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Full Length Research Paper

Haplotype analysis of Ethiopian bread wheat (*Triticum aestivum*) cultivars and elite lines for yellow rust resistance genes using linked molecular markers

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Yellow rust, caused by *Puccinia striiformis* f. sp. *tritici,* is one of the most important diseases of wheat in Ethiopia. Poor knowledge of resistance genes deployed in Ethiopian wheat cultivars is one major factor for recurrent epidemics of rust diseases. Molecular marker based gene identification showed the presence of *Yr1, Yr4, Yr6, Yr7, Yr9, Yr10, Yr17* and *Yr18* in various frequencies, whereas *Yr8* was not detected in any of the tested 74 bread wheat genotypes. *Yr7* was the most frequent (74%) followed by *Yr6* (56%) and *Yr* 18 (37%) whereas *Yr1* and *Yr4* were detected in lower frequency (7 and 14%), respectively. The contribution of each *Yr* gene was evaluated in yellow rust differential lines possessing various genes. The differential lines carrying *Yr9* and *Yr8* had the highest average coefficient of infection (ACI) value (83 each) followed by *Yr6* and *Yr7* with ACI values of 82 and 80, respectively. The lowest ACI value (46.4) was exhibited on a differential line that carried *Yr4*. The number of *Yr* genes identified from the tested genotypes varied from 0 to 5. The ACI value exhibited by varieties possessing the maximum number of five resistance genes was 42. The majority (26 genotypes representing 35%) of the genotypes possessed three genes with an average coefficient of infection of 42. Pyramiding of the identified genes does not provide sufficient protection against yellow rust in Ethiopia. Thus, there is urgent need for searching for more effective resistance genes to be incorporated in Ethiopian bread wheat cultivars.

Key words: Average coefficient of infection (ACI), bread wheat, molecular markers, wheat genotypes, *Yr* genes.

INTRODUCTION

Wheat is one of the staple food crops cultivated by 5 million small scale farmers in Ethiopia. It is ranked fourth in land coverage and total production after teff, maize and sorghum (CSA, 2017). Yellow rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases of wheat that incurs 30 to 69% yield loss in Ethiopia (Badebo et al., 2008). Growing yellow rust resistant cultivars is widely recognized as the most eco-friendly and economically feasible approach. Since the 1970s, more than 100 bread wheat varieties have been

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Table 1. Details of molecular markers and primer sequences used for *Yr*- gene screening.

Gene	Marker/Primer	Type	Distance to gene (cM)	Primer sequence	Anneling Temperature (°C)	Fragment size (bp)	Reference
Yr1	BU099658	EST-SSR	-	TGAATCAATACAAGGACGCC GTCACAACACTGGCAACCC	54	≥206	Hasancebi et al. (2014)
Yr1	Stm673acag	STM	1.1	TAACTCACAACACGTTCTGGTCGT ACACACACACACACAGAGAGAG	56	120-124	Bansal et al. (2009)
Yr4	barc75	SSR	5.3	AGGGTTACAGTTTGCTCTTTTAC CCCGACGACCTATCTATACTTCTCTA	50	132-139	Bansal et al. (2009)
Yr5/18	Wms120	SSR	-	GTGAAGCAGACCCAACAC GACGGCTGCGACGTAGAG	46	472	Singh (1992a)
Yr6	Wmc76	SSR	-	CTTCAGAGCCTCTTTCTCTACA CTGCTTCACTTGCTGATCTTTG	51	256	Li and Niu (2007)
Yr6	Wmc276	SSR	-	GACATGTGCACCAGAATAGC AGAAGAACTATTCGACTCCT	47	292	Li and Niu (2007)
Yr7	Xgwm526	SSR	5.3	CAATAGTTCTGTGAGAGCTGCG CCAACCCAAATACACATTCTCA	48	212, 217	Cabuk et al. (2011)
Yr8	Xgwm157	SSR	-	GTCGTCGCGGTAAGCTTG' GAGTGAACACACGAGGCTTG	60	120	-
Yr9	Xgwm582	SSR	-	AAGCACTACGAAAATATGAC TCTTAAGGGGTGTTATCATA	55	-	Cabuk et al. (2011)
Yr9	iag95	Gene specific	-	CTCTGTGGATAGTTACTTGATCGA CCTAGAACATGCATGGCTGTTACA	55	1100	Mago et al. (2002)
Yr9	P6M12-P	STS	0.35	GTACTAGTATCCAGAGGTCACAAG CAGACAAACAGAGTACGGGC	56	56 250/350	Mago et al. (2005)
Yr10	Yr10F & Yr10R	Gene specific	-	TCAAAGACATCAAGAGCCGC TGGCCTACATGAACTCTGGAT	51	543	Liu et al. (2014)
Yr15	Xgwm273	SSR	-	ATTGGACGGACAGATGCTTT AGCAGTGAGGAAGGGGATC	-	167	Yaniv et al. (2015)

Table 1. Contd.

Yr17	VENTRUP-F, LN2	Gene specific	-	AGGGGCTACTGACCAAGGCT TGCAGCTACAGCAGTATGTACACAAAA			Qamar et al. (2008)
Yr17	URIC/LN2	*T. ventricosum* chromosom specific	-	GGTCGCCCTGGCTTGCACCT TGCAGCTACAGCAGTATGTACACAAAA	64	275/285	Jia et al. (2011)
Yr17	SC-385	SCAR	3.4	CTGAATACAAACAGCAAACCAG ACAGAAAGTGATCATTTCCATC	50	385	Jia et al. (2011)
Yr18	Wms295	SSR	-	GTGAAGCAGACCCACAACAC GACGGCTGCGACGTAGAG	-	-	Cabuk et al. (2011)
Yr18	L34DINT9F, L34PLUSR	Gene specific	-	TTGATGAAACCAGTTTTTTTTCTA GCCATTTAACATAATCATGATGGA	58	517	Krattinger et al. (2009)
Yr16/Yr24	Xgwm18	SSR	-	GTGGTATTTCAGGTGGAGTTGTTTTA CGGAGGAGCAGTAAGGAAGG	60	200	Ahmad et al. (2015)
Yr15 &Yr26	Xgwm11	SSR	-	GGATAGTCAGACAATTCTTGTG GTGAATTGTGTCTTGTATGCTTCC	55	215	Ma et al. (2001)
Yr65	Xgwm11	SSR	2.1	GATAGTCAGACAATTCTTGTG GTGAATTGTGTCTTGTATGCTTCC	50	213 /202	Cheng et al. (2014)
Yr29	Xgwm44	SSR	3.6/10.9	GGTCTTCTGGGCTTTGATCCTG TGTTGCTAGGGACCCGTAGTGG	57	260	Rosewarne et al. (2006)
Yr32	Xgwms198	SSR	-	CACGCTGCCATCACTTTTAC TTGAAGTGGTCATTGTTGCT	51	160	Eriksen (2004)
Yr33	Xgwm111	SSR	-	TCTGTAGGCTCTCTCCGACTG ACCTGATCAGATCCCACTCG	52	184/206	Zahravi et al. (2003)
Yr35	Xgwm508	SSR	7.5	GTTATAGTAGCATATAATGGCC GTGCTGCCATGATATTT	51	135	Dadkhodaie et al. (2011)
Yr46	Xgwm165	SSR	0.4	TGCAGTGGTCAGATGTTTCC CTTTTCTTTCAGATTGCGCC	449	~236 bp	Herrera-Foessel et al. (2014)
Yr46/Yr62	Xgwm192	SSR	0.4/2	GGTTTTCTTTCAGATTGCGC CGTTGTCTAATCTTGCCTTGC	54/51	~130/222	Herrera-Foessel et al. (2014); Liu et al. (2014)

Table 1. Contd.

Yr 49	Xgwm161	SSR	1	GATCGAGTGATGGCAGATGG TGTGAATTACTTGGACGTGG	57	145/154	McIntosh et al. (2016)
Yr52	Barc182	SSR	1.2	CCATGGCCAACAGCTCAAGGTCTC CGCAAAACCGCATCAGGGAAGCACCA AT	59	≥75	Ren et al. (2012)
Yr57	Xgwm389	SSR	2	ATCATGTCGATCTCCTTGACG TGCCATGCACATTAGCAGAT	51	117-128	Radhawa et al. (2015)
Yr62	Xgwm251	SSR	3.3	GGTTTTCTTTCAGATTGCGC CGTTGTCTAATCTTGCCTTGC	51	133	Liu et al. (2014)

released in Ethiopia. However, the resistance is not lasting long due to the development of new virulent races of the pathogen. Poor knowledge of resistant genes deployed in Ethiopian wheat cultivars is one of the major reasons for recurrent epidemics of rust diseases. Those cultivars may be protected by the same resistance gene(s) or combinations, which could increase selection pressure for the corresponding virulent races. To date, more than 67 yellow rust resistant genes have been reported in wheat and its wild relatives (McIntosh et al., 2013). Most of these genes condition race-specific resistance and many have been overcome by the emergence of new races.

The most effective strategy for protecting wheat from rust is to deploy cultivars with combinations of different resistance genes. For this, information on the resistance genes in major cultivars is of paramount importance. The traditional way of gene postulation requires multi-pathotypes testing in which a host cultivar is evaluated against a collection of isolates carrying different avirulence/ virulence gene combinations (pathotypes) on the basis of phenotypic expression in the form of infection types (ITs). As an alternative to gene postulation, presence of resistance genes can be determined by testing host cultivars with molecular markers linked to resistance genes. This approach overcomes gene interactions and plant stage dependent gene expression problems associated with traditional gene postulation (Vanzetti et al., 2011).

Currently, there have been advances in development and mapping of molecular markers that are diagnostic for major *Yr* genes (Mago et al., 2002; Eriksen, 2004; Li and Niu, 2007; Bansal et al., 2009; Krattinger et al., 2009; Jia et al., 2011; Cabuk et al., 2011; Liu et al., 2014; Yaniv et al., 2015; Ahmad et al., 2015). These markers provide an important tool to plant breeders for marker assisted wheat breeding and also for pyramiding resistance genes in the absence of distinguishable rust virulences (Kaur et al., 2008). This study was conducted to identify yellow rust resistant gene (s) that are present in commercial bread wheat cultivars and elite lines using molecular markers linked to *Yr* genes, assess the effectiveness of the identified *Yr* genes to the prevailing races under field conditions in Ethiopia, and to evaluate genetic variations among the wheat cultivars and elite lines.

MATERIALS AND METHODS

Plant materials

A total of 58 commercial bread wheat cultivars, 16 elite lines and 15 reference lines with known *Yr* genes and a negative check, *Morocco,* with unknown *Yr* genes and *PBW345* were included in this study. All the wheat materials were obtained from the Ethiopian Institute of Agricultural Research (EIAR), KARC, Ethiopia. The list of wheat genotypes together with their additional information is shown in Supplementary Table 1.

Field testing

All wheat genotypes were evaluated for yellow rust reaction in three locations, namely, Mararo, Arsi Robe and Kulumsa under natural infection in 2016 and 2017. The materials were sown in two rows of 1 m length with 0.20 m spacing between rows. For scoring yellow rust severity in the field, the modified Cobb Scale (Peterson et al., 1948) was used to determine the percentage of tissue infected with rust. The host response to infection in the field was scored using ‘‘R’’ or resistant (small uredinia surrounded by

chlorosis or necrosis); ‘‘MR’’ or moderately resistant (medium sized uredinia surrounded by chlorosis or necrosis); ‘‘MS’’ or moderately susceptible (medium large compatible uredinia without chlorosis and necrosis); and ‘‘S’’ or susceptible (large, compatible uredinia without chlorosis and necrosis). Disease severity and host response data were combined in a single value called the coefficient of infection (CI). The average coefficient of infection (ACI) was used for host response: immune = 0.0, R = 0.2, MR = 0.4, MS = 0.8, and S = 1.0.

Molecular markers

Two closely linked (usually flaking) markers of each of the major *Yr* genes were chosen to identify its presence/absence in wheat genotypes/materials, except a few genes for which only one closely linked marker was reported. A total of 31 markers that are linked to yellow rust resistant genes were used to identify *Yr* genes from the test materials as well as for genetic diversity analysis. Primer names, forward and reverse primer sequences, expected amplified fragment size in base pairs, and annealing temperature are shown in Table 1.

DNA extraction

DNA was extracted from 2 weeks old fresh leaves harvested and pooled from five seedlings of each accession and stored cryogenically at -80°C. Extraction from frozen leaves was performed using the modified cetyl trimethylammonium bromide (CTAB) method described by Doyle and Doyle (1990).

PCR amplification and fragment analysis

Polymerase chain reactions (PCR) were performed in Perkin-Elmer (Norwalk, CT) thermocyclers in a total volume of 25 µl containing 50 to 100 ng each template DNA, 250 nM cy5-labelled forward primer, 250 nM unlabelled reverse primer, 0.2 mM dNTPs, 2.5 µl PCR buffer (10x), 1.5 mM $MgCl_2$, and 1U *Taq* DNA polymerase. After 3 min at 94°C, 45 cycles were performed with 1 min at 94°C, 1 min at either 50, 55, or 60°C (depending on the individual primer), 2 min at 72°C, and a final extension step of 10 min at 72°C. The PCR product was denatured at 94°C for 2 min and placed on a cold block until use. Each sample of 6 µl (4 µl PCR product and 2 µl internal markers) and one external standard marker of 6 µl were loaded in the preheated gel. For SSR markers, fragments were detected by an Automated Laser Fluorescence (ALF express) sequencer (Amersham Biosciences) and fragment sizes were calculated using the computer program Fragment Analyser 1.02 (Amersham Biosciences) by comparison with internal size standards. The EST-SSR, STM and SCAR markers were resolved in 2.0% agarose gels by loading 15 µl PCR products and the amplified fragments were stained with ethidium bromide and photographed.

