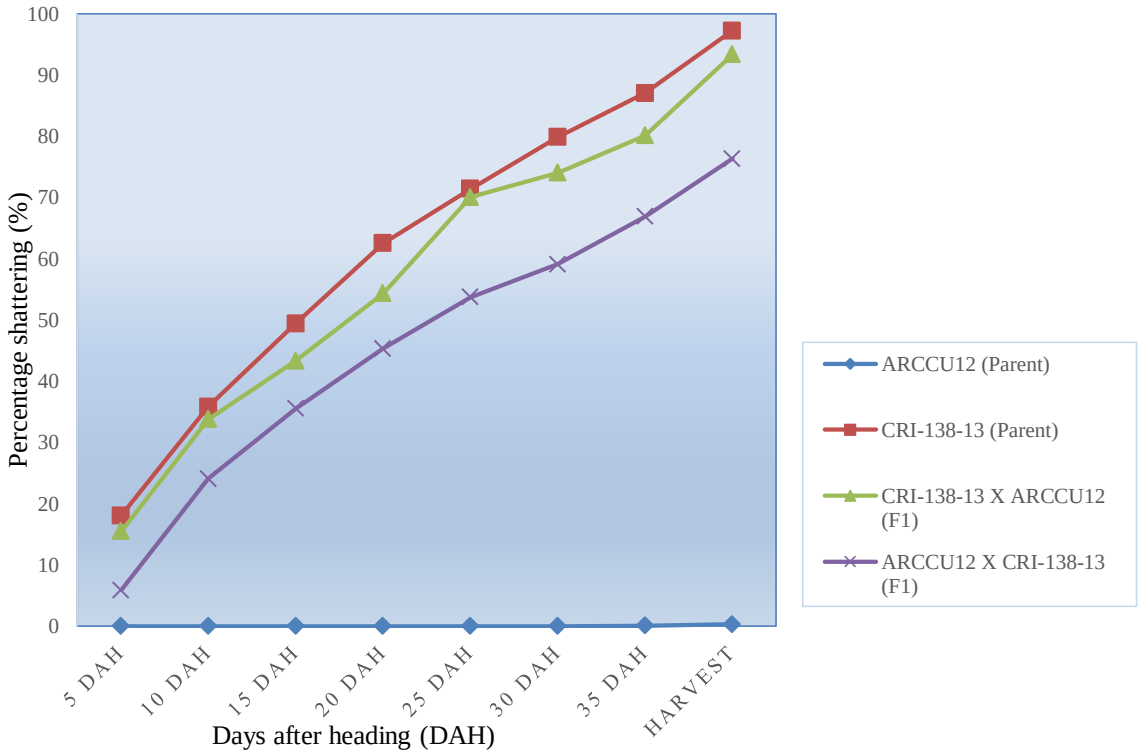

Fig 1:-Percentage shattering of the parentals; ARCCU12 (non-shattering), CRI-138-13 (shattering), and the reciprocal F_1 plants; ARCCU12 x CRI-138 and CRI-138-13 x ARCCU12.

The F_1 reciprocal crosses showed different rates of shattering. At 5 DAH, the F_1 of which the shattering parent, CRI-138-13 was the female (CRI-138-13 x ARCCU12) produced 15.49% average shattering. This rose to 54.34% at 20 DAH. At harvest, it had reached 92.89%. On the other hand, the F_1 of which the non-shattering parent ARCCU12 was the female (ARCCU12 x CRI-138-13) – recorded an average of 5.87% shattering at 5 DAH. At 20 DAH, it recorded an average of 45.32% shattering, and at harvest, the rate of shattering had reached 74.76%.

Student's t-test was performed to determine whether there was significant difference between the average shattering of the two categories of F_1 reciprocals. Table 1 shows the mean shattering data that was taken at harvest from three blocks.

Table 1:-Mean percentage shattering at harvest of 200 F_1 plants produced from reciprocal crosses between CRI-138-13 and ARCCU12.

