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Genetic diversity of elite wheat mutant lines using morphological characters and molecular markers

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Genetic diversity is the material basis for crop improvement. The genetic diversity of 17 wheat genotypes was evaluated using 25 agro-morphological characters and 10 simple sequence repeat (SSR) markers. The objective of this study was to determine the genetic diversity of elite stem rust resistant mutant lines in comparison with their adaptable but susceptible parent varieties using morphological traits and molecular markers. The results obtained showed significant variation in morphological traits and molecular makers existed. Morphological diversity between mutant lines and their parent varieties was mainly separated by grain yield per spike, 1000 grain weight and maturity time period. The dendrogram based on 10 SSR markers grouped the 17 genotypes into three major clusters and six sub-clusters with mutants clustering with their respective parents. 10 SSR primer pairs yielded 13 polymorphic loci with a percentage of 92.86%. The mean number of alleles per locus in each group was 2.0 and the mean number of polymorphic alleles per locus was 1.9286. The gene diversity ranged from 0 to 0.4893 for each sample. Results showed it is possible to classify genetic diversity of elite wheat genotypes and select them for the highest genetic diversity. The results can be used in selecting diverse parents in breeding programs and also in maintaining genetic variation in the germplasm.

Key words: Genetic diversity, molecular markers, morphological traits, wheat.

INTRODUCTION

Wheat (*Triticum aestivum.*L) contributes to food security in Kenya and his ranked second important cereal crop after maize (KALRO, 2016). However, its productivity is low due to abiotic and biotic stresses (Njau et al., 2010). Wheat is a self-pollinating crop that has been bred and developed for specific end-use quality traits and to grow within a specific production environment. Genetic variability holds the potential to deal with multiple biotic and abiotic stresses. Knowledge of genetic diversity of a crop is important in the development and improvement of a particular crop species. Evaluation of genetic diversity among adapted germplasm provides predictive estimates of genetic variations among segregating progeny for new varieties development. It is desirable therefore to have a large genetic diversity for the creation of new genotypes.

Morphological traits and molecular markers play an important role in the analysis of variance in genetic diversity studies. Use of morphological traits alone is unreliable because they are greatly influenced by the

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environment (Takumi et al., 2009). A genotype may exhibit different morphological traits for two different locations (Stepien et al., 2007). Molecular markers increase the breeding progress for traits that are difficult to select under field conditions and those that are controlled by multiple genes. Simple Sequence Repeats (SSRs) were considered the best molecular markers because they are able to identify and differentiate genotypes within a particular species. Their co-dominant inheritance and the high level of polymorphism in a large sample of elite germplasm make them useful (Prasad et al., 2000). Genetic diversity is important for adaptability and survival of genotypes against the threats of biotic and abiotic stresses. Wheat breeding in Kenya has attempted to develop resistant wheat varieties through utilization of resistant genes, but virulence is still being reported in most these varieties (Njau et al., 2009). The objective of this study was to assess genetic diversity of eight pre-selected elite mutant lines in comparison with their adaptive but stem rust susceptible parents and seven other commercial checks using morphological traits and molecular markers.

MATERIALS AND METHODS

Experimental local sites

Three major wheat growing sites were used in Kenya; the first site was at University of Eldoret, on 0°34'N; 35° 18 'E, at 2,153 m above sea level. The average temperature is 18°C with average annual rainfall of 1,100 mm. Second site was at KALRO-Kitale, on 0°33'S; 35° 55'E, at 2,900 m above sea level with average temperatures of 15°C and average rainfall of 1,800 mm. Third site was KALRO-Njoro, on 0°20'S; 35° 56'E, at 2,185 m above sea level with average temperatures of 20°C and average annual rainfall of 900 mm. The experiments were carried out in 2012/2013.

Plant materials

Seventeen wheat genotypes were used in this study comprising of eight pre-selected mutant lines as illustrated in Table 1, from University of Eldoret: SP-9, SP-16, SP-20, SP-21, SP-26, SP-29, SP-31 and SP-34. Two parental varieties: Njoro II (SP-N) and Kwale (SP-K) and seven other commercial checks: Duma (SP-D), Pasa (SP-P), Simba (SP-S), Farasi (SP-F), Robin (SP-R), KS Mwamba (SP-M) and Chozi (SP-C) all sourced from KALRO Njoro Seed Unit. The two parents and the seven commercial check varieties used in this study are popular and moderately susceptible commercial wheat varieties grown in Kenya.

Field experimental procedures

The 17 genotypes were established based on Complete Randomized Block Design with three replications per location. Field experimental plots were 6 rows by 2 m in length with 20 cm inter-row by 5 cm intra-row spacing. Seeds were hand planted with Di-ammonium Phosphate (DAP 18:46:0) at a rate of 125 kg/ha, followed by an application of Urea at 75 kg/ha at tillering and booting stages. Irrigation was carried out when the soils were dried to maintain soil moisture. Wheat agronomic practices were carried out as recommended by Kinyua and Ochieng (2005).