Data analysis

Presence of *Yr* genes in the wheat genotypes was counted for each yellow rust resistance gene based on presence of the fragment sizes of both flaking markers, except for a few genes where one closely linked marker has been reported. This was explained by comparing with the reference lines with known *Yr* genes. For genetic diversity analysis, amplification profile of markers was recorded with each band representing a different allele, with a particular primer pair. Each allele was scored on the basis of presence of a band (scored as 1) and its absence (scored as 0) for generating a binary matrix which was further used to calculate Jaccard’s similarity coefficients for each pair of parents following NTSYS-PC program (Rohlf, 2000). Allelic Polymorphic Information Content (PIC) for each primer locus and genetic diversity was analysed with Power Marker version 3.25 Software (Liu and Muse, 2005).

RESULTS

Yellow rust reactions in field test

Average coefficient of infection of yellow rust for the wheat genotypes tested at three locations (Kulumsa, Arsi robe and Meraro) during 2016 and 2017 is shown in Table 2. Of the 74 test genotypes, nine cultivars showed resistance (R) with 0 to 10 ACI, 11 cultivars exhibited resistance to moderate resistance (R-MR) with ACI value of 10 to 20 and 14 genotypes showed moderately resistance (MR) with ACI value of 20 to 30, whereas 8, 9 and 23 of the genotypes exhibited moderately resistant to moderately susceptible (MR-MS), moderately susceptible (MS) and susceptible (S) with ACI values in ranges of 30 to 40, 40 to 50, and >50, respectively.

Identification of yellow rust resistance (*Yr)* genes using molecular markers

Yellow rust resistant gene, Yr1

Yr1 is a seedling resistance gene which is located on chromosome 2AL. An EST-SSR marker, BU099658 and STM marker, Stm673acag (1.1 cM linkage with *Yr1* gene) were used to identify this gene in 74 wheat genotypes. The expected fragment size for BU099658 marker was 206 bp (Hasancebi et al., 2014). In the present study, the marker amplified DNA fragment sizes of 157 to 206 bp. The amplified fragment at 206 bp was monomorphic and detected in all the tested materials including the reference line (*Yr1*/6*Avocet S). The polymorphic DNA fragment size, 162 bp, which was produced in the reference line was used for identification of *Yr1* gene. In this regard, the presence of *Yr1* was detected in 11 (15%) of the genotypes. The expected fragment size for the second marker, Stm673acag was 120 to 124 bp (Bansal et al., 2009). This marker amplified fragment sizes from 118 to 129 bp in this study. The polymorphic fragment size of 129 bp which was produced in the reference line was used to predict the*Yr1* gene. *Yr1* was identified in 11 (15%) of the tested genotypes. Only 5 (6.7%) of the genotypes exhibited similar fragment size to the reference lines for both markers.

Yellow rust resistant gene, Yr4

Yr4 which is synonymous with *Yr4a*, and *Yr4b* was

Table 2. Identified *Yr* gene in released and advanced Ethiopian bread wheat genotypes using linked molecular markers. Numeric values are the fragment sizes (bp) of PCR amplicons for the respective marker and wheat line.

Genotype	YR	*Yr1*		Genotype	YR	*Yr4*	Genotype	YR	*Yr6*	
		BU099658	Stm673acag			Barc075			WMC078	Xgwm278
*Yr1/6*Avocet S*	62	162	129	*Hybrid 46*	46.4	105	*Yr6/6*Avocet S*	82	256	289
Dereselign	-	162	129	Abola	-	105	Pavon 76	40.7	256	292
Digalu	-	162	129	HAR 1018	-	105	Abola	51.7	256	292
Dure	-	162	129	HAR 723	-	105	Alidoro	32.9	256	289
ETBW5890	-	162	129	Hawi	-	105	Bolo	76.7	256	289
ETBW5879	-	162	129	HAR 820	-	105	Bonde	55.2	256	292
Alidoro	-	156	129	Hoggana	-	105	Danda'a	20.4	256	292
Bolo	-	164	129	Jafersson	-	105	Dashen	25.7	256	289
Bonde	-	160	129	Mada Walabu	-	105	Dereselign	63.5	256	289
ETBW6094	-	159	129	PBW343	-	105	Digalu	81.5	256	289
ETBW6098	-	159	129	Simba	-	105	Batu	25.9	256	292
Tura	-	159	129	Sulla	-	105	Enkoy	13.2	256	292
Dinknesh	-	162	NA	Alidoro	-	112	ET13A2	34.7	256	292
ETBW6130	-	162	120	Batu	-	113	ETBW6093	50	256	289, 292
Galil	-	162	120	Bobicho	-	109	ETBW7698	2.7	256	292
Menze	-	162	120	Bolo	-	112	FH4-2-11	14.4	256	289, 292
Shorima	-	162	120	Bonde	-	113	Galema	26.7	256	292
Watera	-	162	124	Danda'a	-	113	Gambo	29.2	256	289
Abola	-	157	120	Dashen	-	113	Gassay	8.9	256	292
Batu	-	159	120	Dereselign	-	109	HAR 1018	9	256	289
Bobicho	-	157	120	Digalu	-	113	HAR 1331	10	256	289
Danda'a	-	156	120	Dinknesh	-	110	HAR 723	68.3	256	292
Dashen	-	157	118	Dodota	-	113	HAR 820	11.4	256	289
Dodota	-	159	120	Dure	-	110	HAR 934	12.1	256	292
Enkoy	-	157	120	Enkoy	-	113	Hidassie	32.7	256	292
ET13A2	-	157	120	ET13A2	-	113	Hoggana	50	256	289
ETBW5800	-	157	120	ETBW5800	-	113	Huluka	26.7	256	289
ETBW6093	-	159	120	ETBW5890	-	112	K6290 Bulk	55	256	292
ETBW6496	-	157	120	ETBW6093	-	112	Kakaba	55.7	256	289
ETBW6647	-	159	123	ETBW6094	-	112	Katar	60	256	289, 292
ETBW6696	-	158	124	ETBW6098	-	112	KBG-01	66.2	256	289
ETBW6861	-	157	123	ETBW6130	-	112	Kulkulu	58.4	256	289
ETBW6939	-	157	124	ETBW6496	-	112	Menze	73.4	256	289
ETBW7698	-	159	120	ETBW6647	-	112	Millennium	42.3	256	289

Table 2. Contd.

FH4-2-11	-	157	124	ETBW6696	-	113	Ogolcho	28.4	256	292
Galema	-	159	120	ETBW6861	-	112	PBW343	70	256	289
Gambo	-	157	124	ETBW6939	-	112	Shorima	28	256	289
Gassay	-	157	125	ETBW7698	-	112	Simba	30.9	256	286
HAR 1018	-	157	120	FH4-2-11	-	NA	Sirbo	30	256	292
HAR 1331	-	157	118	Galema	-	113	Sofumar	65	256	289
HAR 719	-	157	120	Galil	-	113	Sulla	71.7	256	292
HAR 723	-	159	120	Gambo	-	114	Tossa	65	256	292
HAR 727	-	157	118	Gassay	-	112	ETBW5879	30.5	256	292
HAR 820	-	158	120	HAR 1331	-	112	ETBW7255	32.1	256	292
HAR 934	-	158	120	HAR 719	-	112	Bobicho	48.4	NA	292
Hawi	-	157	120	HAR 727	-	112	Dodota	0.3	258	292
Hidassie	-	157	120	HAR 934	-	113	ETBW5890	16.3	258	292
Hoggana	-	156	120	Hidassie	-	113	ETBW6094	53.4	258	292
Huluka	-	156	120	Huluka	-	113	ETBW6098	56.7	258	292
Isreal	-	156	120	Isreal	-	113	ETBW6130	9.1	258	292
Jafersson	-	157	119	K6290 Bulk	-	113	ETBW6696	14.6	258	289, 292
K6290 Bulk	-	157	120	K6295-4A	-	113	ETBW6861	23.7	258	289, 292
K6295-4A	-	155	120	Kakaba	-	113	ETBW6939	29.3	258	289
Kakaba	-	156	120	Katar	-	113	Galil	20.9	253	292
Katar	-	156	120	KBG-01	-	113	Hawi	58.4	253	289
KBG-01	-	158	121	Kubsa	-	113	Jafersson	34.2	254	292
Kubsa	-	158	120	Kulkulu	-	113	K6295-4A	58.4	249	292
Kulkulu	-	160	127	Mandoyu	-	113	Kubsa	70	264	289
Mada Walabu	-	160	120	Menze	-	113	Mada Walabu	55	258	289
Mandoyu	-	156	120	Meraro	-	113	Mandoyu	14.5	258	292
Meraro	-	159	120	Millennium	-	110	Meraro	22.7	262	289
Millennium	-	158	120	Mitike	-	113	Mitike	50	253	289, 292
Mitike	-	156	120	Ogolcho	-	113	Sanate	4.5	265	289
Ogolcho	-	158	120	Pavon 76	-	113	Shinna	75	NA	289
Pavon 76	-	156	120	Sanate	-	110	Watera	40.8	268	292
PBW343	-	157	120	Senkegna	-	113	Dure	17.1	256	250
Sanate	-	156	120	Shinna	-	113	ETBW5800	15.9	256	250
Senkegna	-	156	120	Shorima	-	112	HAR 719	26	256	NA
Shinna	-	156	120	Sirbo	-	112	Isreal	48	256	299
Simba	-	158	121	Sofumar	-	110	Senkegna	8.4	256	295
Sirbo	-	158	120	Tossa	-	112	Tura	21.3	256	291

Table 2. Contd.

Sofumar	-	156	120	Tura	-	112	Tusie	15.4	256	299
Sulla	-	157	120	Tusie	-	112	Dinknesh	66.9	254	296
Tossa	-	153	120	ETBW5879	-	112	ETBW6496	13.1	258	287
Tusie	-	152	120	Watera	-	112	ETBW6647	49.4	258	282
ETBW7255	-	164	124	ETBW7255	-	112	HAR 727	3.5	253	296
Frequency (%)	-	5 (7)		-	-	-	-	-	43 (57)	

Genotype	YR	*Yr7*	Genotype	YR	*Yr8*	Genotype	YR	*Yr9*	
		WMC078			WMC078			Xwms582	iag95
*Yr7/6*Avocet S*	80	157	*Yr8/6*Avocet S*	83	120	*Yr9/6*Avocet S*	83	142	1100
Lee	-	159	Abola	51.7	130	Batu	48.4	142	1100
Abola	51.7	159	Alidoro	32.9	130	Bobicho	66.9	142	1100
Batu	48.4	157	Batu	48.4	130	Dashen	25.7	142	1100
Bobicho	66.9	157	Bobicho	66.9	130	ETBW6496	13.1	142	1100
Bolo	76.7	157	Bolo	76.7	130	ETBW6861	23.7	142	1100
Bonde	55.2	157	Bonde	55.2	130	FH4-2-11	14.4	142	1100
Danda'a	20.4	157	Danda'a	20.4	130	Galil	20.9	142	1100
Dashen	25.7	159	Dashen	25.7	130	HAR 1018	9	142	1100
Dereselign	63.5	157	Dereselign	63.5	130	HAR 1331	10	142	1100
Digalu	81.5	157	Digalu	81.5	130	HAR 723	68.3	142	1100
Dinknesh	0.3	159	Dinknesh	0.3	130	HAR 727	3.5	142	1100
Dodota	25.9	157	Dodota	25.9	130	HAR 820	11.4	142	1100
Dure	17.1	157	Dure	17.1	130	HAR 934	12.1	142	1100
ETBW5800	15.9	159	Enkoy	13.2	130	Hawi	58.4	142	1100
ETBW5890	16.3	159	ET13A2	34.7	130	Katar	60	142	1100
ETBW6094	53.4	157	ETBW5800	15.9	130	Mada Walabu	55	142	1100
ETBW6130	9.1	157	ETBW5890	16.3	130	Meraro	22.7	142	1100
ETBW6496	13.1	157	ETBW6093	50	130	Millennium	42.3	142	1100
ETBW6647	49.4	159	ETBW6094	53.4	130	PBW343	70	142	1100
ETBW6696	14.6	159	ETBW6098	56.7	130	Senkegna	8.4	142	1100
ETBW6861	23.7	159	ETBW6130	9.1	130	Simba	30.9	142	1100
ETBW6939	29.3	157	ETBW6496	13.1	130	Sirbo	30	142	1100
ETBW7698	2.7	159	ETBW6647	49.4	130	Tura	21.3	142	1100
FH4-2-11	14.4	159	ETBW6696	14.6	130	Tusie	15.4	142	1100
Galema	26.7	157	ETBW6861	23.7	130	Watera	40.8	142	1100
Galil	20.9	159	ETBW6939	29.3	130	ETBW7255	32.1	146	1100
HAR 1331	10	157	ETBW7698	2.7	130	Dinknesh	0.3	147	1100
HAR 719	26	159	FH4-2-11	14.4	130	ETBW6696	14.6	147	1100

Table 2. Contd.

