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Introgression of Stem Rust Resistance into Popular Kenyan Wheat Varieties to Improve Production

Tubei E. Jackline[1], Fredrick M. Njoka[2*], Omwoyo Ombori[1] and Peter N. Njau[3]

[1]Department of Plant Sciences, Kenyatta University, P.O.Box 43844, Nairobi, Kenya.
[2]Department of Agricultural Resource Management, University of Embu, P.O.Box 6-60100 Embu, Kenya.
[3]Kenya Agricultural and Livestock Research Organization, Njoro, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Authors TEJ and FMN designed the experiment. Authors OO and PNN prepared the original manuscript. All authors have read and approved the manuscript.

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ABSTRACT

Aim: The objective of this study was to assess the introgression of the stem rust resistance genes into the BC_1F_1 plants using molecular markers.
Place and Duration of Study: Field experiments were carried out in a nursery, at Kenya Agricultural and Livestock Research Organization (KALRO) Njoro Centre, Nakuru, Kenya at 0.3ºS and 35.9ºE and 2185 m above sea level from June 2012 to December 2013. Laboratory work was carried out in the Biotechnology laboratory, KALRO Njoro.
Methodology: Polymerase Chain Reaction (PCR) amplifications were carried out to assess the introgression of stem rust resistance genes into the BC_1F_1 plants. Analysis of genetic variation was done at each locus in terms of the number of alleles observed, heterozygosity, expected heterozygosity, Shannon's diversity index, genetic diversity and gene frequency using genetic analysis package POPGENE Version 1.32. The neighbouring joining method was used to construct a dendrogram from a distance matrix based on Sokal and Michener's genetic distances, using MEGA4 software 1in Power Marker 3.25.

**Corresponding author: E-mail: njokafm@gmail.com;*

Results: Amplification using PCR showed that wheat BC_1F_1 plants accessions NP3, NP12, NP89, NP163, NP192 and Kingbird were positive for the *Lr34* gene while the susceptible plants and Njoro Bw_2 were negative. Evaluation done on three molecular markers showed that CSLV34 was polymorphic across the BC_1F_1 plant accessions with a total of 2 alleles per locus. The 17 wheat BC_1F_1 plant accessions and their parents (Njoro Bw_2 and Kingbird) were placed into 2 clusters using the markers as a result of introgression, considering that the clustering in this study was purposively based on the presence or absence of the resistance genes. This indicates that the *Lr34* gene which is an APR gene was successfully introgressed into five BC_1F_1 accession plants (NP3, NP12, NP89, NP163 and NP192).

Conclusion: The resistant BC_1F_1 accession plants (NP3, NP12, NP89, NP163 and NP192) showed success in the introgression of the *Lr34* gene that gives resistance to stem rust at adult plant stage together with other minor genes including *Sr2* and *Lr46.* These can further be developed in the field, multiplied and the seeds distributed to the farmers as they showed low disease severity to stem rust disease.

Keywords: Stem rust; Ug99; resistance; backcross; improvement; disease severity.

1. INTRODUCTION

Wheat is the most significant source of carbohydrates in a majority of countries worldwide [1]. Wheat flour is used for making most baked food products and alcoholic drinks [2,3]. Wheat straw can be used as a source of fiber and forage for livestock, though to a limited extent [2]. The entire wheat grain is a source of vitamins, minerals and proteins, whereas starch is commonly used to replace some cosmetics and pharmaceutical products [4]. In order to cope with the rising rate of consumption, it is essential to breed for new high yielding cultivars, tolerant against biotic and abiotic stresses to ensure food security and to meet the challenges of the 21st century [5].

Wheat (*Triticum sp*) is an allopolyploid species containing three distinct homologous genomes A, B and D. Through spontaneous hybridization, the three genomes were brought together [6]. Based on the number of chromosomes, wheat has been divided into three groups (diploid, tetraploid and haploid). The basic number of chromosomes in wheat is n=7. For the diploid wheat (AA genome, 2n=14), the cultivated varieties are occasional with einkorn being the only example that is known to be cultivated [7].

