

**IDENTIFICATION OF THE GENE SRWD2 INVOLVED IN SALT TOLERANCE IN
SELECTED RICE (*Oryza sativa* .L.) LANDRACES AND VARIETIES IN NIGERIA**

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ABSTRACT

Salinization of soil is one of the major factors limiting crop production particularly in saline prone coastal regions. It reduces the growth of glycophytes and occupies a prominent place amongst soil problems that threaten the sustainability of Agriculture. Among PCR based markers, -SSR markers are more popular in rice because they can detect high level of allelic diversity and have been extensively used for selection of salinity tolerant genotypes of rice. This study is based on identifying salinity tolerant genotypes using Simple Sequences Repeat marker(SRWD2) was used to identify salinity tolerance in two conventional varieties (faro 44 and faro 67) in addition to three landraces; (C-price, CHI and Iron). The marker- SRWD2 was designed and used to identify the candidate gene SRWD2 at 150 bp in the local landrace; iron. Others did not reveal any amplification for the allele carrying the resistant gene (SRWD2). The landrace is then recommended for use as a potential donor for rice breeding programmes. The genotypes could be further tested in saline areas to observe yield potential for developing high yielding genotypes suitable for saline areas. The markers used in this study is capable of detecting major gene locus for plants breeders to develop new salt tolerant genotypes.

INTRODUCTION

Rice (*Oryza sativa* .L.) is the most important staple food crop of the world. This is because it is the major source of calories of more than half of the total global population and occupies almost one-fifth of the total land area covered under cereals (Sasaki,1999; Vaughan et al, 2003). The centre of origin of rice is believed to be South East Asia for *Oryza sativa* and *Oryza glaberrima* which originated in Africa (IRRI 2008). Rice belongs to the grass family *Oryzaeae* and is one of the leading food crops in the worlds population in Asia, and it provides 20% of the per capita energy, and 13% of the protein consumed worldwide (Juliano,1994). Approximately 11% of the worlds arable land is planted to rice annually and ranks next to wheat Rice is grown in more that 100 countries under widely differing climatic conditions and is particularly productive in tropical regions. However, one of the most significant factors in the vegetative and reproductive stages that hinder the growth and production of rice is arable soil salinity

(Majakkir et al; 2015). Stalinization has a detrimental effects on agricultural production farmers loving conditions, the economy at various levels and ecosystem balance including the quality of natural resources (FAO 2019).

Salinity decreases the ability of plants to absorb water, resulting in growth reduction. Mukta *et al* (2017) and Yichie *et al* (2018) reported that modern rice varieties are highly sensitive to salinity- thereby reducing the production of rice. Rice yield in salt affected land is significantly reduced with an estimation of 30-50% yield losses annually (Enyard *et al* 2005). Salinity can lead to sterility in rice, particularly if imposed during pollen development and fertilization hence, high yielding salt tolerant rice varieties must have tolerance at the reproductive stage. Technologies that minimize the spread of salinization decrease salinity levels in crop fields or increase the salt tolerance of crops. To enhance the salt tolerance of crops, including rice, plants will have to undergo a variety of changes from physiological adaptations to gene expressions hence new genetic sources of tolerance. Khuat *et al*; 2017. Salinity tolerance breeding was possible after identifying Quantitative trait loci (QTLs) which allowed first integration of traits into modern high yielding varieties. Recent advancement in the field of molecular markers have made it possible to identify the individual salinity tolerance control genes which could promote rice selection especially for low inherited traits such as salinity (Aliyu et al, 2011).saline tolerance of rice is derived from the genes that limit the rate of salt uptake from the soil, and transport the salt throughout the plants. This adjusts the ionic, and osmotic balance of cells in roots and shoots and regulate leaf development and the onset of senescence (Munns 2005). SRWD (salt responsive WD 40 protein genes have been identified to be involved in rice salt tolerance. The SRWD2 sub family is highly expressed in the ovary, embryo, endosperm and seed-(Huang et al, 2008). Other genes in rice have also been identified to confer salinity tolerance in rice such as salt-L, RSSL, SCKL, Saltol etc. it is then relevant to develop salt tolerant genotype(s) that can sustain optimum yield level in saline stressed environment. The aim of the study is to identify prospective genotypes tolerant to salinity

by using SSR primers, screen the varieties for the presence of resistant gene-SRWD2.