HAR 727	3.5	157	Galema	26.7	130	Galema	26.7	147	1100
HAR 820	11.4	157	Galil	20.9	130	Dereselign	63.5	149	NA
HAR 934	12.1	159	Gambo	29.2	130	Digalu	81.5	145	NA
Hawi	58.4	157	Gassay	8.9	130	Abola	51.7	147	NA
Hidassie	32.7	159	HAR 1018	9	130	Alidoro	32.9	147	NA
Hoggana	50	157	HAR 1331	10	130	Bolo	76.7	145	NA
Huluka	26.7	157	HAR 719	26	NA	Bonde	55.2	149	NA
Isreal	48	157	HAR 723	68.3	130	Danda'a	20.4	147	NA
Jafersson	34.2	159	HAR 727	3.5	130	Dodota	25.9	145	NA
K6290 Bulk	55	157	HAR 820	11.4	130	Dure	17.1	149	NA
Kakaba	55.7	159	HAR 934	12.1	130	Enkoy	13.2	149	NA
Kubsa	70	159	Hawi	58.4	130	ET13A2	34.7	149	NA
Kulkulu	58.4	157	Hidassie	32.7	130	ETBW5800	15.9	147	NA
Mandoyu	14.5	157	Hoggana	50	130	ETBW5890	16.3	147	NA
Menze	73.4	157	Huluka	26.7	130	ETBW6093	50	147	NA
Meraro	22.7	159	Isreal	48	130	ETBW6094	53.4	147	NA
Pavon 76	40.7	159	Jafersson	34.2	130	ETBW6098	56.7	147	NA
PBW343	70	159	K6290 Bulk	55	130	ETBW6130	9.1	147	NA
Sanate	4.5	159	K6295-4A	58.4	NA	ETBW6647	49.4	147	NA
Senkegna	8.4	157	Kakaba	55.7	NA	ETBW6939	29.3	147	NA
Shinna	75	159	Katar	60	130	ETBW7698	2.7	147	NA
Shorima	28	157	KBG-01	66.2	130	Gambo	29.2	147	NA
Simba	30.9	157	Kubsa	70	130	Gassay	8.9	147	NA
Sirbo	30	159	Kulkulu	58.4	130	HAR 719	26	147	NA
Sofumar	65	159	Mada Walabu	55	130	Hidassie	32.7	NA	NA
Tossa	65	159	Mandoyu	14.5	130	Hoggana	50	147	NA
Tura	21.3	157	Menze	73.4	130	Huluka	26.7	147	NA
Tusie	15.4	157	Meraro	22.7	130	Isreal	48	149	NA
Alidoro	32.9	141	Millennium	42.3	130	Jafersson	34.2	149	NA
Enkoy	13.2	142	Mitike	50	130	K6290 Bulk	55	149	NA
ET13A2	34.7	155	Ogolcho	28.4	130	K6295-4A	58.4	145	NA
ETBW6093	50	158	Pavon 76	40.7	130	Kakaba	55.7	147	NA
ETBW6098	56.7	156	PBW343	70	130	KBG-01	66.2	147	NA
Gambo	29.2	145	Sanate	4.5	130	Kubsa	70	147	NA
Gassay	8.9	141	Senkegna	8.4	130	Kulkulu	58.4	147	NA
HAR 1018	9	143	Shinna	75	130	Mandoyu	14.5	147	NA
HAR 723	68.3	143	Shorima	28	130	Menze	73.4	145	NA

Table 2. Contd.

K6295-4A	58.4	143	Simba	30.9	130	Mitike	50	147	NA
Katar	60	158	Sirbo	30	130	Ogolcho	28.4	147	NA
KBG-01	66.2	158	Sofumar	65	130	Pavon 76	40.7	147	NA
Madda Walabu	55	141	Sulla	71.7	130	Sanate	4.5	147	NA
Millennium	42.3	143	Tossa	65	130	Shinna	75	147	NA
Mitike	50	151	Tura	21.3	130	Shorima	28	147	NA
Ogolcho	28.4	141	Tusie	15.4	130	Sofumar	65	147	NA
Sulla	71.7	143	ETBW5879	30.5	130	Sulla	71.7	147	NA
ETBW5879	30.5	158	Watera	40.8	130	Tossa	65	147	NA
Watera	40.8	158	ETBW7255	32.1	NA	ETBW5879	30.5	147	NA
ETBW7255	32.1	158	Frequency (%)	-	0	Frequency (%)	-	25 (33)	
Frequency (%)	-	55 (73%)	-	-		-	-	-	-

Genotype	YR	*Yr10*	Genotype	YR	*Yr17*		Genotype	YR	*Yr18*	
		YR10F & YR10R			SC-385	VENTRUP			WMS295	L34DINTF9F L34PLUSR
*Yr10/6*Avocet S*	60	543	*Yr17/6*Avocet S*	77	378	258	*Yr18/6*Avocet S*	78	255	517
Morro		543	Abola	51.7	378	258	Alidoro	32.9	255	517
Alidoro	32.9	543	Alidoro	32.9	378	258	Bobicho	66.9	255	517
Enkoy	13.2	543	Batu	48.4	378	258	Dodota	25.9	255	517
ET13A2	34.7	543	Bobicho	66.9	378	258	Dure	17.1	255	517
Galema	26.7	543	Bolo	76.7	378	258	ETBW6093	50	255	517
Hidassie	32.7	543	Bonde	55.2	378	258	ETBW6130	9.1	255	517
K6290 Bulk	55	543	Digalu	81.5	378	258	ETBW6496	13.1	255	517
K6295-4A	58.4	543	Dinknesh	0.3	378	258	ETBW6647	49.4	255	517
KBG-01	66.2	543	ETBW6093	50	378	258	ETBW6861	23.7	255	517
Madda Walabu	55	543	ETBW6094	53.4	378	258	FH4-2-11	14.4	255	517
Meraro	22.7	543	HAR 934	12.1	378	258	HAR 1331	10	255	517
Mitike	50	543	Hoggana	50	378	258	HAR 719	26	255	517
Shinna	75	543	Huluka	26.7	378	258	HAR 727	3.5	255	517
Abola	51.7	NA	KBG-01	66.2	378	258	HAR 820	11.4	255	517
Batu	48.4	NA	Kulkulu	58.4	378	258	HAR 934	12.1	255	517
Bobicho	66.9	NA	Madda Walabu	55	378	258	Hidassie	32.7	255	517
Bolo	76.7	NA	Menze	73.4	378	258	Hoggana	50	255	517
Bonde	55.2	NA	Meraro	22.7	378	258	K6295-4A	58.4	255	517
Danda'a	20.4	NA	Sofumar	65	378	258	Kakaba	55.7	255	517
Dashen	25.7	NA	ETBW5879	30.5	378	258	Katar	60	255	517
Dereselign	63.5	NA	ETBW7255	32.1	378	258	Madda Walabu	55	255	517

Table 2. Contd.

Digalu	81.5	NA	Hawi	58.4	378	250	Sanate	4.5	255	517
Dinknesh	0.3	NA	Isreal	48	378	250	Sirbo	30	255	517
Dodota	25.9	NA	Jafersson	34.2	378	NA	Tura	21.3	255	517
Dure	17.1	NA	Danda'a	20.4	393	258	Tusie	15.4	255	517
ETBW5800	15.9	NA	ETBW6098	56.7	393	258	Digalu	81.5	255	517
ETBW5890	16.3	NA	Katar	60	393	258	Watera	40.8	255	517
ETBW6093	50	NA	Kubsa	70	393	258	Bolo	76.7	247	517
ETBW6094	53.4	NA	Millennium	42.3	393	258	Bonde	55.2	255	NA
ETBW6098	56.7	NA	Dashen	25.7	393	250	Dinknesh	0.3	255	NA
ETBW6130	9.1	NA	Dereselign	63.5	379	260	Batu	48.4	255	NA
ETBW6496	13.1	NA	Dodota	25.9	393	273	Millennium	42.3	255	NA
ETBW6647	49.4	NA	Dure	17.1	393	250	PBW343	70	255	NA
ETBW6696	14.6	NA	Enkoy	13.2	393	250	ETBW5879	30.5	255	NA
ETBW6861	23.7	NA	ET13A2	34.7	393	250	ETBW7255	32.1	255	NA
ETBW6939	29.3	NA	ETBW5800	15.9	393	250	Abola	51.7	253	NA
ETBW7698	2.7	NA	ETBW5890	16.3	393	268	Danda'a	20.4	247	NA
FH4-2-11	14.4	NA	ETBW6130	9.1	393	250	Dashen	25.7	248	NA
Galil	20.9	NA	ETBW6496	13.1	393	287	Dereselign	63.5	253	NA
Gambo	29.2	NA	ETBW6647	49.4	393	273	Enkoy	13.2	247	NA
Gassay	8.9	NA	ETBW6696	14.6	393	250	ET13A2	34.7	253	NA
HAR 1018	9	NA	ETBW6861	23.7	393	250	ETBW5800	15.9	253	NA
HAR 1331	10	NA	ETBW6939	29.3	393	250	ETBW5890	16.3	253	NA
HAR 719	26	NA	ETBW7698	2.7	393	250	ETBW6094	53.4	253	NA
HAR 723	68.3	NA	FH4-2-11	14.4	393	250	ETBW6098	56.7	247	NA
HAR 727	3.5	NA	Galema	26.7	393	268	ETBW6696	14.6	248	NA
HAR 820	11.4	NA	Galil	20.9	393	281	ETBW6939	29.3	253	NA
HAR 934	12.1	NA	Gambo	29.2	393	NA	ETBW7698	2.7	253	NA
Hawi	58.4	NA	Gassay	8.9	393	250	Galema	26.7	253	NA
Hoggana	50	NA	HAR 1018	9	393	NA	Galil	20.9	252	NA
Huluka	26.7	NA	HAR 1331	10	393	250	Gambo	29.2	248	NA
Isreal	48	NA	HAR 719	26	393	NA	Gassay	8.9	253	NA
Jafersson	34.2	NA	HAR 723	68.3	393	250	HAR 1018	9	253	NA
Kakaba	55.7	NA	HAR 727	3.5	393	250	HAR 723	68.3	253	NA
Katar	60	NA	HAR 820	11.4	393	250	Hawi	58.4	251	NA
Kubsa	70	NA	Hidassie	32.7	393	250	Huluka	26.7	252	NA
Kulkulu	58.4	NA	K6290 Bulk	55	393	NA	Isreal	48	252	NA
Mandoyu	14.5	NA	K6295-4A	58.4	393	250	Jafersson	34.2	253	NA

Table 2. Contd.

Menze	73.4	NA	Kakaba	55.7	NA	250	K6290 Bulk	55	253	NA
Millennium	42.3	NA	Mandoyu	14.5	393	250	KBG-01	66.2	253	NA
Ogolcho	28.4	NA	Mitike	50	393	NA	Kubsa	70	253	NA
Pavon 76	40.7	NA	Ogolcho	28.4	393	250	Kulkulu	58.4	253	NA
PBW343	70	NA	Pavon 76	40.7	393	252	Mandoyu	14.5	253	NA
Sanate	4.5	543	PBW343	70	393	250	Menze	73.4	253	NA
Senkegna	8.4	NA	Sanate	4.5	393	277	Meraro	22.7	248	NA
Shorima	28	NA	Senkegna	8.4	393	NA	Mitike	50	253	NA
Simba	30.9	NA	Shinna	75	393	280	Ogolcho	28.4	253	NA
Sirbo	30	NA	Shorima	28	393	284	Pavon 76	40.7	247	NA
Sofumar	65	NA	Simba	30.9	393	250	Senkegna	8.4	253	NA
Sulla	71.7	NA	Sirbo	30	393	250	Shinna	75	253	NA
Tossa	65	NA	Sulla	71.7	393	272	Shorima	28	253	NA
Tura	21.3	NA	Tossa	65	393	276	Simba	30.9	253	NA
Tusie	15.4	NA	Tura	21.3	393	250	Sofumar	65	253	NA
ETBW5879	30.5	NA	Tusie	15.4	393	250	Sulla	71.7	252	NA
Watera	40.8	NA	Watera	40.8	393	278	Tossa	65	247	NA
ETBW7255	32.1	NA	Frequency (%)	-	21 (28%)		Frequency (%)	-	28 (37%)	
Frequency (%)	-	12 (16%)	-	-	-			-		

originally derived from common wheat and it is located on chromosome 3B. An SSR marker, Barc075 was located at 2.4 ± 1.2 cM from *YrRubl*/*Yr4* (Bansal et al., 2010). This marker amplified a single 105 bp band in the reference line (Hybrid46) and the band was absent in wheat lines without *Yr4*. After evaluation by this marker, 10 (14%) cultivars, namely, *Abola, HAR1018, HAR723, HAR820, Hawi, Hoggana, Jafersson, Madda Walabu*, *Simba,* and *Sulla* were genotyped to carry *Yr4* (Table 2).

Yellow rust resistance gene, Yr6

Li and Niu (2007) reported that the SSR markers Wmc076 and Wmc276 were linked to the *Yr6* gene. These two markers amplified 256 and 292 bp fragments, respectively in most lines carrying *Yr6* (Li and Niu, 2007). Marker Wmc76 amplified 256, 267 and 271 bp bands in the reference line (*Yr6*/6*Avocet S). The expected fragment size (256 bp) that indicates for the presence of this gene was used to genotype for *Yr6* genes in the tested materials. Based on this marker, 70% of the wheat lines possessed this gene. The second marker, Wmc276 amplified a single band of size 289 bp in the *Yr6*/6*Avocet S and 292 bp in Pavon 76 (with known *Yr6* gene). In this regard, both fragment sizes were considered to identify this gene from the tested lines. Based on this marker, around 63 (85%) of the wheat lines possess *Yr6* (Table 2). Of the identified genotypes that possess this gene, 42 (56%) of them showed similar results for both markers.

Yellow rust resistant gene, Yr7

Yr7 was first identified in wheat (*Triticum aestivum* L.) cultivar 'Lee'. It is located on the chromosome 2B (Deng et al., 2004). It is one of the *Yr* genes that was used widely by CIMMYT during the1970s and 1980s, and has been deployed in many commercial cultivars in Ethiopia. An SSR marker, Xgwm556, which is linked at 5.3 cM to the target gene was used to genotype for *Yr7*. The marker allowed null allele on nine of the tested genotypes. The fragment sizes produced by other genotypes varied between 131 and 160 bp. The

marker amplified two different fragment sizes on two of the reference lines (Lee and *Yr7*/6*Avocet S). Thirty-two of the genotypes showed a fragment size of 157 bp, similar to *Yr7*/6*Avocet S. A fragment size of 159 bp was observed in 26 genotypes, which was similar to Lee. Six genotypes showed fragment sizes similar to the reference lines. Therefore, 55 (74%) of the genotypes that showed fragment sizes similar to the two reference lines were considered to possess *Yr7* gene.

Yellow rust resistant gene, Yr8

Yr8 is located on chromosome 2D. SSR marker Xgwm157 was linked to *Yr8* with a map distance of about 1.1 cM. The marker amplified a fragment size of 120 bp in wheat lines that possessed *Yr8* genes. In this study, this marker amplified the expected fragment size (120 bp) in the reference line, *Yr8*/6*Avocet S only, whereas none of the tested wheat genotypes amplified fragment sizes similar to the reference lines, indicating the absence of this gene in all the tested genotypes (Table 3).