Among the most threatening disease of wheat is stem or black rust, caused by the fungus *Puccinia graminis* [8], which causes a yield reduction of up to 100% during its outbreaks. Barley, wheat and triticale are affected by stem rust [5,9]. It causes a lot of damage as it attacks the leaf blade, the leaf sheath, stem and spike (head) of the plant. The fungus feeds on the sugar produced by the host plant, thus reducing yield and even causing plant death under severe infection [10]. The rust fungi are pathogens that constantly evolve to new races through migration, mutation and recombination among the existing genotypes [10]. The gene for gene hypothesis by [11] shows how stem rust resistance occurs. The host-parasite was first demonstrated by [11] who worked on the rust (*Melampsora lini*) of flax (*Linum usitatissimum*). Gene for gene relationship is a widespread and very important aspect of plant disease resistance. In every gene that confers pathogenicity in a pathogen, there is always a corresponding gene that confers resistance in the host [12-14]. The inheritance of both resistance in the host and parasite's ability to cause a disease is controlled by pairs of matching genes.

Use of genetic resistance for some decades, controls stems rust disease in wheat. If the spread is left unchecked though, it could bring about food shortages and famines in Africa, the Middle East and Asia [5,15]. The best strategy to enhance wheat production in these regions is the replacement of the currently prevalent cultivars which are susceptible, with resistant and high-yielding cultivars [5].

More than 50 stem rust resistance genes in wheat have been characterized and described [5]. Many of these genes are race-specific and several of them are currently used by breeders to develop new cultivars. The resistance provided by these genes is, however; short-lived as new races of stem rust pathogen (*Puccinia graminis tritici*) are continuously evolving and acquiring virulence against these genes [5]. In addition, cultivars with resistance based on a single race-specific gene are of limited agricultural use. An

example is that of wheat stem rust race Ug99, which has overcome almost every race-specific stem rust resistance gene in commercial varieties grown throughout the world [16]. Healthy plant up to three weeks before harvest has been reported to be reduced into a black tangle of broken stems and shrivelled grains when infected by these race (Ug99) [5]. This, therefore, requires the identification of additional genes which are resistant to Ug99. Since 70s of the last century, the stem rust problem in wheat has been solved through breeding varieties resistant to stem rust. The short-term solution, however, has been applying fungicides to the wheat crop to restrict the development of the pathogen which is often damaging to the environment [16]. Breeding to incorporate resistance genes into high yielding adapted varieties and new germplasm is necessary to reduce risks of major pandemics, and this requires knowledge on the sources of resistant genes, epidemiology and race differentiation of the pathogen [5].

The TTKSK (Ug99) race is currently a major threat to world wheat production and it is predicted to spread to the world's most important wheat growing regions in the near future [5,17]. Plant breeders and pathologists, however, still have time to identify resistant genotypes, introgress the genes and increase the seed for farmers before major problems occur in the Saudi Arabian Peninsula, South Asia and East Asia. Introgression of stem rust resistance genes into Njoro Bw_2 variety, using the Kingbird variety derived from CIMMYT germplasm as the donor parent has the potential to produce good results. The Kingbird variety has some adult plant resistance genes (non- race specific, polygenic genes) to Ug99 and matures early [18]. Njoro Bw_2 (recipient) is high yielding, drought resistant, but late maturing and susceptible to Ug99 [19].

Adult Plant Resistance (APR) genes are those which offer resistance at post-seedling stages [20] and the adult stage, offers resistance to several races of the stem rust pathogen and slows the development of rust in the field [21]. Since APR genes show partial responses, combinations of more than three genes are often required to attain the commercially acceptable level of resistance. Considering the occurrence of the new race of stem rust, with virulence for many of the known major genes for resistance in Eastern Africa, there is need to focus on finding new sources of adult plant resistance genes which are resistant to stem rust [22]. Combinations of *Sr2* APR gene with other resistance genes that slow the development of rust, usually known as the ''*Sr2*-Complex,'' provide the basis for long-lasting resistance [17,23]. The *Lr34* and *Lr46* genes are known as slow rusting genes to stem and leaf rust and in the Thatcher background, *Lr34* is associated with increased resistance to stem rust [17,24]. They provide durable and non-race specific adult plant resistance but their effect is more reduced than that of race-specific genes [24]. The *Lr34* is a gene present in many parent lines that have been used for breeding commercial varieties around the world.