Green house experimental procedure

Seeds (10) of each genotype were planted in the greenhouse in pots using completely randomized design (CRD).

Data collected for morphological analysis

Plants were selected at random for 25 morphological characters and were evaluated to determine morphological diversity. They were divided into qualitative and quantitative traits and were measured or observed as follows: germination (%), plant height (cm), spike length (cm) and flag leaf area (cm^2). Growth habit, seed shape, re-curved flag leaf, spike shape, flag leaf attitude, straw pith, spike density, grain colour, lower glume, sprouting, awns, and shriveling were observed. Number of tillers (no.), lodging (no.), number of grains per spikelet (no.), number of spikelet's per spike (no.), awn length (cm), seed diameter (cm), 50% heading (Days), 50% maturity period (Days), grain yield per spike (g), and 1000 seed weight (g).

Statistical analyses

Analyses of variance (ANOVA) were performed on the quantitative traits using Genstat computer software (Gensat 15th Edition, 2012). Data were subjected to general linear model and analyzed as a RCBD:

$$Y_{ijkl} = \mu + \lambda_i + \pi_{(j)i} + t_k + \lambda t_{jk} + \varepsilon_{ijkl}$$

where Y_{ijkl} = plot observations, μ = overall mean of experiment, λ_i = Season effect, $\pi_{(j)i}$ = Replication within season effect, t_k = genotype effect, λt_{jk} = Interaction of genotype effect, ε_{ijkl} = Residual effect.

Qualitative data were subjected to frequency distribution analyses and assigned numerical values and computed in excel using Shannon-Weavers diversity index (H') = ($\log_e Pi$) / $\log_e n$; where H' = Shannons-weaver diversity index, Pi = Frequency proportion of each qualitative trait, n = number of classes per qualitative trait; H' value ranges from 0-1 (0 = absences of diversity and 1 maximum diversity).

Molecular analysis

SSR markers as seen in Table 2 were used to investigate the relationship among the 17 genotypes. 10 seeds of each genotype were planted in pots in the greenhouse and after 4 weeks, leaf tissues were selected randomly from each genotype, cut and crushed together in the laboratory using a mortar and pestle. DNA extraction was performed following modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle, 1990). 45 µl of SDS was added and mixed thoroughly. Samples were then incubated at 65°C in a water bath for 1 h and cooled down for 5 min before adding 220 µl of 5M Potassium Acetate. Samples were then put in ice for 15 min and later on centrifuged for 10 min at 13000 rpm. 700 µl of the supernatant was transferred into a microfuge tube and 600 µl of Chloroform and Isoamyl Alcohol in the ratio (24:1) added and centrifuged again for 10 min at 13000 rpm. 600 µl of the supernatant was transferred into a new tube and ice cold isopropanol added to the samples. They were centrifuged again for 10 min at 13000 rpm to pellet the DNA. The supernatant was poured leaving the pellet. 500 µl of 70% ethanol was added to the DNA pellets and centrifuged at 6500 rpm for 5 min and then gently poured off. The pellets were air dried for 1 h and re-suspended in50 µl distilled water then stored at 4°C.

Table 1. Samples identities and their descriptions.

Sample No.	Sample abbreviation	Sample description
1	SP-D	Duma
2	SP-P	Pasa
3	SP-S	Simba
4	SP-F	Farasi
5	SP-R	Robin
6	SP-N	Njoro II (Parent)
7	SP-M	KS Mwamba
8	SP-C	Chozi
9	SP-K	Kwale (Parent)
10	SP-9	Njoro II Mutant
11	SP-16	Njoro II Mutant
12	SP-20	Njoro II Mutant
13	SP-21	Njoro II Mutant
14	SP-26	Kwale Mutant
15	SP-29	Kwale Mutant
16	SP-31	Kwale Mutant
17	SP-34	Kwale Mutant

Concentration and purity of the extracted DNA was determined using Nanodrop200 spectrophotometer (Thermo Fisher Scientific Inc.) and Gel electrophoresis. All samples exhibited good quality and quantity of DNA for PCR amplification. 1 g of agarose was added to 100 ml of TBE buffer (Tris Boric Edta) and casted to make the gel that was used to quantify the DNA samples (1% gel). Nanodrop spectrophotometer 200 (Applied Biosystems) was used to quantify the extracts. The extracted total nucleic acid was suspended in distilled water and 1 ul of each sample loaded on the spectrophotometer pedant and its absorbance measured. Extracts were run in a 1% agarose gel containing Ethidium bromide staining dye at voltage of 100 V and a current of 400 mA for 30 min and visualized on a UV Trans illuminator.