2.0. MATERIALS AND METHODS

2.1. LOCATION:

Seeds were raised in nursery and transferred to polybags in field at the teaching and research farm of the faculty of Agriculture Imo state University-Owerri, which lies within 4°45'N and 7°15'N latitude and (6°50'E and 7°25'E) longitude of the South eastern rain forest agriecological zone of Nigeria (NMET2009). Owerri has an average annual rainfall of 2500mm, relative humidity of 26-28°C. leaves extracted from growing plants in field after two months of establishment were collected and taken to the regional centre for Biotechnology and Bioresources Research Centre-University of Port Harcourt Laboratory, Rivers State.

2.2. SEED MATERIALS;

Two varieties of upland rice faro 44 and faro 67 were sourced from National Cereals Research Institute-Badeji, in addition to three other landraces; iron, C-price and CHI landraces cultivated in Abakaliki area of Ebonyi State-Nigeria were sourced from local market in the area.

2.3. DNA EXTRACTION

DNA was extracted from the leaves of five rice cultivars using Zyro-Quick DNA plant/seed mini prepkit Beta-Mecaptoethanol was added to the genomic lysis beiffer to a formal dilution of 0.5% (v/v) i.e (500 uL per 100ml). 150mg of finely cut plant sample was added to a 1.5ml. Eppendorf tube containing Bashing Bead™ Buffer. The Eppendorf tube was centrifuged at 10,000xg for 1 minute. A volume of 400uL supernatant was transferred to Zymo-spin™ IIF filter in a collection tube and centrifuged at 800gm for 1 minute. After which the Zymo spin™ IIF filter was discarded. Geneomic Lyjis buffer (1200uL) was added to the filtrate in the collection tube from step 5 was transferred to Zymo-spin™ IIC at 10000g for 1 minute. Precisely 500uLg -DNA wash buffer was added to the Zymo-spin™ IIC column and centrifuged at 10,000x g for 1 minute. The Zymo-spin™ IIC column was transferred to a clean 1.5 NL micro centrifuge tube and 60uL of DNA

elution buffer was added directly to the column matrix. The content was centrifuged at 10,000 x g for 30 seconds to elute the DNA using a filter paper in a clean 1.5mL micro centrifuge and centrifuged at exactly 16000g for 3 minutes. The filtered DNA now suitable was used for PCR and other downstream applications. The qualitative and quantitative tests of DNA genomic were evaluated by agarosegel electrophoresis (1.5%) at the end of the running time, the DNA fragments were visualized under (UV) transiluminater (Gel Docementation micro DOCTM, Cleaver Scientific Ltd U.K) and nanophotometer (Nanodrop 2000c Spatrophotometer- thermofisher Scientific USA) with a wave length of 260nm. Then the DNA was calculated for purity with an absorbance ratio of 260/280 (A_{260}/A_{280}) and were documented.

2.4. DNA AMPLIFICATION ANALYSIS:

The PCR- DNA amplification used the master mix PCR kit (New England Biolabs, USA) with a final volume of 12.5 ul. the PCR program was set as follows; 35x cycles of amplification with initial denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute and extension at 72°C for 5 minutes, the thermocycle was set to hold the PCR mix at 4°C (Hanafiah et al 2021; Singh et al 2020). The primer set used (Amplicon size approximately 200bp in 5' → 3') comprised forward primer (F)5' C A T A A T T G T A C G G T A C T T C C T T 3' and Reverse (R) 5 T G C T G A A G T A A C A T G T G T A T G G -3'. The primers used in the study were custom-synthesized by inqaba Biotec, West Africa. Specific primer to detect salinity resistance (SRWD2) genes were identified after a through literature survey. The sequences of the primer as shown above, were evaluated using primer Blast tools (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>) to identify any non specific targets and to determine the specificity of the PCR assay.