Resistance gene pyramiding, planting of multiline cultivars and cultivar mixtures are strategies to deter evolution of virulent pathogens [25]. Wheat breeders can use slow rusting genes as a complement to race-specific genes. The available molecular markers can greatly facilitate the pyramiding process. The main objective of this study was to assess the introgression of the stem rust resistance genes into the BC_1F_1 plants using molecular markers.

2. MATERIALS AND METHODS

2.1 Experimental Site

Field experiments were carried out in a nursery, at Kenya Agricultural and Livestock Research Organization (KALRO) Njoro Centre, in Nakuru, Kenya at 0.3ºS and 35.9ºE and 2185 m above sea level from June 2012 to December 2013. It has an average annual rainfall of 939 mm (an average of 61 years) (Kenya Meteorological Station Identification Number 9031021) with an average daily minimum and maximum temperature of 9.7 and 23.5ºC respectively. Laboratory work was carried out in the Biotechnology laboratory, KALRO Njoro.

2.2 Plant Materials

The seeds of the 'Kingbird' variety were obtained from CIMMYT and Njoro Bw_2 from KALRO, Njoro Centre. The Njoro Bw_2 variety grown in Kenya is susceptible to Ug99, though it contains the *Sr2* gene. Kingbird is resistant to stem rust and known to have the *Sr2* gene with other slow rusting resistance genes that form the "*Sr2* complex" [26,27]. Kingbird was crossed with Njoro Bw_2 to obtain F_1 plants. The F_1 plants were backcrossed with Njoro Bw_2 to obtain a BC_1F_1 progeny [28].

2.3 DNA Extraction

Ten grams of fresh leaves of the parents and individual BC_1F_1 (240 plants) and parent plants grown in the nursery were harvested at three to four-leaf stage. The leaf samples were preserved in medical envelopes using silica pellets. Frontana and Hope varieties were used as positive controls for the presence of *Lr34* and *Lr46* gene, respectively. Samples of the eight most resistant and eight most susceptible leaves earlier harvested were selected from those plants scored as resistant 'R' and susceptible 'S' in the field experiments. DNA was extracted from the leaf samples of the parent plants, and from eight resistant and eight susceptible BC_1F_1 plants. Leaf tissue samples of 0.4 g were used to extract DNA using a modified method from [29]. The quality of the extracted DNA was checked by running in 0.8% agarose gel stained with ethidium bromide. The extracted DNAs were dissolved in 100 µl sterile deionized water and stored at -20ºC.

2.4 PCR Amplifications for Stem Rust Resistance Gene Markers

The DNA of the selected BC_1F_1 wheat plants and parents were subjected to polymerase chain reaction (PCR) and the markers GWM533, BARC80 and CSLV34, which are diagnostic for genes *Sr2, Lr46* and *Lr34* respectively, were used to assess introgression of stem rust adult plant resistance (APR) genes (Table 1). PCR amplification was carried out in a 12.5 µl volume reaction comprising of 10x PCR buffer, 6.25 µl *Taq* polymerase, 0.25 µl of 0.2 µM forward primer, 0.25 µl of 0.2 µM reverse primer, 0.75 µl of 1.5 mM $MgCl_2$, 4 µl of dH_2O (deionized water) and 1 µl of 50 ng of the template DNA.

The expected band size for *Sr2* was 120 base pairs (bp) [30]. The amplification conditions for *Sr2* gene were initial denaturation step at 94ºC for three minutes and then 45 amplification cycles. Each amplification cycle comprised of the denaturation step at 94ºC for sixty seconds, an annealing step at 60ºC for sixty seconds and extension step at 72ºC for two minutes. The final extension step was set at 72ºC for ten minutes [30].

The expected band size for *Lr34* gene was 150 bp and a 229 bp band amplified in non-*Lr34* germplasm [31]. The expected band size for *Lr*46 was 105 bp [32]. The amplification conditions for *Lr34* and *Lr46* were initial denaturation step at 94ºC for five minutes and then forty five amplification cycles. Each amplification cycle comprised of the denaturation step at 94ºC for forty five seconds, an annealing step at 55ºC for thirty seconds and extension step at 72ºC for sixty seconds. The final extension step was set at 72ºC for seven minutes and the products were stored at 4ºC.