Yellow rust resistant gene, Yr9

Yr9 was transferred to wheat through chromosomal translocation of 1B/1R and is linked to the *Lr26, Sr31* and *Pm8* resistance genes (Zhou et al. 2004). It is common in CIMMYT-originated bread wheat cultivars. SSR marker Xgwm582 and a resistance gene analogue (RGA) clone marker (iag95) were linked to the *Yr9* gene (Cabuk et al., 2011; Mago et al., 2002). Marker Xgwm582 amplified three bands (142, 149 and 152 bp) on the reference line. The observed fragment size, of 152 bp, was monomorphic, and it was observed in all the tested wheat genotypes. The fragment size of 142 bp which was amplified on chromosome 1B/1R where the target gene (*Yr9*) was located was used to haplotype this gene in the tested materials. Based on this, marker *Yr9* was detected in 34% of the genotypes. On the other hand, based on RGA iag95 marker (gene specific marker), 36% of the wheat genotypes possess *Yr9*. Of the identified wheat genotypes, 25 (32%) of them were amplified by both markers (Figure 1).

Yellow rust resistant gene, Yr10

Yr10 was originally found in wheat line PI 178383 and located on the short arm of chromosome 1B. Singh et al. (2009) designed two primer pairs (*Yr*10 F/R and *Yr10* F1/R1) based on the *Yr10* sequence and produced markers completely linked to *Yr10*. Genotyping with this marker indicated that *Yr10* was identified in 12 (16%) of the commercially released old as well as recently released cultivars such as *Alidoro, Enkoy, ET13A2, Galama, Hidassie, K6290 Bulk, K6295-4A, KBG-01, Mada Walabu, Meraro*, Mitike and *Shina.*

Yellow rust resistant gene, Yr17

Wheat yellow rust resistance gene, *Yr17*, in combination with *Lr37* (leaf rust resistant gene) and *Sr38* (stem rust resistant gene) are located within a segment of *Triticum ventricosum* chromosome 2NS translocated to the short arm of wheat chromosome 2A (Helguera et al., 2003). VENTRIUP-LN2 primers were 2NS specific and the 259 bp PCR amplification product was observed only in plants carrying the 2NS translocation. This marker amplified a fragment size of 259 bp on 26 (35%) entries to have *Yr17* similar to the reference line (*Yr17/6*Avocet S). Likewise, SC-385 amplified a fragment size of 378 pb in the reference line. Based on this marker, *Yr17* was identified in 24 (32%) of the tested genotypes. These two markers exhibited a different output for nine of the genotypes. Based on these two markers, 21 (28%) of the genotypes carry this gene.

Yellow rust resistant gene, Yr18

The adult plant resistance gene *Yr18* was located on the same chromosome segment containing the *Lr34* gene and is tightly linked with it (Singh, 1992b). Additionally, their co-segregation with other traits such as leaf tip necrosis (*Ltn1*), powdery mildew resistance gene (*Pm38*), and tolerance to barley yellow dwarf virus (*Bdv1*) has been reported (Liang et al., 2006; Singh, 1992a; Spielmeyer et al., 2005). *Wms*298 (Cabuk et al., 2011) and a gene specific marker L34DINT9F, L34PLUSR (Krattinger et al., 2009) were used to haplotype this gene. Of the 74 entries, 33 (45%) and 28 (36%) were found to carry *Yr18* according to these markers, respectively. All the wheat genotypes which were genotyped by L34DINT9F, L34PLUSR marker showed the presence of this gene by the second marker, Wms298.

Contribution of the identified yellow rust resistance (Yr) genes

The contribution of each *Yr* gene to yellow rust resistance was evaluated in differential lines that possessed the individual *Yr* gene. As shown in Table 2 and Figure 2, the differential lines carrying *Yr9* and *Yr8* had the highest ACI value (83 each) followed by *Yr6* and *Yr7* with ACI values of 82 and 80, respectively. The lowest (46.4) value was exhibited on a differential line that carried *Yr4*. The ACI values in the other differential lines that carried *Yr1*, *Yr10*, *Yr17* and *Yr18* were 62, 60, 77 and 78, respectively. This indicates that the identified resistance genes do not provide sufficient protection to wheat yellow rust if

Table 3. Total and average number of alleles, polymorphism information content (PIC), allele frequency, and rare alleles for Ethiopian commercial bread wheat and elite lines for *Yr* linked markers.

Marker	Linked to *Yr* gene	Major allele frequency	Allele No.	Gene diversity	PIC
Bu099658	*Yr1*	0.4615	6	0.6972	0.6557
Stm673acag	*Yr1*	0.1923	9	0.9191	0.9143
Barc075	*Yr4*	0.7308	3	0.4464	0.4253
WMS120	*Yr5/18*	0.2308	10	0.8830	0.8744
Xgwm76	*Yr6*	0.2949	7	0.8202	0.8000
Xgwm276	*Yr6*	0.3462	5	0.7768	0.7489
Wms526	*Yr7*	0.1026	9	0.9596	0.9581
Xgwm582	*Yr9*	0.3974	5	0.7163	0.6732
P6M12-P	*Yr9*	0.0769	15	0.9796	0.9792
iag95	*Yr9*	0.6154	1	0.4734	0.3613
Yr10F & R	*Yr10*	0.8333	1	0.2778	0.2392
Wmc273	*Yr15*	0.3846	5	0.7429	0.7053
SC-385	*Yr17*	0.7692	5	0.3744	0.3342
URIC-N2	*Yr17*	0.4359	2	0.7587	0.7373
VENTRUP	*Yr17*	0.1667	8	0.9313	0.9276
Wms295	*Yr18*	0.3077	2	0.8024	0.7766
L34DINT9F, L34PLUSR	*Yr18*	0.6538	1	0.4527	0.3502
Xgwm011	*Yr18*	0.0641	16	0.9790	0.9785
Xgwm44	*Yr18*	0.3205	11	0.7959	0.7681
Xgwc198	*Yr32*	0.8333	2	0.2817	0.2486
Xgwm18	*Yr24*	0.2436	7	0.8695	0.8576
Wmc410	*YrN19*	0.1410	11	0.9421	0.9395
Xgwm111	*Yr33*	0.0641	12	0.9711	0.9703
Xgwm508	*Yr35*	0.6154	9	0.6042	0.5910
Xgwm192	*Yr46*	0.0641	9	0.9691	0.9682
Xgwm165	*Yr46*	0.1282	6	0.9349	0.9312
Barc1182	*Yr1*	0.4359	4	0.6815	0.6242
Xgwm161	*Yr47*	0.5128	5	0.6453	0.5929
Xgwm389	*Yr57*	0.0513	17	0.9832	0.9830
Xgwc251	*Yr62*	0.1282	8	0.9415	0.9386
Mean	-	0.3530	7.24	0.7540	0.7288

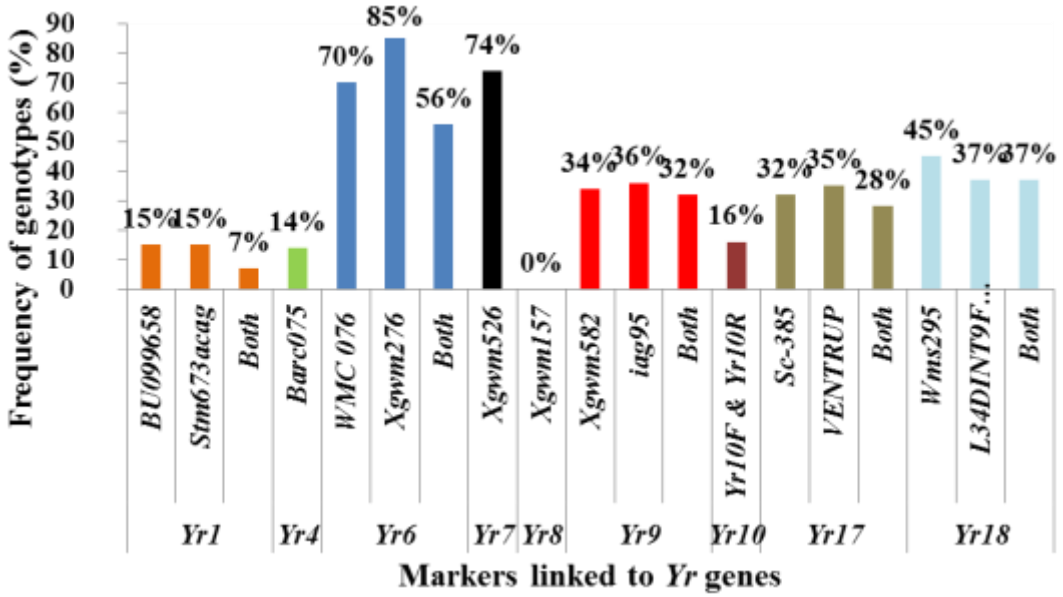

Figure 1. Frequency of wheat genotypes identified with *Yr* genes using linked markers.

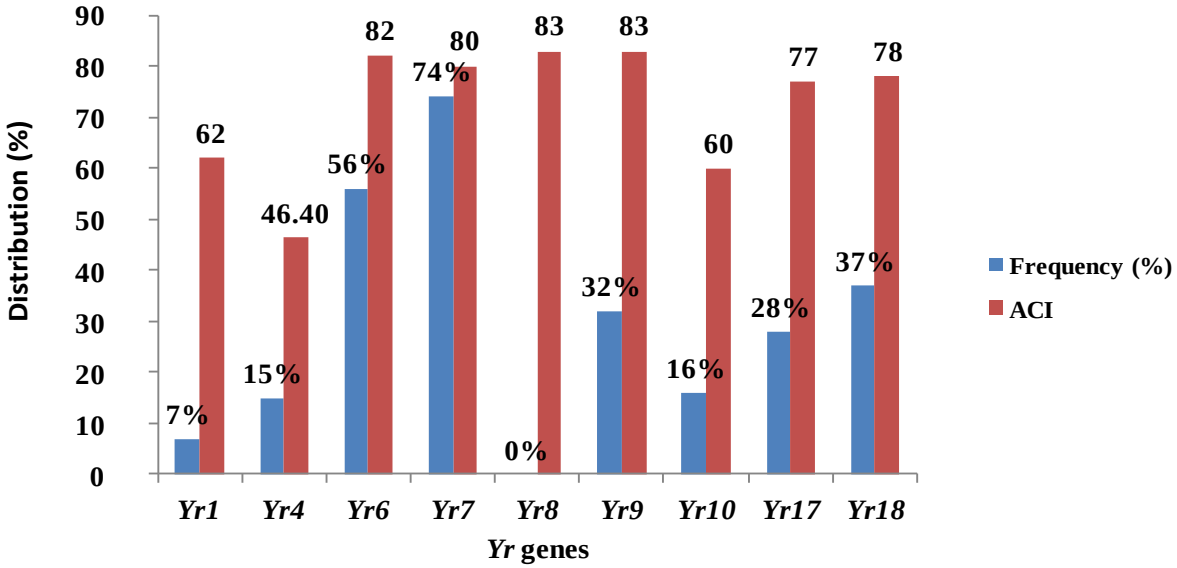

Figure 2. The distribution (%) of each *Yr* gene in 74 wheat genotypes and its ACI values.

they are used as single genes.

Relationship between the number of pyramided genes and yellow rust resistance

Relationship between the number of pyramided genes and yellow rust resistance is as shown in Figure 3. The number of *Yr* genes identified from the tested genotypes varied from 0 to 5 genes. Five varieties (*Digalu, HAR 820, HAR 934, Hoggana* and *Mada Walabu*) possessed the maximum number of five resistance genes. Among these varieties, the highest (82) and lowest (11.4) ACI values were recorded on *Digalu* and *HAR 820*, respectively. These five varieties exhibited ACI values of 42. On the other hand no gene was identified from bread wheat elite line, *ETBW6098*. The field study indicated that this variety exhibited an ACI value of 66.2. Twenty-six (35%) genotypes possessed three genes with an ACI value of 42 followed by 23 (31%) genotypes possessing

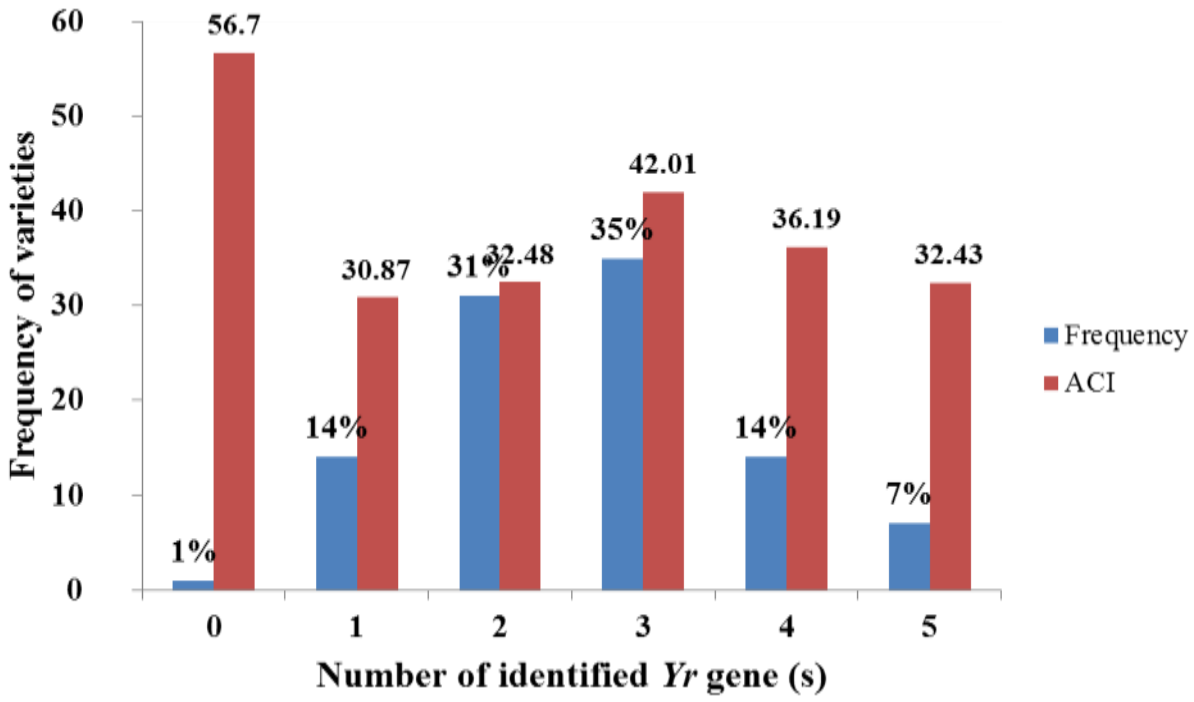

Figure 3. Number and ACI of *Yr* genes detected in 74 bread wheat genotypes.

two genes with an ACI value of 32.