The study used a scoring method where (1) represented presence of expected band, while (0) was absence of band. Genetic variation at each locus was characterized in terms of observed number of alleles (na), observed heterozygosity (HO), expected heterozygosity (HE), gene diversity and Shannon's diversity index (I) using the genetic analysis packages POPGENE Version 1.32 (Yeh et al., 2000).

In addition, Hardy-Weinberg equilibrium (HWE) was tested by the Chi-squared test. Gene diversity (GD) and polymorphic information content (PIC) were measured by calculating the shared allele frequencies (Weir, 1996) using PowerMarker 3.25 (Liu and Muse, 2005). UPGMA algorithm was used to construct an unrooted phylogram from a distance matrix based on (Nei, 1973) genetic distances, using MEGA4 software implemented in PowerMarker 3.25 (Liu and Muse, 2005).

RESULTS

Morphological diversity of the wheat genotypes

Qualitative traits

Results of qualitative traits showed 94% of the genotypes had erect growth habit, 34% had shriveled grains, 24% had hard red grains, while 36% were soft white grains. Computed diversity ranged from 0.27 to 0.85. Low diversity values for straw pith (0.27) indicated a low variation while shriveled grains showed high variation (0.85) among the 17 genotypes (Table 3).

Quantitative traits

The combined analysis of variance (ANOVA) showed the 17 genotypes were significantly different ($P < 0.05$) for seed weight, seed diameter, spike length, grain yield per spike and number of grains per spike. The seasonal effects were significant ($P < 0.05$) for seed weight, seed diameter, days to maturity, spike length, plant height and grain yield per spike. But no significant difference ($P < 0.05$) was observed from awn length and days to 50% ear emergence. Significant ($P < 0.001$) genotype × season (G × S) interaction were observed for seed weight, seed diameter, spike length, grain yield per spike and number of grains per spike (Table 4).

Pearson's moment correlation

Pearson's correlation (r) showed significant positive correlation between seed weight and seed diameter, seed weight and grain yield per spike. However, significant negative correlation was between seed weight and number of tillers, seed weight and maturity period (Table 6).

Genetic diversity of 17 wheat genotypes based on SSR markers

Total of 10 polymorphic SSR primers were detected after

Table 2. 10 sets of specific stem rust resistance gene marker primers were used.

Resistance gene	Linked marker	Nucleotide sequence	Expected band size	Reference
Sr2	gmw533	F 5'-AAGGCGAATCAAACGGAATA-3' R 5'-GTTGCTTTAGGGGAAAAGCC-3'	120	Börner et al. (2000)
RSr22	WMC633	F 5'- ACA CCA GCG GGG ATA TTT GTT AC -3' R 5'- GTG CAC AAG ACA TGA GGT GGA TT -3'	117	Bhavani et al. (2008)
Sr28	wmc332	F 5'- CAT TTA CAA AGC GCA TGA AGC C -3' R 5'- GAA AAC TTT GGG AAC AAG AGC A -3'	214,217 and 220	Rouse and Jin (2011)
Sr25	BF145935	F 5'- CTT CAC CTC CAA GGA GTT CCA C -3' R 5'- GCG TAC CTG ATC ACC ACC TTG AAG G -3'	180,198 and 202	Liu et al. (2010)
Sr25	Gb	F 5'- CAT CCT TGG GGA CCT C -3' R 5'- CCA GCT CGC ATA CAT CCA -3'	130	Liu et al. (2010)
Sr26	Sr26#43	F 5'- AAT CGT CCA CAT TGG CTT CT -3' R 5'- CGC AAC AAA ATC ATG CAC TA -3'	207 +ve, 303 – ve	Mago et al. (2005)
Lr34	CsLV34	F 5'-GTTGGTTAAGACTGGTGATGG-3' R 5'-TGCTTCCTATTGCTGAATAGT-3'	150	Lagudah et al. (2006)
Lr67	CFD71	F 5'- CAA TAA GTA GGC CGG GAC AA -3' R 5'- TGT GCC AGT TGA GTT TGC TC -3'	214	Bhavani et al. (2008)
Sr 33	Xcfd15	F 5'- CTC CCG TAT TGA GCA GGA AG -3' R 5'- GGC AGG TGT GGT GAT GAT CT -3'	150-220	Lagudah et al. (2006)
Sr31	csSr32# 1	F 5'- CTC CCG TAT TGA GCA GGA-3' R 5'- CCA GCT CGC ATA CAT CCA -3'	210	Bhavani et al. (2008)

screening 20 markers on 17 genotypes. Most primers had 2 alleles and the alleles sizes were within the expected range. The 10 SSR primer pairs yielded a total 13 polymorphic loci with a percentage of 92.86%. The mean number of different alleles per locus in each group was 2.0 and the mean number of polymorphic alleles per locus was 1.9286 (Figure 1).