Cross	Mean (%)	s.d	s.e
ARCCU12 x CRI-138-13	74.76	2.24	0.224

CRI-138-13 x ARCCU12	92.89	1.22	0.122

sd: Standard deviation of n= 100, se: Standard error of n= 100.

The mean percentage shattering of the 100 F_1s from which the non-shattering parent ARCCU12 was the female produced 74.76% whilst the cross from which the shattering parent CRI-138-13 was the female produced 92.89% shattering at harvest. The cross between ARCCU12 and CRI-138-13 had 2.24 and 0.224 as standard deviation and standard error respectively, whilst the cross between CRI-138-13 and ARCCU12 had 1.22 and 0.122 as standard deviation and standard error respectively.

Student's t-test was performed on the recorded data to find out whether the observed differences among the F_1 plants in terms of percentage shattering was significant. The results of the test are shown in table 2.

Table 2:-Results of t-test performed on the 100 CRI-138-13 x ARCCU12 F_1 plants and the 100 ARCCU12 x CRI-138-13 F_1 plants in terms of mean percentage shattering at harvest.

Test Statistic	**CRI-138-13 x ARCCU12**	**ARCCU12 x CRI-138-13**
Mean	92.89	74.76
Variance	1.4897	5.0061
Observations	100	100
Hypothesized Mean Difference	0	
Df	153	
t Stat	71.14150645	
P(T<=t) two-tail	3.8006E-119	
t Critical two-tail	1.975590315	

There was highly significant difference ($P<0.01$) between the rate of shattering of the F_1 plants (ARCCU12 x CRI-138-13) and that of the F_1 plants (CRI-138-13 x ARCCU12) in both the one-tailed and two-tailed t-tests.

The results obtained showed that the two parental lines CRI-138-13 and ARCC12, exhibited shattering characteristics that allows the study of the shattering trait among the two accessions. According to Beavis (1994, 1997), quantitative trait analysis must have parental lines that exhibit extremes of the trait(s) under study.

The F_1 reciprocals shattered per the results obtained (Fig 1), but the rate of shattering differed among the two reciprocals. The student's t-test performed from the harvest data obtained from the reciprocal crosses showed highly significant difference ($P<0.01$) in terms of grain shattering. This suggests that there are maternal effects associated with the inheritance of the grain shattering trait in rice.

Two main organelles in the cell harbour cytoplasmic chromosomes. Thus, the chloroplast and mitochondria (Nakazono and Hirai, 1993). The differences in the expression of the shattering trait is therefore attributed to the influence certain genes on the mitochondria or the chloroplast or both have on the expression of the trait.

The chloroplast genome of rice comprise a single circular DNA that is divided into large single copy (LSC) and small single copy (SSC) which together contain over 162 predicted genes and open reading frames (ORFs). Comparing the chloroplast genomes of the Asia rice (*Oryza rufipogon*) which tends to shatter to that of cultivated rice (*O. sativa*) shows the two varieties have 68 nucleotide changes either in the form of SNPs or indels (Waters et al., 2011). It is therefore be possible that some of these nucleotide changes might contribute to differences in the rate of shattering among rice varieties.

The mitochondrion of the rice plant also contains 490,520 bp which comprise 3 rRNAs, 17 tRNAs, 5 pseudo tRNAs, 11 ribosomal protein genes and 2 pseudo ribosomal proteins (Notsu et al., 2002). Thus, changes in the expression of any of the above-mentioned genes can contribute to the changes in the expression of the shattering trait in rice. The results of this research will help open into future research, to find out the specific gene(s) in the cytoplasm of the rice plant that influence(s) the expression of the rice grain shattering trait.

Conclusion:-

There is a highly significant difference ($P< 0.01$) among the reciprocal F_1 plants in terms of percentage grain shattering. Since the F_1 plants that had the shattering parent (CRI-138-13) as the female parent shattered more than

the F_1 plants that had the non-shattering parent (ARCCU12) as the female parent, it can be concluded that genes other than nuclear genes also influence the expression of the rice grain shattering trait. It will therefore help a great deal if further research is conducted to ascertain the exact types of genes and their location in the cytoplasm that influence the expression of the rice grain shattering trait.

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