Genetic diversity

A total of 212 alleles were detected with the 29 markers linked with *Yr* genes reported on A, B and D genomes. The number of alleles per locus ranged from 1 to 17 with an average of 7.24 alleles per locus. The highest number of alleles (17) was detected for marker Xwms389, which was linked to *Yr57* (Table 3). Genetic diversity values ranged from 0.28 to 0.97, with the highest value of 0.98 detected for marker Xwmc389. The lowest genetic diversity value was observed for marker Xwmc198. The mean genetic diversity value was observed to be 0.77. In this study, the polymorphism information (PIC) content value ranged from 0.31 to 0.98 with an average of 0.75. The highest PIC was detected by Xgwm389, which is linked to *Yr57,* whereas the lowest was detected by SC-385, which is linked to *Yr17*.

DISCUSSION

The development of molecular markers for mapping resistance genes to yellow rust and of marker-assisted selection (MAS) has been among the most active areas of research in wheat. The accuracy of MAS is affected by the distance between the target gene and the linked markers. Randhawa et al. (2014) stated that markers used to map a gene may not be suitable for detecting the gene in diverse genetic backgrounds. In the present study, genotyping was performed only for those yellow rust resistance genes where reference lines were available as positive controls. The presence of *Yr* genes in the wheat genotypes was counted for each yellow rust resistance gene based on the presence of the fragment sizes of two flanking markers, except for a few genes where only one closely linked marker has been reported.

Molecular marker based gene identification showed the presence of *Yr1, Yr4, Yr6, Yr7, Yr9, Yr10, Yr17* and *Yr18* in various frequencies. On the other hand, *Yr8* gene was not detected in any of the tested wheat genotypes. *Yr*7 is the most frequent (74%) followed by *Yr6* (56%), *Yr18* (37%) and *Yr9* (32%) whereas *Yr1* was detected at the lowest frequency (7%), followed by *Yr4* (14%). *Yr6* and *Yr7* are located on chromosomes 7BS and 2BL, respectively, and they confer all stage resistance (ASR) to yellow rust. *Yr9* was transferred to wheat through chromosomal translocation of 1B/1R (Zhou et al., 2004). These three genes were used widely by CIMMYT and have been deployed in many commercial cultivars (Van and Rajaram, 1993). Badebo et al. (1990) reported that *Yr9* gene was the most frequently (67%) identified gene from Ethiopian commercial and advanced bread wheat genotypes. But in this study, this gene was only detected in 35% of the genotypes, which may indicate the reduction of the previously widely applied *Yr9* gene in the country. However, yellow rust resistance in wheat is dominated by few *Yr* genes, such as *Yr7* and *Yr6,* which

were present in 74 and 56% of the tested varieties and lines, respectively. A diversity reduction of resistant varieties is unfavorable for breeding varieties with durable resistance. With extensive deployment of numerous commercial cultivars throughout the world, virulence for these genes was wide spread. Chen et al. (2002) reported occurrence of high virulence frequency to *Yr2, Yr6, Yr7,* and *Yr9* in most wheat producing areas of the world. Virulence frequency as high as 90% was recorded for *Yr6* and *Yr7* to *Pst* isolates collected internationally (Sharma-Poudyal et al., 2013). In Ethiopia, virulence frequencies of 92 to 100% were recorded among 107 isolates collected in 2005 from different parts of the country (Dawit et al., 2012). The average coefficients of infection (ACI) exhibited in the present study were 82 (*Yr6*), 80 (*Yr7*) and 83 (*Yr9*), indicating the inefectiveness of these genes in the country.

The adult plant resistance gene *Yr18* is located on chromosome 7DL and is tightly linked with leaf rust resistant gene, *Lr34* (Singh, 1992a). Additionally, their co-segregation with other traits such as leaf tip necrosis (*Ltn1*), powdery mildew resistance gene (*Pm38*), and tolerance to barley yellow dwarf virus (*Bdv1*) has been reported (Liang et al., 2006; Singh, 1992a; Spielmeyer et al., 2005). This multi-pathogen resistance locus is a valuable source of resistance in wheat breeding. The use of the slow rusting gene pair *Lr34/Yr18* in combination with other slow rusting genes has been suggested to contribute to near immunity to leaf and yellow rust infections (Singh et al., 2000). Using a sequence specific marker (L34DINT9F, L34PLUSR) that indicated the presence of the cloned *Yr18* in the genomic DNA accurately, this gene was detected in 37% of the tested bread wheat genotypes. Singh (1992a) reported that despite the contribution of this gene in many countries, wheat genotypes with *Yr18* displayed inadequate resistance in some locations in Ecuador and Kenya. In the present study, an ACI value of 78 was recorded on the differential line that possessed this gene, which may indicate the ineffectiveness when used alone in Ethiopia. However, more than 67% of the bread wheat genotypes that possessed *Yr18* in different *Yr* gene combinations exhibited ACI values lower than 78. This study is the first report on the presence of the adult plant resistant gene, *Yr18,* in Ethiopian bread wheat genotypes. Those genotypes that were identified to possess *Yr18* may also possess leaf rust resistance gene (*Lr34*) and other genes linked to *Yr18* such as *Pm38*.

Yellow rust resistance gene, *Yr17* together with other genes such as *Lr37* and *Sr38* are located within a segment of *T. ventricosum* chromosome 2NS translocated to the short arm of wheat chromosome 2A (Helguera et al., 2003). The resistant gene *Yr17* was used in many breeding programs to develop resistant cultivars (Eugene et al., 2015). The yellow rust reaction on wheat seedlings with this gene is influenced by environmental conditions (for example temperature) and genetic background (Eugene et al., 2015). Thus, it is very difficult to apply gene postulation to identify this gene in wheat genotypes. The VENTRIUP-LN2 marker, which is 2NS specific and amplifies a 259 bp PCR product only in plants carrying the 2NS translocation, was used together with the second marker SC-385 for genotyping of this gene. These markers identified 24 (32%) of the entries to have *Yr17* in the present study. Those wheat genotypes that are genotyped to have *Yr17* may also carry leaf rust (*Lr37*) and stem rust (*Sr38*) resistance genes. Earlier reports indicated that *Yr17* was postulated to be present only in three (7%) of the tested Ethiopian bread wheat genotypes (Badebo et al., 2008). On the contrary, *Yr17* was widely deployed in European wheat cultivars and a virulence frequency close to 100% has been reported in Northern European countries (Villaral et al., 2002). Yellow rust resistance gene *Yr17* was effective with regard to the prevailing races in East Africa Badebo and Stubbs, 1995). Similarly, Dawit et al. (2012) reported the effectiveness of *Yr17* gene under Ethiopian conditions. In the same report, a virulence frequency of 14% was exhibited on this gene by isolates collected from Ethiopia, but there was no indication for the presence of these genes in the tested Ethiopian wheat cultivars. In the present study, the exhibited ACI on *Yr17* was 77, which may indicate that this gene is no more effective to the prevailing races in the country.

In addition to the aforementioned yellow rust resistant genes, this study identified the seedling resistant genes *Yr1, Yr4* and *Yr10*. *Yr1* is located on chromosome 2AL. Virulence to *Yr1* has been found in several countries of the world (Wan et al., 2017). But, the virulence frequency is high in East Africa where 166 Chinese wheat cultivar originated (Zhan et al., 2016). For instance, virulence frequencies of 50 and 74% were recorded in Kenya and Ethiopia, respectively (Sharma-Poudyal et al., 2013). Similarly, high yellow rust disease intensity with ACI value of 62 was exhibited in the present study. Bansal et al. (2009) reported a molecular marker, stm673acag, that amplified 120 and 124 bp products in most lines carrying *Yr1* gene, but in the present study the marker amplified a 129 bp fragment size, which may be due to technical reasons. Hasancebi et al. (2014) reported the second marker, bu099658 that is linked to *Yr1* gene. This marker amplified 206 bp only in those lines that possessed *Yr1* genes, including *Yr1*/6*Avocet S. However, this marker amplified 162 bp product on the positive control (*Yr1**Avocet S). Both markers were used to haplotype *Yr1* in the present study but showed different results. Thus, only 5 (7%) of the genotypes that exhibited a fragment size similar to both markers were considered to possess this gene. According to Bansal et al. (2009) stm673acag is used to haplotype both *Yr1* and *Sr48*, thus those wheat genotypes identified to possess *Yr1* may have also stem rust resistant gene *Sr48*.

Yr4 is originally derived from common wheat and is synonymous with *Yr4a* and *Yr4b,* and it is located on

chromosome 3B A microsatellite marker, Barc075, which is 2.4 ± 1.2 cM distal to *YrRub/Yr4* on chromosome 3BS, was used for genotyping. This marker was initially linked to uncharacterized resistant gene (*YrRub*) in Australian wheat cultivar Rubric. Later, the marker was tested in genotypes known to carry *Yr4* (Hybrid 46 and Avalon). Based on the amplification of the Rubric-specific PCR products at Barc075 loci, it was concluded that *YrRub* could be *Yr4* (Bansal et al., 2010). In the present study, the marker amplified a single 105 bp band in the reference line, *Yr4*/6*Avocet S. After evaluation by this marker, 11 (14%) of the bread wheat cultivars showed a similar band size to the reference line. Two of the cultivars (*HAR723* and *HAR82*0), which were previously reported/postulated to have the *Yr4* gene (Badebo, 1990) exhibited a similar fragment size to the reference line. This further confirms the probability of *YrRub* to be *Yr4*. Yellow rust resistant gene *Yr4* was effective in East Africa with regard to the prevailing races (Badebo and Stubbs, 1995). A low virulence frequency of 6% was recorded in Ethiopia (Dawit et al., 2012). In the present study, *Yr4* exhibited an ACI of 50, which may indicate that this gene is no more effective if it is used alone. *Yr10* originated from bread wheat and is located on the short arm of chromosome 1B (It is one of the resistant genes that confers high resistance to *Pst* races in Pakistan (Farrakh et al*.,* 2016) and China (Zheng et al., 2017). In Ethiopia, a yellow rust severity as high as 40% was recorded on the differential lines that possessed this gene. By using a gene specific marker, *Yr10* is confirmed to be present in 16% of the tested wheat genotypes.

Gene pyramiding, combining multiple *Yr* genes in a single genotype is an important strategy to develop durable rust resistant cultivars. The ACI value that was recorded on the genotypes that possessed the maximum number (five) of resistance genes was 42. Cultivars with four and three genes exhibited 36.19 and 42.01 ACI, respectively. This may indicate that pyramiding of those identified genes may not provide sufficient protection against the prevalent races in the country. Thus, there is an urgent need to search for more effective resistance genes to be incorporated in Ethiopian bread wheat cultivars. On the contrary, ACI values ranging from low (8.9) to high (70) were exhibited in cultivars *Gassay* and *Shina,* respectively with one gene. Those cultivars with low average coefficient of infection may indicate the presence of additional *Yr* genes that were not identified in the present study.

The number of alleles per locus ranged from 1 to 17 with an average of 7.24. Genetic diversity values ranged from 0.28 to 0.98, with the highest value of 0.98 detected for markers Xwmc389 and P6M12-P. The mean genetic diversity values were observed to be 0.75. The polymorphic information content (PIC) value ranged from 0.24 to 0.98 with an average of 0.73. The highest PIC value was detected by Xgwm389, which is linked to *Yr57,* whereas the lowest was by Yr10F & R, linked to *Yr10*.

The evidence in this study on the basis of genetic diversity and the presence of *Yr* genes in the improved wheat genotypes will be helpful for developing appropriate breeding strategies to broadening the genetic base in future wheat breeding programs in Ethiopia.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Supplementy Table 1. Particulars of Ethiopian-grown bread wheat varieties and lines used in the study.