2.5 Gel Electrophoresis

The products of the PCR reaction were separated in an MGU-502T electrophoresis tank (C.B.S. scientific) using 1.2% agarose gel electrophoresis in TBE (Tris Borate Edta) buffer. The gel was stained with 0.2 µg/ml ethidium bromide and run at 80 volts and a current of 400 mA for 80 minutes. The gel was visualized and photographed using BIORAD gel documentation system. A simple numerical scoring method was used where 1 was used to represent the occurrence of the expected band while 0 was used to represent the absence of the band. The scoring was done for the purposes of genotyping. The bands visualized were used to perform genotyping to determine the number of BC_1F_1 plants whose DNA showed introgression of the adult plant resistance genes. The size of the amplified gene markers was determined using GeneRuler™ 100 bp DNA Ladder (Thermo Scientific).

Table 1. Primer sequences of molecular markers utilized in the study

Name of genes	Linked marker	Nucleotide sequence	Reference
Sr2	GWM533	F 5'-AAGGCGAATCAAACGGAATA-3' R 5'-GTTGCTTTAGGGGAAAAGCC-3'	[28]
Lr34	CSLV34	F 5'-GTTGGTTAAGACTGGTGATGG-3' R 5'-TGCTTCCTATTGCTGAATAGT-3'	[29]
Lr46	BARC80	F 5'-GCGAATTAGCATCTGCATCTGTTT GAG-3' R 5'-CGGTCAACCAACTACTGCACAAC-3'	[30]

F, Forward primer, R, Reverse primer.

2.6 Data Analysis

Analysis of the genetic variation at each locus in terms of number of alleles observed heterozygosity (HO), expected heterozygosity (HE), Shannon's diversity index (I), genetic diversity flow (H) and gene frequency were carried out using genetic analysis package POPGENE Version 1.32 [33]. The neighboring-joining method was used to construct a dendrogram from a distance matrix based on Sokal and Michener's [34] genetic distances, using MEGA4 software implemented in Power Marker 3.25 [35].

3. RESULTS

The DNA of the parents (Njoro Bw_2 and Kingbird), positive control and all the BC_1F_1 plants selected for molecular work were positive for the *Sr2* gene except for Lane 5 where there was no amplification (Fig. 1). The marker GWM533 amplified a 120 bp band. The DNA in Lanes 1 to 16 for BC_1F_1 plants and those of the parents (Njoro Bw_2 and Kingbird) were all positive for *Lr46* gene marker (Fig. 2). The DNA in Lanes 1, 2, 4, 6 and 7 from the most resistant BC_1F_1 plants accessions numbers NP3, NP12, NP89, NP163 and NP192 respectively, with those of the resistant parent Kingbird and positive control, were positive for the *Lr34* gene which had a DNA fragment of 150 bp. The susceptible BC_1F_1 plants (Lanes 9-15) and the susceptible parent Njoro BW_2 were negative for the *Lr34* gene but a 229 bp fragment was amplified (Fig. 3).

Analysis on the genetic variation indicated that the observed number of alleles per locus ranged from 1.0 to 2.0 while the effective number of alleles per locus ranged from 1.0 to 1.8 and the mean number of polymorphic alleles per locus was 1.67 (Table 2).

The highest genetic diversity value (0.44) was detected in loci CSLV34 while the lowest (0.00) was detected in loci BARC80. The mean diversity value was 0.18 (Table 2). The expected heterozygosity (He) and observed heterozygosity (Ho) ranged from 0.10 to 0.55 and from 0.08 to 0.55 respectively (Table 2). As a measure of the informativeness of the markers used in this study (Shannon's information index), genetic diversity within the 18 wheat plants used was investigated and marker CSLV34 showed the highest diversity (0.64).