3.0 RESULTS AND DISSCUSSION

3.1 DNA ISOLATION TESTS

The qualitative and quantitative tests of extracted genomic DNA was estimated using 1.5% agarose gel electrophoresis and this revealed a thick band in the upper positive just below the gel electrophoresis well (plate I)

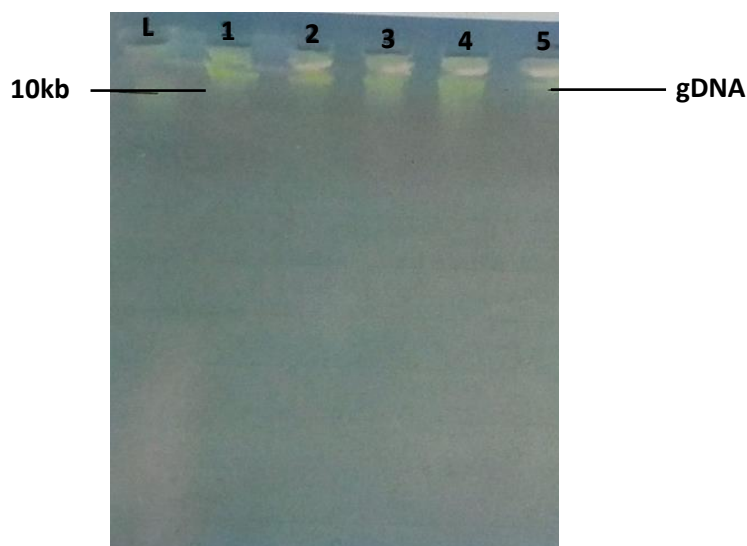


Plate I gel electrophoresis image of genomic DNA (gDNA) from five rice varieties, L=Ladder, Lanes 1, 2, 3, 4 and 5 represent rice varieties, Iron, faro 44, C-price, CHI and faro 67 respectively.

The integrity of bands obtained from the electrophoretic gel revealed the success of DNA genomic isolation. The presence of single band indicates that isolated genomic DNA is good enough for PCR template in the analysis of saline resistant gene (SRWD2) fragments. The quantity and purity of the DNA genomic is as shown below (table 1).

Table 1 Quality and Purity of Total DNA of Rice Varieties

S/N	Code	DNA Conc ng/uL	A260	A280	Purity index 260/280
1.	IRON	71.14	1.427	0.811	1.76
2.	FARO 44	95.6	1.912	1.105	1.73
3	C-Price	90.5	1.809	1.022	1.77
4	CHI	91.5	1.829	1.028	1.78
5	FARO 67	126.9	2.537	1.417	1.79

Table 1 reveals that genomic isolation yielded a purity of 1.73-1.79 and a concentration of 71.4-126.9 ng/uL implying that DNA genomes are free of contamination for PCR analysis.

3.2 DETECTING SALINITY RESISTANCE GENE (SRWD2)

Plate 2 below shows the results of gel image amplification of (SRWD2) gene in one of the varieties of rice.