S/N	Variety/line	Origin/Source	Year released/ Registered	Cross/selection
1	Abolla (HAR 1522)	CIMMYT/ Ethiopia	1997	BOBWHITE/BUCKBUCK
2	Alidoro	Ethiopia/USA	2007	(HK-14-R251)
3	Batu	CIMMYT	1984	GLL/CUC//KVZ/SX (SUNBIRD)
4	Bobicho (HAR2419)	CIMMYT	2002	PEG/PF70354/KAL/BB/ALD/3/MRNG
5	Bolo (HAR 3816)	CIMMYT	2009	VEE/LIRA//BOW/3/BCN/4/KAUZ
6	Bonde	Ethiopia	landrace	landrace
7	Danda'a (DANPHE#1)	CIMMYT	2010	KIRITATI//2*PBW65/2*SERI.1B
8	Dashen	CIMMYT	1984	KAVKAZ/(SIB)BUHO//KALYANSONA/BLUEBIRD
9	Dereselign	CIMMYT	1974	CI8154/2*FR
10	Digalu	CIMMYT	2005	SHA7/KAUZ
11	Dinknesh (HAR3919)	CIMMYT	2007	CARACARA/4/CORYDON/3/PELOTAS 72380/ARTHUR-71*2/H567.1
12	Dodota (HAR2508)	CIMMYT	2001	BJY/COC//PRL/BOW
13	Dure (HAR1008)	CIMMYT	2001	BOW"S"/YD"s"/ZZ'S" CM62045-1Y-1M-1Y-1M-6Y-1M-OY
14	Enkoy	Kenya	1974	[HEBRAND sel./(WIS 245/ SUP51)]//[FR-FN/Y)2 .A)
15	ET-13A2	Ethiopia	1981	UQ105 Sel. x ENKOY
16	ETBW5800	CIMMYT	Line	WAXWING*2/TUKURU
17	ETBW5890	ICARDA	Line	EALME4SA - 167
18	ETBW6093	CIMMYT	Line	CROC_1/AE.SQUARROSA (205)//KAUZ/3/ENEIDA/4/PSN/BOW//MILAN
19	ETBW6094	CIMMYT	Line	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1
20	ETBW6098	CIMMYT	Line	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1
21	Wane (ETBW6130)	CIMMYT	2016	SOKOLL/EXCALIBUR
22	ETBW6496	CIMMYT	Line	CROC_1/AE.SQUARROSA (205)//FCT/3/PASTOR
23	ETBW6647	CIMMYT	Line	MARCHOUCH*4/SAADA/3/2*FRET2/KUKUNA//FRET2
24	ETBW6696	CIMMYT	Line	PBW343*2/KUKUNA//SRTU/3/PBW343*2/KPASTOR
25	Lemu (ETBW6861)	CIMMYT	2016	WAXWING*2/HEILO
26	ETBW6939	ICARDA	Line	UTIQUE 96/FLAG-1
27	ETBW7698	CIMMYT	Line	FRNCLN/4/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1
28	FH4-2-11	Ethiopia/Germany	Line	Arb//295//SM/3/149/SM//150/M
29	Galama (HAR 604)	CIMMYT	1995	4777*2//FLN/GB/3/PVN
30	Galil	Israel	2010	HORK/YAMHILL//KALYANSONA/BLUEBIRD/3/BOBWHITE
31	Gambo (QUIAU 2)	CIMMYT	2011	BABAX/LR42//BABAX*2/3/VIVITSI
32	Gassay	CIMMYT	2009	PFAU/SERI//BOBWHITE
33	HAR1018	CIMMYT	Line	2109.36?VEE/4WRM//KAL/BB/3?KAL/BB//ALD
34	HAR1331	CIMMYT	Line	L2266-1406101/BUC'S'//VPM-MOS83.11.4.8/NAC
35	HAR 719	CIMMYT	Line	L1RA 'S'
36	HAR723	CIMMYT	Line	CHIL'S'
37	HAR727	CIMMYT	Line	PEG'S'
38	HAR820	CIMMYT	Line	CHIL'S'
39	HAR934	CIMMYT	Line	TJB788.1039/PVN76
40	Hawi (HAR2501)	CIMMYT/ Ethiopia	2000	CHIL/PRL

Supplementy Table 1 Contd.

41	Hidasse (ETBW5795)	CIMMYT/ Ethiopia	2012	YANAC/3/PRL/SARA//TSI/VEE#5/4CROC-1/AE.SQUAROSA(224)//OPATTA
42	Hoggana (ETBW5780)	No inf.	2011	PYN/BAU//MILAN
43	Huluka (ETBW5496)	ICARDA/ Ethiopia	2010	UTQUE96/3/PYN/BAU//MILAN
44	Israel	Unknown	Pre-1949	NA
45	Jefferson	USA	2012	Imported by Morell PLC
46	K 6290-Bulk	Kenya	1977	AF.M2*ROMANY
47	K 6295-4A	Kenya	1980	ROMANZ x GB -GAMENYA
48	Kakaba (Picaflor#1)	CIMMYT	2010	KIRITATI/SERI/RAYO
49	Katar (HAR 1899)	CIMMYT	1999	COOK/VEE//DOVE/SERI/3/BIY/COC
50	KBG-01	Ethiopia/Germany	2001	300-SM-501-M/HAR-1709
51	Kubsa (HAR 1685)	CIMMYT	1994	ND/VG9144//KAL/BB/3/YACO /4/VEERY #5 (ATTILA)
52	Kulkulu	ICARDA	2009	PYN/BAU//MILAN
53	Mada-Welabu (HAR1480)	ICARDA	2000	TL/3/FR/Th/Nar59*2/4/BOL'S'C M56569-/AP-1AP-5AP-2AP-OAP
54	Mandoyu	CIMMYT	2014	WORRAKATTA/PASTOR
55	Menze (HAR 3008)	CIMMYT	2007	MILAN/SHANGHAI#7
56	Meraro (11-6-24)	CIMMYT	2005	M/4/HAR1709/3/M//24/E
57	Millennium (ETBW4921)	CIMMYT	2007	ALONDRA/CEP 75630//CEP 75234/PAT 7219/3/BUCKBUCK/BIY/4/
58	Mitike (HAR1709)	Ethiopia	1994	BOW 28 / RBC
59	Ogolcho (ETBW5520)	CIMMYT	2012	WORRAKATTA/2*PASTOR
60	Pavon 76	CIMMYT	1982	VCM//CNO/7C/3/KAL/BB
61	PBW343	CIMMYT/India	1995	NORD-DESPREZ/VG-1944//KALYANSONA//BLUEBIRD/3/YACO(SIB)/4/VEERY-5
62	Sanate	CIMMYT parent	2014	14F/HAR 1685
63	Senkegna (HAR3646)	CIMMYT	2005	CHUAN-MAI-18/BAGULA
64	Shina (HAR 1868)	CIMMYT	1999	GOV9/AZ//MUS/3/R37/GHL21//KAL/BB/4/ANI
65	Shorima (ETBW5483)	ICARDA	2011	UTIQUE-96/3/PAYNE/BAGULA//MILAN
66	Simba (HAR 2536)	CIMMYT	2000	PARULA/VEERY-6//MYNA/VULTUREPRINIA
67	Sirbo (HAR 2192)	CIMMYT	2001	VS-73-600/MIRLO/3/BOBWHITE/YECORA-70//TRIFON
68	Sofumer (HAR1889)	CIMMYT	200	LIRA'S'/TAN'S'
69	Saulla	Ethiopia	2007	HAR710/RBC
70	Tossa (HAR 3123)	CIMMYT	2004	ATTILA
71	Tura (HAR 1775)	Ethiopia	1999	AROYR Sel.60/1989
72	Tusie (HAR 1407)	CIMMYT	1997	COOK/VEE//DOVE/SERI
73	Honqolo (EBW5879)	CIMMYT	2014	NJORO SD-7
74	Wetera (HAR 1920)	CIMMYT	2000	MON/VEE//SARA
75	ETBW7255	ICARDA	Line	AGUILAL/FLAG-3

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Evaluation of the morphological and quality characteristics of new papaya hybrid lines in Kenya

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Papaya (*Carica papaya* L.) is among the most grown fruit crops worldwide with high economic and nutritional value. In Kenya, the papaya industry relies heavily on imported varieties and farmers' selected seed whose quality is not known. Therefore, the morphological and quality characteristics of mature fruits of eight newly developed papaya hybrids and their control, Sunrise solo were assessed using papaya descriptors (International Board for Plant Genetic Resources). The results showed significant differences in fruit sizes among the newly developed papaya hybrid lines and the control, Sunrise solo with Line 4 having the longest and heaviest fruits. Fruits from Sunrise solo, lines 2, 3, 7 and 8 ranged from small to medium in size, while those of lines 4 and 6 were large. Line 1 had the shortest shelf life of 4 days while Line 7 had the longest shelf life of 11 days. The total soluble solids (TSS) varied from 7.4 in Line 8 to 12.3% in Lines 5 and 7. Hence, most newly developed papaya hybrids Lines showed traits that were comparable to or exceeded those of Sunrise and could be suitable for both local and export markets. However, there is a need to evaluate and characterize the newly developed papaya hybrid lines in different agro-ecological zones in order to monitor the influences of the environment, pests and diseases.

Key words: *Carica papaya* L., new papaya hybrid lines, morphological characteristics, shelf life, fruit quality.

INTRODUCTION

Papaya (*Carica papaya* L.) belonging to the family Caricaceae and order Brassicales, is among the most widely grown fruit crops worldwide. Papaya is native to tropical America but it is currently grown in all tropical and subtropical countries (Nakasone and Paull, 1998; OECD, 2005; FAOSTAT, 2018). It is a trioecious medium sized crop plant with the potential to produce fruits throughout the year (Nakasone and Paull, 1998; OECD, 2005; Teixeira da Silva et al., 2007). Papaya fruits range from 10 to 50 cm in length and the shapes may vary according

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to the varieties (Storey, 1969). The fruit weight also varies substantially and may range from 0.2 to 12 kg depending on the environment and variety (Imungi and Wabule, 1990; OECD, 2005; Chan and Paull, 2008; Nakasone and Paull, 1998; Das, 2013; Yogiraj et al., 2014; Ayele et al., 2017). In Kenya, fruit weights of between 0.23 and 1.3 kg have been reported (Imungi and Wabule, 1990).

Papaya fruits have high economic and nutritional value (Imungi and Wabule, 1990; Nakasone and Paull, 1998; OECD, 2005; Ming et al., 2008). It is grown for a variety of products including juice, wine, jams, candies and dried fruits. The ripe fruits are eaten fresh while the green fruits are cooked as vegetables. The latex of green fruits contains papain which is a proteolytic enzyme used in beverage, food and product of chewing gum, chill-proofing beer, tenderizing meat and for treating digestive disorders (Nakasone and Paull, 1998; Workneh, 2012; Rahman, 2013; Azad et al., 2014). Papaya is a very wholesome fruit and an excellent source of vitamins A and C (Imungi and Wabule, 1990; Nakasone and Paull, 1998; Wijaya and Chen, 2013). The intake of 100 g per day from any papaya variety would satisfy more than recommended dietary allowances of Vitamin C for all age groups (NAS, 1980).

Papaya shows a wide variation in many traits including fruits, plant stature and leaf characteristics (Ocampo et al., 2006; Aikpokpodion, 2012), some of which are exploited in the development of commercial papaya cultivars. The commercial papaya cultivars are generally classified as inbred gynodioecious lines, typified by the Hawaiian Solo lines (Storey, 1969) out-crossing dioecious populations, such as the Australian papaws; F1 hybrids, including the Tainung series (Taiwan), Eksotika II (Malaysia), and Rainbow (Hawaii); or occasionally even clones, such as Hortus Gold in South Africa (Kim et al., 2002). Many commercial papaya cultivars developed in different parts of the world were introduced into Kenya. These include ‘Kapoho solo’ (Storey, 1969), ‘Waimanalo’, ‘77’, ‘116’, ‘273’ from Hawaii, ‘Cavite’, introduced from the Philippines, ‘417’, ‘418’ and ‘455’ from India, 457 from Indonesia and ‘Kiru’ from Tanzania. Locally developed papaya cultivars included ‘Kitale’, ‘Malindi’ and ‘PP1’ (Imungi and Wabule, 1990; Asudi et al., 2013). Recent evidence also indicates that various commercial cultivars such as ‘US’, ‘Redlady’, ‘Sunrise’, ‘Sunrise-Solo’ and ‘Honey dew’ originating from Asia and America, are regularly imported as seeds by commercial papaya growers in Kenya. Some of the commercial papaya cultivars reported in the 1990s (Imungi and Wabule, 1990) no longer exist (Asudi et al., 2010) probably due to the disappearance or selection or importation of new cultivars into the country.

Globally, Asia is the leading papaya producing continent with 56.27% of the global production, followed by America (33.12%) and Africa with 10.50% production (FAOSTAT, 2018). In Kenya, papaya is popular and economically important and it is grown for domestic use as well as for commercial purpose on both small and large scale with majority of growers being small-scale farmers (Asudi, 2010). Papaya is ranked sixth after banana, mangoes, pineapples, avocado and watermelon, and accounts only for 4% of the revenue generated by the fruit’s subsector in the country (Horticultural Crops Directorate, 2016). The area under production and yields have also decreased rapidly from 9,346 to 8,112 ha and from 127,782 to 107,591 tons representing a 13 and 16% drop, respectively. The decline is due to lack of quality planting materials arising from genetic erosion due to open pollination in papaya, lack of established seed producers, insect pests and diseases such as ringspot viruses. Papaya fruit production in Kenya also relies on imported varieties and farmers’ selected seeds (Asudi, 2010; Horticulture Crops Directorate, 2016) whose quality is not known. In addition, since the introduction of papaya fruits in Kenya, little attempts have been made to develop improved papaya variety with superior quality attributes and that are adapted locally. Hence, the researchers have developed new papaya hybrid lines using some of the commercial papaya cultivars and accessions collected locally with divergent morpho-agronomic traits in Kenya (Asudi et al., 2010) with good quality fruits. However, the quality characteristics of these new papaya hybrids have not been documented. Therefore, the objectives of this study were to evaluate the morphological and quality characteristics of the fruits of the newly developed papaya hybrid lines.

MATERIALS AND METHODS

Study area

The study was carried out at the JKUAT main campus situated in Juja (1°5′ 29″ S, 37°0′39″ E and 1521.3 m above sea level), 36 km northeast of Nairobi, Kenya.

Source of papaya fruits

Eight papaya hybrid lines and the control (‘Sunrise solo’) were used in the experiment. The papaya hybrids were developed as a result of selection of papaya seeds collected all over Kenya by Asudi et al. (2010). The seeds were extracted, germinated and grown in screen house and then cross-bred. Line 1 was developed from a cross between a local papaya from Manyani (MAN1) and Sunrise solo. Line 2 was from a cross of local papaya from Voi (VOI4) and local papaya from Kilifi (ST2). Line 3 was bred from a cross between a local papaya from Voi (VOI5) and a local papaya collected from JKUAT farm (BLOCK A). Line 4 was developed as a result of a cross between VOI5 and Sunrise solo, Line 5 between a local papaya from Mombasa (MT/M7) and (VOI4), Line 6 between a local papaya from Voi (KIBBELEPTIC) and Sunrise solo, Line 7 between (VOI4) and (BLOCK A), and Line 8 from a cross between a local papaya from Manyani (MAN2) and Sunrise Solo.