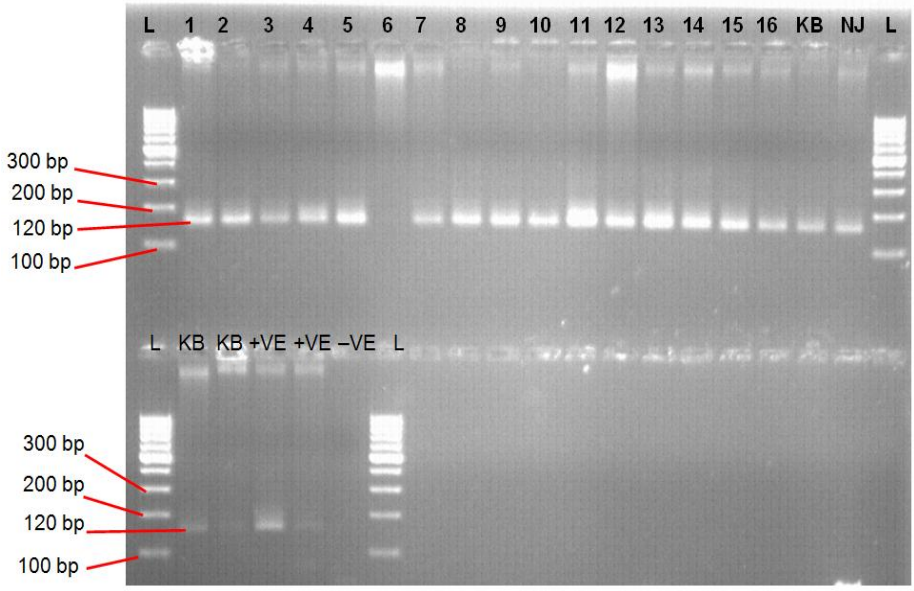

Fig. 1. Gel electrophoresis showing direct PCR with GWM533 marker for *Sr2* gene; concentration of agarose gel used 1.2 %; L, GeneRuler™ 100 bp DNA Ladder (Thermo Scientific); NJ, Njoro Bw_2; Lanes 1-8, Resistant plant accessions (NP3, NP12, NP49, NP89, NP142, NP163, NP192 and NP231); Lanes 9-16, Susceptible plants accessions (NP9, NP17, NP21, NP22, NP25, NP121, NP123 and NP217); KB, Kingbird; +VE, Positive control; -VE, Negative control

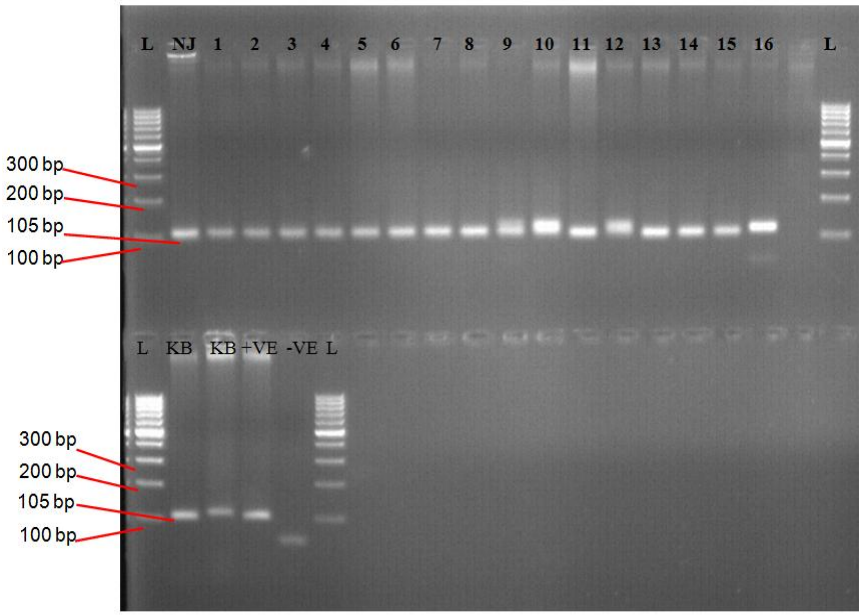

Fig. 2. Gel electrophoresis showing direct PCR with BARC80 for *Lr46* gene; concentration of agarose gel used 1.2 %; L, GeneRuler™ 100 bp DNA Ladder (Thermo Scientific); NJ, Njoro Bw_2; Lanes 1-8, Resistant plant accessions (NP3, NP12, NP49, NP89, NP142, NP163, NP192 and NP231); Lanes 9-16, Susceptible plant accessions (NP9, NP17, NP21, NP22, NP25, NP121, NP123 and NP217); KB, Kingbird; +VE, Positive control; -VE, Negative control.