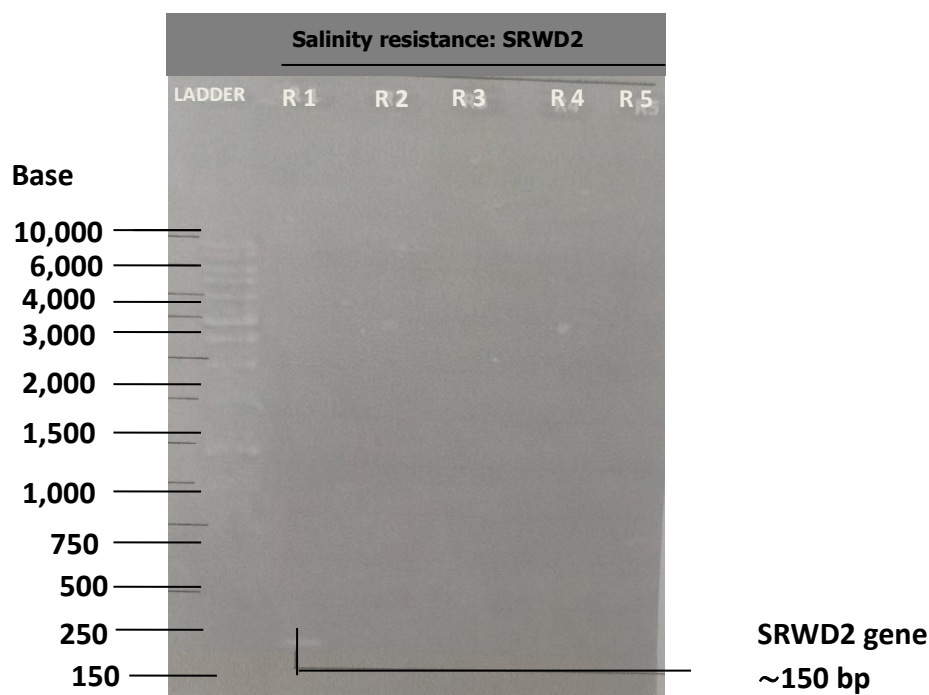


Plate 2 : Gel electrophoresis image of amplification of SRWD2 genes in five rice varieties R₁,R₂,R₃,R₄ and R₅ representing IRON, Faro 44, C-price CHI and Faro 67 respectively.

In examining of the candidate gene(SRWD2) in the five rice varieties, only one variety ;Iron, A landrace in Ebonyi State Nigeria, was found to have the candidate gene at 150 bp; while the other landraces did not reveal the presence of the gene. In a similar work, Khuat *et al.*, (2017) working on salt tolerances of Vietnamese rice landraces, reported that it is possible to amplify the candidate gene segment by 150 bp. They further observed the appearance of band 150 bp in only one variety. Motbui that carried the candidate gene SRWD2 in homozygote while the other three rice landraces were shown to be heterozygous for the genes and so were amplified at about 130 bp. using some SSR markers in screening rice genotypes for salinity tolerance Khatab et al (2022) observed that of their 21 SSR markers screened, three primers were monomorphic (RM1384, RM6990 and RM 6193) while their rest 18 primers were polymorphic. Some primers (RM6976, RM6193 and RM5565) that separated the tolerant and sensitive genotypes also distinguished five highly moderate salinity tolerant Egyptian genotypes including FL478, their Salt tolerance control adding that their rice genotype tolerant to salinity generate similar bands to FL478 and IRR1148- their salt tolerant control genotypes. Their bands had a molecular weight of 140, 170 and 150 bp that were found in the salt tolerant varieties, Titov et al., (2009) in their study used three primers in genotypic

screening of rice for salt tolerance, out of the three primers, only RM 336 distinguished nine out of 11 genotypes used and two as susceptible.

However, in this study, amplification of tolerance gene in iron was observed at 150bp so, the marker was monomorphic as it did not reveal any other amplification of the gene at 136 or 160 bp. Khuat et al (2019) reported polymorphism of the marker SRWD2 in their study since their tolerance gene showed amplification at 150bp and 136 bp bands for one and three varieties respectively, out of the 26 accessions in their study. In a similar review Randiv et al (2019) noted that some markers Rm 140 detected Saltol QTL at 260bp in some of their genotypes .Rm 1287 at 160 bp while Rm 3412 at 211 bp but that the marker Rm 562 at 243 bp indicated absence of resistance alleles in all their genotypes. They suggested that further study may confirm that some of their genotypes might have saltol QTL that can be used as alternative donors in tolerances rice breeding programmes.

CONCLUSION

DNA isolation yielded a purity range from 1.73-1.79 and a concentration of 71.4- 126.9 indicating good quality DNA free of contaminant. The marker (SRWD2) revealed amplification of the tolerance gene in only one landrace (IRON) at 150bp, while others did not. The marker would be a candidate

marker and the genotype an ideal one for further production of improved varieties that would be used to breed salt tolerant cultivars and higher potential yields in saline stressed environments. Additional marker for further evaluation of more landraces will most likely reveal genotypes with promising Saltol QTL genes that will all act as donor races for salinity breeding programmes in rice.

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