Experimental design

The plants were planted in an open field in a complete randomized

Table 1. The morphological and quality characteristic of new papaya hybrids.

Hybrid	Fruit weight (g)	Fruit length (cm)	fruit diameter (cm)	Internal cavity length (cm)	Internal cavity diameter (cm)	TSS (°brix)
Sunrise solo	544 ± 56.3^{cd}	12.3 ± 0.6^{e}	9.4 ± 0.6^{def}	8.5 ± 0.5^{f}	5 ± 0.4^{cd}	7.7 ± 0.2^{e}
Line 1	430 ± 45.3^{d}	13.8 ± 0.5^{d}	8.5 ± 0.5^{f}	10 ± 0.5^{ef}	4.4 ±0.4^{cd}	11.2 ± 0.1^{b}
Line 2	813.7 ± 72.2^{bc}	16.8 ± 0.5^{c}	10.5 ± 0.4^{cd}	11 ± 0.5^{de}	5.8 ± 0.3^{bc}	11.6 ± 0.1^{b}
Line 3	898.5 ± 62.5^{b}	17.2 ± 0.5^{bc}	11.4 ± 0.3^{bc}	11.6 ±0.4^{cde}	6.3 ±0.3^{bc}	8.7 ± 0.2^{d}
Line 4	1246.7 ± 70.3^{a}	21.2 ± 0.5^{a}	11.9 ±0.2^{b}	15.6 ± 0.9^{a}	6.7 ±0.2^{b}	8.6 ± 0.2^{d}
Line 5	586.7 ± 58.2^{cd}	16.6 ± 0.6^{c}	10 ± 0.5^{de}	13.7 ± 0.6^{b}	7 ± 0.5^{b}	12.3 ± 0.2^{a}
Line6	1240.8 ± 93.9^{a}	18.5 ± 0.6^{b}	13.3 ± 0.6^{a}	15.7 ± 0.6^{a}	11 ± 0.7^{a}	10 ± 0.2^{c}
Line7	586.3 ± 36.2^{cd}	16.5 ± 0.5^{c}	9.2 ± 0.4^{ef}	12.7 ± 0.5^{bc}	3.1 ± 0.3^{e}	12.3 ± 0.2^{a}
Line8	626.7 ± 44.9^{c}	17.5 ± 0.4^{bc}	9 ± 0.3^{ef}	12.3 ± 0.4^{bcd}	5.2 ± 0.1^{cd}	7.4 ± 0.2^{e}
LSD	171.9	1.5	1.22	1.6	2	0.5
CV%	43.6	17.2	23.1	25.3	19.1	10.6

The data are expressed as means ± standard error of the mean. The means followed by the same letters in the same column are not significantly different at P≤ 0.05 (n=30).

block design and the set-up replicated three times. The normal agriculture and agronomic practices were performed for the plants. Ten fruits were hand picked randomly at colour break stage from 11 months' old papaya tree from the farm with three replications for each hybrid. The fruits were wrapped with newspapers and placed gently in crates in single layers, then transported to the laboratory, sorted, washed and dried at room temperature (25°C±2) for about 30 min. The fruits were then stored at room temperature and relative humidity of 65 to 70% for four days.

Morphological and quality characterization of the fruits

Phenotypic characterization of the new papaya hybrids and the control was determined using papaya descriptors (International Board for Plant Genetic Resources, 1988). The weights of papaya fruits were determined by using an electronic weighing balance (Dahongying, SKU model) and then grouped into small, medium or large based on the fruit's weight, length and diameter. Small fruits consisted of fruits weighing less than 500 g, 15 cm long or less and up to 10 cm in diameter. The medium fruits weighed between 500 and 1000 g and were between 15 and 25 cm long and between 10 and 13 cm in diameter while large fruits consisted of fruits weighing greater than 1000 g or ≤3000 g, >25 cm in length and >13 cm in diameter. The papaya fruits were classified into extra class, class I or class II according to the guidelines of the Codex standard for fresh papaya fruits (Codex Alimentarius, 2007). Data for fruit length, diameter and fruit cavity dimensions were collected using a set of Vernier calipers. Longitudinal sections of the harvested fruits per tree were made and then the fruits lengths were measured from the base of calyx to the tips of fruits using digital Vernier caliper. The diameters of the fruits were measured at the broadest part from the equator. The longitudinal and transversal sections of the harvested fruits per tree were also made for determining the central cavity sizes and shapes. Fruit skin and fresh colour were determined using the Royal Horticultural Society Colour Chart (RHS, 2015). The colours were arranged in four fans with each fan having specific colour group with numbers and letters. Then, a hole was placed on fruits surface or fresh in the presence of natural light and the corresponding colour recorded.

Fruit shelf life was evaluated for the fruits at interval of two days from the beginning of ripening until the end of edible life at room temperature (25±2°C) and relative humidity of 65 to 70%. The number of days the fruits lasted at room temperature before softening was recorded. The total soluble solid (TSS) was determined for the fruits using an Atago hand held refractometer (Model RX5000, Tokyo, Japan).

Data analysis

Quantitative data on the fruit weights, diameter and length, internal cavity length and diameter, % brix and shelf life were subjected to a one way analysis of variance using GenStat software 14th edition (VSN International Ltd.) to assess any differences between commercialized hybrid, sunrise solo and the newly developed hybrid lines. Statistical significance was determined at 95% and means separated by the Duncan's Multiple Range test. Qualitative data on fruit colour, shape, texture and ridging on the fruit surfaces were summarized using cross tabulations and processed descriptively using means, frequencies and percentages and chi-square (χ^2) using the Statistical Package for Social Sciences (SPSS) version 18 (SPSS Inc. Chicago, USA) with a statistical significance of 95%.

RESULTS

Morphological characteristics of fruits of the new papaya hybrids

The weights of the fruits varied significantly (Table 1; P < 0.05) between the new papaya hybrid lines and Sunrise solo and ranged from 430 g in Line 1 to 1246.7 g in Line 4. The lightest hybrid line was 110 g lighter than control, Sunrise solo (Table 1). Averagely, papaya hybrid Line 4 also had the longest fruits, while the control, Sunrise solo had the shortest fruits. The mean fruit length varied significantly (Table 1; P< 0.05) between the hybrids and the control. The longest mean fruit diameter of 13.3 cm was recorded in Line 6 while the shortest mean fruit diameter of 8.5 cm was observed in Line 1. The mean fruit internal cavity length varied significantly (Table 1; P <

Figure 1. Morphology of new papaya hybrid lines. **(A)** Sunrise solo with small fruits; **(B)** Line 1 with small fruits; **(C)** Line 2 with small and medium fruits; **(D)** Line 3 medium to large fruits; **(E)** Line 4 with large fruits; (F) Line 5 with small and medium fruits; **(G)** Line 6 with large fruits; **(H)** Line 7 with small and medium fruits; **(I)** Line 8 with small and medium fruits.

0.05) between the new hybrid lines and the control with shortest length in control (8.5 cm) and the longest length in Line 6 (15.7 cm). The mean fruit internal cavity diameter also varied widely and significantly between the hybrids and the control from 3.1 cm in Line 7 to 11 cm in Line 6. Generally, TSS varied significantly from 7.4 to 12.3° Brix in the new papaya lines with lines 5 and 7 having the highest TSS and Line 8 with the lowest TSS (Table 1; $P < 0.05$).

Qualitative characterization of the new papaya hybrids

The shapes of the fruits varied widely and significantly ($\chi 2 = 1137.2$; df = 96, $P < 0.01$) among the new papaya hybrid lines (Figure 1) and the Sunrise solo with 13 different shapes being observed. However, Line 1 had the highest number of varied shapes consisting of 56.7% of fruits with oval shape, followed by round-shaped fruits with 26.7%, elliptic (6.7%), and globular, high round and pear-shaped each with 3.3% fruits. Fruits belonging to Line 2 were divided into five different shapes with 56.7% being turbinate inferior, followed by elongated fruits with 20%, elliptic (16.7%), and club and globular each with 3.3% of fruits. Majority of the fruits (70%) belonging to the Line 3 were oblong-blocky shaped but a few were elongated (13.3%), club-shaped (10%) or rounded (6.7%). Fruits from Sunrise solo had three different shapes with majority (70%) being pear-shaped, a few were oval (16.7%) or round (13.3%) in shape. Fruits belonging to Lines 4, 5, 6, 7 and 8 varied widely but were

divided only into two shapes. Hence, fruits belonging to Line 6 were equally grouped into globular or Oblong-ellipsoid, while 36.7, 73.3 and 46.7% of fruits in Lines 5, 7 and 8, respectively were elongated. Pear-shaped fruits were the majority observed in Line 4 (70%) and Line 5 (63.3%) while 26.7% of fruits in Line 7, 30% of fruits in Line 4 and 53.3% of fruits in Line 8 were elliptic, plum-shaped and blossom-end tapered, respectively.

The skin texture of ripened fruits in most hybrids (50.7%) was intermediate or smooth (40.7) with few hybrids, namely Lines 4, 7 and 8 having rough skin texture (Table 2). The texture of ripened fruits varied significantly (χ^2 =126.7; df =16, P <0.01) in all the papaya hybrids (Table 2). The ridging on fruits' surfaces varied significantly (χ^2 = 115.3; df = 16, P < 0.01) among the new papaya hybrids and the control. Intermediate ridging was common in all the hybrids while superficial and deep ridging types were not observed in Lines 6 and 1, 2 and 8, respectively (Table 2). The majority of all fruits had slightly (56.7%) or star-shaped (39.65%) central cavity. However, the central cavities of a few fruits in Line 7 were irregular (0.7%) and a few fruits in Lines 2 and 7 and majority in Line 8 were angularly shaped (3.0%) (Table 2). Significant variation in skin colour was observed (χ^2 = 768.7; df= 32; P< 0.01) (Table 2) with vivid yellow (38.9%), vivid yellowish green (21.9%) and strong orange yellow (19.3%) being the most dominant in all hybrids fruits. The flesh colour of the fruits (Figure 2) also varied significantly in papaya lines with the control (χ^2 =768.78; df = 32, P < 0.001). Five different flesh colours were found among the newly papaya hybrids and the Sunrise solo (Table 2). The study also found diversity (χ^2 = 183.4; df = 24, P <0.001) in fruit stalk end shape including depressed (30.4%), flattened (28.1%), inflated (16.7%), and pointed (24.8%).

Classification of new papaya hybrids based on fruit size

Among the evaluated new papaya hybrids, Line 1 showed the highest proportion of fruits with small size (70%), followed by Sunrise solo with 50% and Line 5 with 46.7%. The highest proportion of medium sized fruits was recorded in Line 7 with 63.3% fruits, followed by Lines 8 and 3 each with 60% and Line 5 with 40% fruits (Table 3). Majority of large fruits were however recorded in Lines 6 and 4 with 63.3 and 76.7% of large fruits, respectively (Table 2). All the assessed fruits belonging to Lines 5 and 7 were grouped into extra class, fruits belonging to the Sunrise solo, Lines 1, 2, 4 and 6 under class I and those from Lines 3 and 8 felt in Class II (Table 3).

The new papaya fruit hybrids storage characteristics

A gradual decline in eating quality among all papaya fruits was noticed (Figure 3). A distinctness in papaya fruits ripening, shriveling and senescence was recorded between the new papaya hybrid and control. Line 7 had the longest shelf life of 11 days, while Line 1 had the shortest shelf life of 4 days. Fruit softening and decline in organoleptic quality by the 5th day was recorded in Line 1, Line 7 and the control, whereas, Line 7 maintained the quality until the 11th day (Figure 3).

DISCUSSION

From the findings of this current study, the morphological and quality characteristics of papaya fruits showed significant differences with majority of newly developed hybrid lines recording higher fruit weights and size than Sunrise solo. Hence, Lines 1 and 8 had smaller fruits sizes that were comparable to Sunrise solo while Lines 4 and 6 recorded bigger fruits, which could be explained by heritability or dominance of either parental line with Sunrise solo conferring small fruit traits to Lines 1 and 8 while its influence was subdued in Lines 4 and 6. Lines 2, 5 and 7 also produced fruits with similar size characteristics indicating dominance of large fruits collected from Voi.

Fruit size, shape, smooth skin and absence of blemishes, skin and flesh colour are the major characteristics that determine the market price and export grades for fruits (Barrett et al., 2010; Zhou et al., 2014). Fruit colour gives the first impression of the fruits to the consumers and is an indicator of freshness and flavour quality. Hence, an attractive product can stimulate the desire of purchasing while an inappropriate colour indicates loss of freshness or lack of ripeness (Okoth et al., 2013; Barrett et al., 2010). In papaya, most female plants produce large round-shaped fruits of good quality with a large seed cavity while hermaphrodite plants produce small to medium elongated fruits of good quality but with a smaller seed cavity (Villegas, 1997; Nakasone and Paull, 1998). Researchers observed a significant variation and number in the shapes of the fruits among the newly developed papaya hybrid lines and Sunrise solo, while the fruit skin colour varied from vivid greenish yellow to vivid yellow. The fruit flesh colour also varied from vivid yellow pink to vivid reddish orange. Therefore, the present study corroborates previous findings of variations in papaya fruit shapes and colour in Mexico, Venezuela, Kenya and Nigeria (Ocampo et al., 2006; Asudi et al., 2010; Aikpokpodion, 2012).

The colour of papaya fruit flesh is determined largely by the presence of carotenoid pigments. Red and yellow are the two major papaya fruit flesh colours and are controlled by a single genetic locus with yellow being dominant over red (Storey, 1969). Besides, the yellow-fleshed fruit contains β-carotene while the red-fleshed papaya fruit has high levels of lycopene and the conversion of lycopene to β-carotene is catalyzed by

Table 2. Qualitative description of the new papaya hybrids.