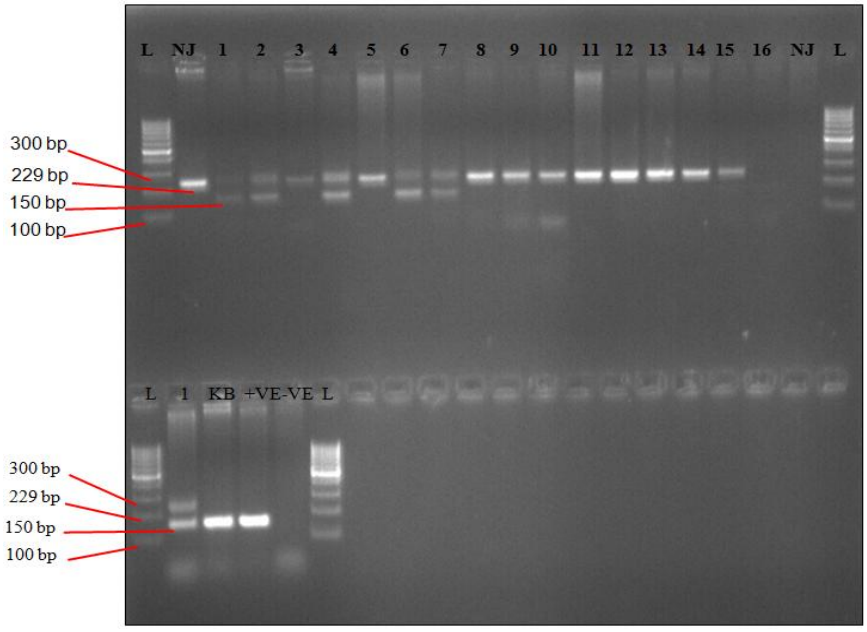

Fig. 3. Gel electrophoresis showing direct PCR with CSLV34 marker for gene *Lr34*; concentration of agarose gel used 1.2 %; L, GeneRuler™ 100 bp DNA Ladder (Thermo Scientific); NJ, Njoro Bw_2; Lanes 1-8, Resistant plants accessions (NP3, NP12, NP49, NP89, NP142, NP163, NP192 and NP231); Lanes 9-16, Susceptible plant accessions (NP9, NP17, NP21, NP22, NP25, NP121, NP123 and NP217); KB, Kingbird; +ve, Positive control; -ve, Negative control; Lanes 1, 2, 4, 6 and 7 represents BC_1F_1 plants accessions (NP3, NP12, NP89, NP163 and NP192), CSLV34 marker positive plants.

The gene frequency for the three markers used ranged from 0.33 for the most polymorphic marker CSLV34 to 1 for the least polymorphic marker BARC80 (Table 3).

The dendrogram grouped the 17 wheat BC_1F_1 plant accessions, Njoro Bw_2 and Kingbird into two clusters (A and B). The resistant cluster A consisted of 7 BC_1F_1 plant accession numbers NP3, NP12, NP19, NP89, NP142, NP163 and NP192 grouped with Kingbird. Cluster A had two sub-clusters with one of the sub-cluster supported by 44% bootstrap. The susceptible cluster B consisted of 10 BC_1F_1 plant accession numbers (NP9, NP17, NP21, NP22, NP25, NP, NP121, NP123, NP217, NP231) group together with Njoro Bw_2. BC_1F_1 plant accession number NP19, deviated from the expected susceptible to resistant cluster and BC_1F_1 plant accession numbers NP49 and NP231 deviated from the expected resistant to susceptible cluster (Fig. 4). It is worth noting that BC_1F_1 plant accession numbers NP49 and NP231 segregated with the susceptible group and not the resistant as expected from the phenotypic data.

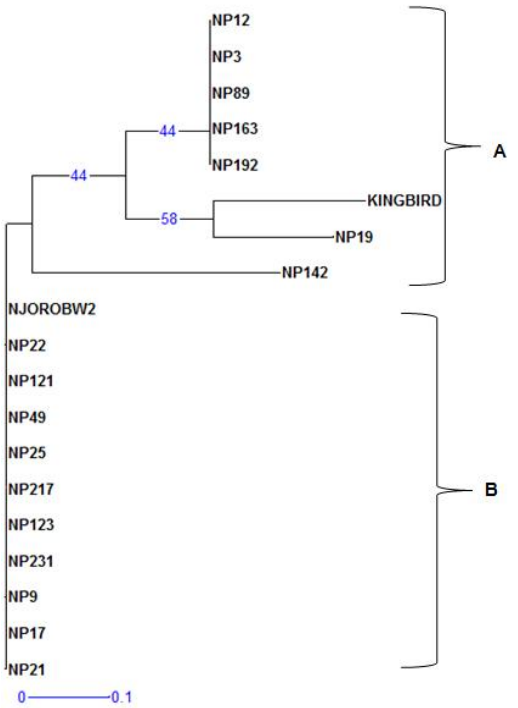

Fig. 4. A dendrogram for the 17 BC_1F_1 plant accessions, the resistant and susceptible parents. The number next to the branches are the bootstrap values. Bootstrap values more than 40 % are shown

4. DISCUSSION

The molecular results show that both parents (Kingbird and Njoro Bw_2) and all the selected wheat plant accessions were positive for the *Sr2* gene marker except for Lane 5 where there was no amplification (Fig. 1). This might have been due to errors during amplification that led to the absence of a band. The eight resistant plants had the pseudo-black chaff (PBC) expression reported previously, which is usually a phenotypic marker for the *Sr2* gene [28]. In the present study, although the eight susceptible wheat plants had the presence of *Sr2* gene it might not offer sufficient resistance to stem rust under severe disease pressure without other minor genes [36]. In the present study, GWM533 marker was successfully used to amplify a 120 bp band for *Sr2* gene similar to the report by [37]. In wheat, evaluation of more than 50 stem rust genes has been reported including *Sr2, Sr30* and *Sr31* among others [38]. Among the designated genes in wheat, *Sr2* is effective in slowing the development of stem rust [26]. However, the resistance conferred by the *Sr2* gene is effective when it is in combination with other minor genes [22,36].

All the sixteen plants with the parents Kingbird and Njoro BW_2 were positive for the *Lr46* gene marker (Fig. 2). The *Lr46* gene is also a slow rusting gene that confers resistance to stem rust in combination with other minor genes. The susceptible BC_1F_1 plant accessions and the susceptible parent plants, however, showed amplification for the *Lr46* gene indicating that the interaction of the minor gene *Lr46* with *Sr2* present in these plants might not offer resistance to stem rust under severe disease pressure. This explains why though NJoro Bw_2 was susceptible in the field; it still showed the presence of *Lr46* which is a slow rusting gene. The DNA of the resistant parent Kingbird and those of five BC_1F_1 plant accessions from the eight resistant categories (Lanes 1, 2, 4, 6 and 7) representing plant accessions NP3, NP12, NP89, NP169 and NP192 respectively, were positive for the *Lr34* gene marker (Fig. 3). The CSLV34 is a co-dominant marker since it is able to discriminate between a heterozygote and a homozygote genotype in F_2 populations. In this study, the DNA marker was able to reveal differences in band sizes between the parents and there was evidence that resistance offered to BC_1F_1 plant accessions by the *Lr34* gene was partial as indicated by the CSLV34 marker in the gel photo (Fig. 3).

Table 2. Genetic variation of the seventeen BC_1F_1 plant accessions and the two parents

Locus	Sample size	NA*	NE*	H*	I*	HO	HE
GMW533	19	2.00	1.12	0.10	0.21	0.55	0.55
CSLV34	19	2.00	1.80	0.44	0.64	0.32	0.34
BARC80	19	1.00	1.00	0.00	0.00	0.08	0.10
MEAN	19	1.67	1.31	0.18	0.28	0.23	0.27
ST. DEV		0.58	0.43	0.23	0.32		

Na, Observed number of alleles: Ne*, Effective number of alleles: H*, Nei's (1973) gene diversity; I*, Shannon's information index; Ho, Observed heterozygozity; He, Expected heterozygozity.*