Descriptor	Papaya hybrids									Mean N = 270	χ^2
	Control	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6	Line 7	Line 8		
Fruit skin texture when riped (%)											
Smooth	26.7	60.0	76.7	6.7	66.7	60.0	10.0	33.3	26.7	40.7	
Intermediate	73.3	40.0	23.3	60.0	33.3	40.0	50.0	63.3	73.3	50.7	126.7***
Large	-	-	-	33.3	-	-	40.0	3.3	-	8.5	
Ridging on fruit surface											
Superficial	50.0	83.3	80.0	3.3	60.0	46.7	-	53.3	56.7	48.1	
Intermediate	40.0	16.7	20.0	46.7	33.3	23.3	53.3	43.3	43.3	35.6	115.3***
Deep	10.0	-	-	50.0	6.7	30.0	46.7	3.3	-	16.3	
Shape of central cavity											
Irregular	-	-	-	-	-	-	-	6.7	-	0.7	
Angular	-	-	6.7	-	-	-	-	20.0	53.3	3.0	63.9***
Slightly star shaped	53.3	50.0	50.0	53.3	73.3	53.3	73.3	50.0	46.7	56.7	
Star shaped	46.7	50.0	43.3	46.7	26.7	46.7	26.7	23.3		39.6	
Skin colour											
Vivid yellow	56.7	83.8	16.7	33.3	16.7	20.0	6.7	66.7	50.0	38.9	
Strong orange yellow	10.0	3.3	-	50.0	73.3	33.3	3.3	-	-	19.3	
Deep green yellow	13.3	-	6.7	3.3	-	16.7	26.7	-	-	7.4	768.7***
Vivid yellowish green	13.3	13.3	76.7	13.3	-	3.3	13.3	30.0	33.3	21.9	
Deep greenish yellow	6.7	-	-	-	10.0	26.7	50.0	3.3	16.7	12.6	
Fruit flesh colour											
Strong orange yellow	-	-	-	-	-	-	-	96.7	10.0	11.9	
Vivid orange yellow	-	-	-	-	-	-	96.7	3.3	-	11.1	
Vivid yellowish pink	-	-	93.3	-	-	-	-	-	-	10.4	768.7***
Vivid reddish orange	40.0	76.7	6.7	86.7	86.7	70.0	3.3	-	60.0	47.8	
Reddish orange	60.0	23.3	-	13.3	13.3	30.0	-	-	30.0	18.9	
Stalk end fruit shape											
Depressed	40.0	13.3	63.3	30.0	56.7	26.7	33.3	3.3	6.7	30.4	
Flattened	40.0	23.3	20.0	50.0	23.3	26.7	30.0	16.7	23.3	28.1	183.4***
Inflated	16.7	63.3	-	10.0	10.0	10.0	33.3	-	6.7	16.7	
Pointed	3.3	-	16.7	10.0	10.0	36.7	3.3	80.0	63.3	24.8	

***Statistically significant (Chi-square analysis) at $P < 0.01$.

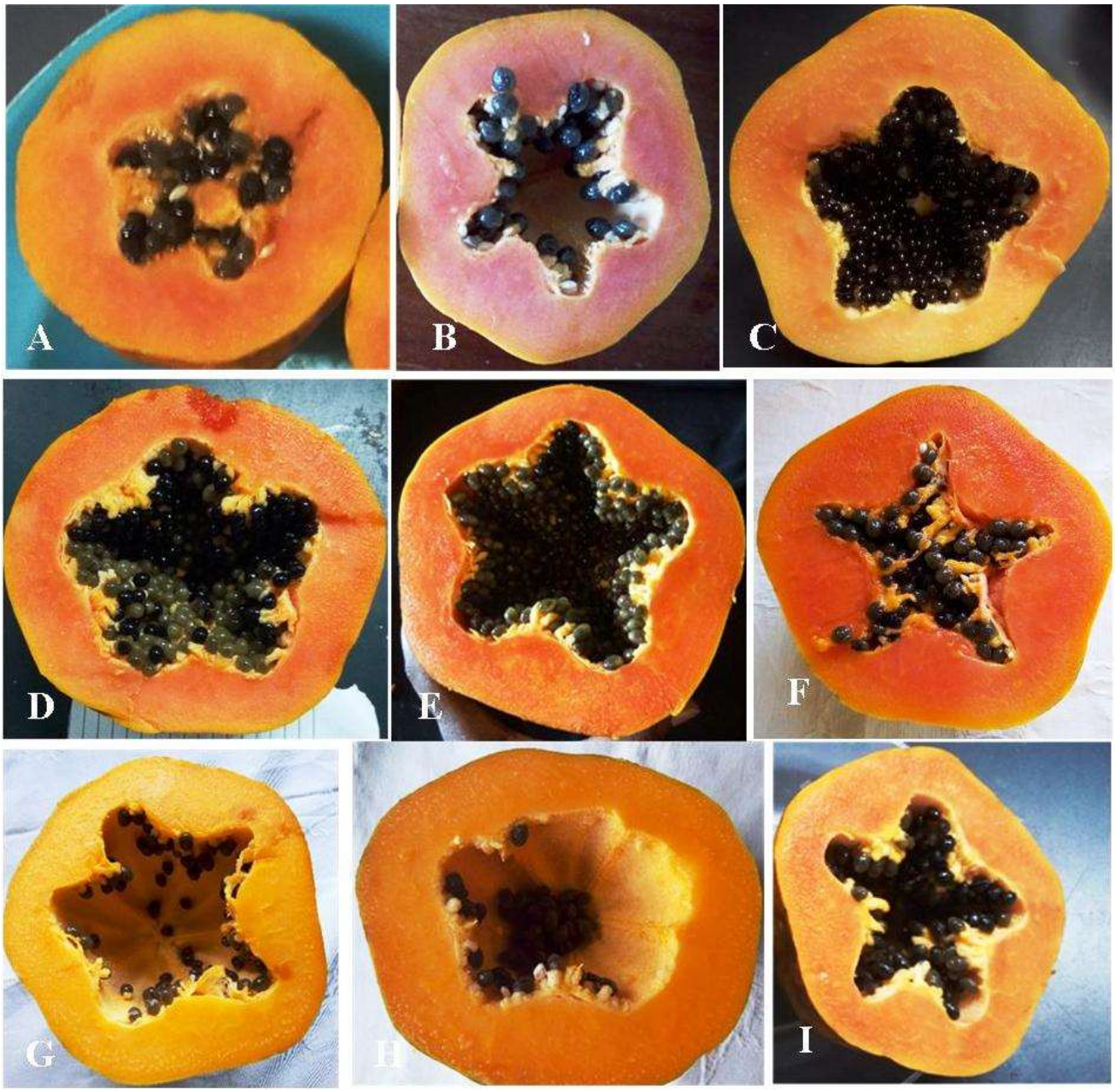

Figure 2. Variations in the fruit central cavity shape and flesh colour among new papaya hybrid lines. **(A)** Sunrise solo with slightly star shaped and vivid reddish orange; **(B)** Line 1 with slightly star shaped and reddish orange flesh colour; **(C)** Line 2 with slightly star shaped and vivid yellowish pink flesh colour; **(D)** Line 3 with slightly star shaped and vivid reddish orange flesh colour; (E) Line 4 with slightly star shaped and vivid reddish orange flesh colour; **(F)** Line 5 with star shaped and vivid reddish orange flesh colour; **(G)** Line 6 with slightly star shaped and vivid orange yellow flesh colour; **(H)** Line 7 with angular shaped and strong orange yellow flesh colour; **(I)** Line 8 with star shaped and vivid reddish orange flesh colour.

lycopene β-cyclase. The carotenoid profile and organization in the cell also differ in yellow and red-fleshed papaya varieties (Yamamoto, 1964; Chandrika et al., 2003; Devitt et al., 2010). Therefore, different papaya fruit flesh colours observed in the present study could be due to differences in the carotenoids content in the newly developed papaya hybrid lines. The variation in skin colour in mature ripen fruits observed among the new hybrid lines and Sunrise solo could also be related with enzymatic degradation or chlorophyll degradation during ripening (Ding et al., 2007; Zuhair et al., 2013).

Besides morphological traits, consumer acceptance of papaya fruit depends on various physicochemical properties including TSS. For instance, TSS of > 11.5°

Table 3. Classification of the new papaya hybrids based on fruits size.

Hybrid	Range (g)	Fruit size classification			CODEX classification
		Small (%)	Medium (%)	Large (%)	
Sunrise solo	200 - 1625	50.0	43.3	6.7	Class I
Line 1	150 - 1200	70.0	23.3	6.7	Class I
Line 2	650 - 930	33.3	36.7	30.0	Class I
Line 3	260 - 2045	6.7	60.0	33.3	Class II
Line 4	685 - 2435	0.0	23.3	76.7	Class I
Line 5	200 - 1400	46.7	40.0	13.3	Extra class
Line6	470 - 2595	6.7	30.0	63.3	Class I
Line7	255 - 1030	33.3	63.3	3.3	Extra class
Line8	320 - 1500	36.7	60.0	3.3	Class II

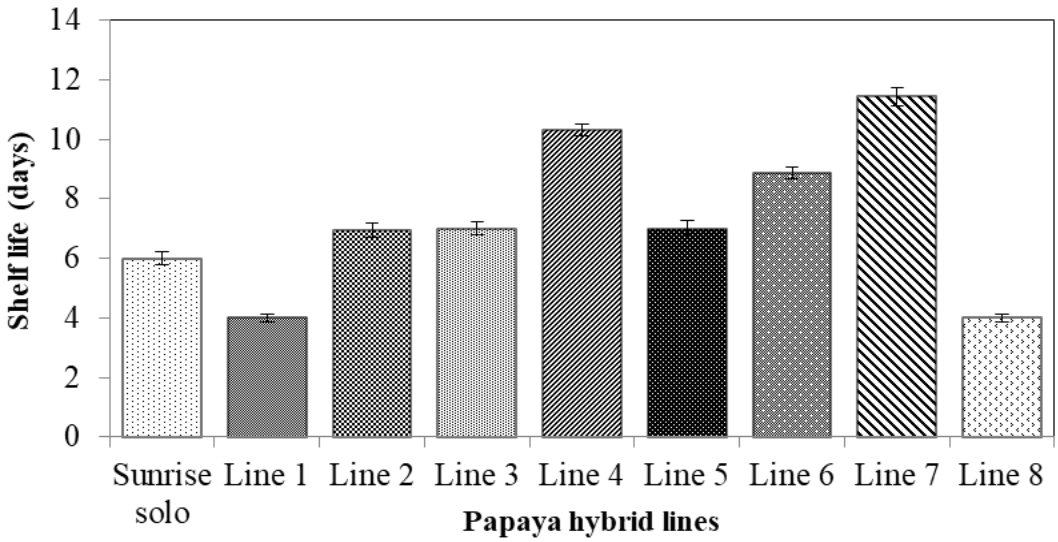

Figure 3. The new papaya hybrids fruit storage characteristics evaluated at interval of two days from the beginning of ripening until the end of edible life at room temperature.

Brix are a minimum grade requirement for traded Hawaiian papayas (Chan and Paull, 2008) while in Jamaica pear-shaped fruits with red flesh, TSS of ≥12° Brix and mass from 385 to 533 g are desired for export. Although similar fruit attributes are required by both the United States (US) and European markets, buyers in the US and the United Kingdom prefer fruits between 274 and 744 g and from 224 to 535 g, respectively (Tennant et al., 2010). In the current study, researcher found desirable TSS ranging from 11.2 to 12.3° Brix in Hybrid Lines 1, 2, 5 and 7 with average weights of 430 to 813 g, which are within the export market limits. However, Hybrid Lines 3, 4 and 6 had large fruits with ≤10° Brix and may be suited for domestic market or local processing industries. However, low °Brix values found in Sunrise solo and Line 8 could have been due to environmental conditions.

The new papaya hybrids fruits were also classified into Extra class, Class I and Class II. The codex standards for fresh fruits and vegetables (Codex Stan 183-1993) indicates different provisions concerning the quality of papaya fruits (Codex Alimentarius, 2007). The Extra class indicates superior quality fruits free of defects; Class I indicates fruits with slight defects in shape or skin due to mechanical, sun spots and/or latex burns with no effect on the fruit's pulp, general appearance and quality of the produce, while Class II includes fruits which satisfy the minimum requirements with defects that may allow them to retain their essential characteristics regarding keeping and presentation qualities. Therefore, this information will assist different actors in papaya value chain to make appropriate decision about the new papaya hybrid lines.

Papaya fruit shows rapid softening and yellowing and has a short-term shelf life due to its climacteric behavior (Archbold and Pomper, 2003; Fernandes et al., 2006).

The storage of papaya fruit at low temperature extends its commercial shelf life, while storage in an inappropriately low temperature results in skin scald, hard lumps in the pulp around the vascular bundles, water soaking of flesh, increased susceptibility to postharvest pathogens and abnormal ripening (Almeida et al., 2005). Therefore, storage conditions in tropics for fresh products are important and essential for quality and shelf life of fruits. In many places of traditional markets and streets in Indonesia in uncontrolled environments, papaya fruits are exposed to high temperatures of up to 30°C, thereby reducing their shelf life (Mohammad et al., 2015). This situation is also common in Kenya where most poor farmers cannot afford such controlled environments to lengthen fruit shelf life. Researchers evaluated the shelf life of newly developed hybrid papaya fruits at room temperature for 14 days and found an average of 4 to 11 days with Line 7 recording the longest shelf life, which could be because of delayed physiological change such as little water loss. This is especially useful for storage, long distance transportation, export and marketing plan for the fruits. Evaluation of morphological and quality characteristics of the fruits of the newly developed papaya hybrid lines has highlighted fruits with small and medium sizes and desirable shapes and TSS that could be suitable for both export and local markets and compared favourably with Sunrise solo, which is an imported papaya variety in Kenya. However, characterization and assessment of distinctness, uniformity and stability of the most performing fruits in different agro-ecological zones is needed in order to monitor the influences of the environment, pests and diseases. There is also need to study the shelf life of newly developed papaya fruits under different temperature conditions or develop new technologies for longer storage to curb postharvest losses.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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