Table 3. Gene frequencies for the different markers

Allele/ locus	GMW533	CSLV34	BARC80
Allele 1			
Allele 2	0.06	0.67	
Allele 3	0.94	0.33	1.00

The analysis carried out on genetic diversity indicated that the mean number of different alleles per locus in each group ranged from 1.0 to 2.0 and the mean number of polymorphic alleles per locus was 1.67 (Table 2). This is consistent with previous studies which made similar observations. [30] reported a range of 2 to 7 alleles per locus with an average of 3.2 alleles per locus whereas [39], observed an average of 7.8 alleles per locus with a range of 3 to as high as 22 alleles per locus. The markers that detected the lower number of alleles had lower gene diversity compared to those which detected a higher number of alleles which revealed higher gene diversity.

In this study, two effective alleles were observed when CSLV34 marker was assessed which were higher compared to other markers making the genetic diversity of this marker to be highest. According to [40] genetic diversity at each simple sequence repeat locus was significantly correlated with the number of alleles detected. The genetic diversity values for this study ranged from 0.0 to 0.44 with a mean value of 0.18 which was lower compared to the findings of [41], where the genetic diversity values ranged from 0.33 to 0.60 with a mean value of 0.47. Analysis done for the seventeen BC_1F_1 plant accessions and the two parents as a measure of informativeness of the microsatellites revealed that there was low genetic diversity within accessions (Table 2). Based on the results presented, CSLV34 was the most polymorphic marker as it exhibited a greater ability to distinguish between the different samples compared to the others while BARC80 was the least polymorphic of the three markers (Table 3).

The dendrogram separated the 17 wheat BC_1F_1 plant accessions and their parents into 2 distinct clusters. The plant accessions in the same cluster were genetically similar while those in dissimilar clusters were genetically different from each other. The deviation of BC_1F_1 plant accession number NP19 from the expected susceptible to resistant cluster, NP49 and NP231 from the expected resistant to susceptible cluster (Fig. 4) might have been due to inhibition or error during PCR amplification of the marker that led to absence of a band.

The segregation of BC_1F_1 plant accession numbers NP49 and NP231 with the susceptible group instead of the resistant as expected from the phenotypic data might have been due to strong phenotypic resistance to stem rust or hybrid vigor (Fig. 4). In this study, it was observed that BC_1F_1 plant accessions that were susceptible shared the same cluster with the susceptible parent (Njoro Bw_2) in cluster B, while those that were resistant shared the same cluster (cluster A) with the resistant parent (Fig. 4).

The clustering pattern of the 17 wheat BC_1F_1 plant accessions and their parents Njoro Bw_2 and Kingbird into two clusters by the markers that were used can be inferred to be a result of introgression, considering that the clustering in this study was purposively based on presence/absence of the resistance genes. This indicates that the *Lr34* gene which is an adult plant resistance gene was successfully introgressed into the BC_1F_1 plant accessions numbers NP3, NP12, NP89, NP163 and NP192 when Kingbird variety was crossed with Njoro Bw_2 variety. Findings already reported indicates that *Lr46* in combination with *Lr34* gives better

results on adult plant stem rust resistance [26]. The *Lr34* is one of the leaves and stem rust resistance genes known as “slow rusting genes” that provide durable and non-race specific adult plant resistance in combination with other minor genes [23,42-45].

5. CONCLUSION

The molecular amplification of stem rust resistance genes in this study shows the success in the introgression of the *Lr34* gene that offers resistance to stem rust at adult plant stage together with other minor genes including *Sr2* and *Lr46* into resistant BC_1F_1 plant accessions (NP3, NP12, NP89, NP163 and NP192). The markers used in this study demonstrated their ability to produce a unique DNA profile and establishment of introgression patterns in wheat genotypes, which is not possible when using phenotypic traits. The resistant BC_1F_1 plants (NP3, NP12, NP89, NP163 and NP192) which showed success in the introgression of the *Lr34* gene that gives resistance to stem rust at adult plant stage together with other minor genes including *Sr2* and *Lr46*, could further be developed, multiplied and used for wheat improvement as far as resistance to stem rust is concerned.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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