The expected and observed moments of heterozygosity was calculated to estimate the number of heterozygous loci. The expected heterozygosity (HE) and observed heterozygosity (HO) ranged from 121.53 to 1.49 and from 22.75 to 0.642, respectively (Figure 2).

The number of alleles obtained was low compared to other studies (Blair et al., 2010). This finding can be attributed to high genetic similarity between the accessions or crossbreeding between the accessions. The gene frequency varied from 0.8824 for *Sr*2 allele 2 to as low as 0.05882 for *Sr*21 allele 3 (Figure 3).

Phylogenetic analysis of the markers was done using DARwin 6.0. 8. Single data dissimilarity was calculated and factorial coordinates calculated from the resulting dissimilarity data to determine segregation of individual samples (Figure 4).

Genotypes were segregated into 4 groups with each group having discrete individuals a. 1, 2, 7, 8, 17, b. 3, c. 6, 9, 10, 13, 14, 15, 16, d. 12, 15. The keys of 17 genotypes based on analysis of 10 SSR markers is as follow: 1-SP-D, 2-SP-P, 3-SP-S, 4-SP-F, 5-SP-R, 6-SP-N, 7-SP-M, 8-SP-C, 9-SP-K, 10-SP-9, 11-SP-16, 12-SP-20, 13-SP-21, 14-SP-26, 15-SP-29, 16-SP-31 and 17-SP-34.

Cluster analysis

Unrooted phylogenetic tree was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) agglomerative hierarchical clustering (Figure

Table 3. Frequency distribution for qualitative traits evaluated in 17 wheat genotypes.

Trait	Frequency %	H'[a]
Flag leaf (recurved)		
Medium to high	6	0.65
Low to medium	94	
Shriveled grains		
Plump	24	
Intermediate	42	0.85
Shriveled	34	
Grain shape		
Oval	48	
Oblong	18	0.75
Elliptical	34	
Flag leaf attitude		
Erect	6	
Semi-erect	74	0.53
Drooping	20	
Sprouting tendency		
None	6	
Low	42	0.79
Medium	52	
Spike density		
Sparse	6	
Medium	18	0.67
Dense	76	
Awns length		
Medium long	97	0.34
Short awns	3	
Grain colour		
white	36	
Intermediate	40	0.71
Hard Red	24	
Spike shape		
Tapering	12	
Parallel sided	82	0.65
Semi clavate	6	
Straw pith at maturity		
Thin	94	0.27
Medium	6	
Brush hair		
Short	64	0.61
Medium	30	

Table 3. Contd.

Long	6	
Growth habit		
Erect	94	
Semi-erect	6	0.57
Intermediate	0	

H'[a] : Shannon-Weaver index.

Table 4. Mean squares from combined ANOVA for different quantitative traits.

Source	D.F	SW	SD	AL	M	SL	H	GY	EE	GS
Rep.	2	12.23	0.02	1.36	15.45	1.81	68.23	5341.90	1.56	490.11
Genotype	16	113.12**	0.85**	1.77	0.98	14.55**	80.32	2072.96**	2.29	547.17**
Season	1	265.86**	11.9**	1.80	63.48**	217.10**	8114.26**	885.53**	0.54	1.84
G × S	16	11.14**	0.29**	0.73	2.96	1.91**	53.18	2858.11**	1.36	416.12**
Error	32	3.22	0.18	0.88	1.94	0.849	53.70	827.44	1.99	86.99

Locus	Sample Size	na*	ne*	h*	I*
A	17	2.0000	1.7101	0.4152	0.6058
B	17	2.0000	1.9931	0.4983	0.6914
C	17	2.0000	1.8408	0.4567	0.6492
D	17	1.0000	1.0000	0.0000	0.0000
E	17	2.0000	1.8408	0.4567	0.6492
F	17	2.0000	1.5622	0.3599	0.5456
G	17	2.0000	1.2620	0.2076	0.3622
H	17	2.0000	1.2620	0.2076	0.3622
I	17	2.0000	1.1245	0.1107	0.2237
J	17	2.0000	1.9396	0.4844	0.6775
K	17	2.0000	1.9931	0.4983	0.6914
L	17	2.0000	1.5622	0.3599	0.5456
M	17	2.0000	1.4098	0.2907	0.4660
N	17	2.0000	1.5622	0.3599	0.5456
Mean	17	1.9286	1.5759	0.3361	0.5011
St. Dev		0.2673	0.3281	0.1545	0.2023

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* h = Nei's (1973) gene diversity
* I = Shannon's Information index [Lewontin (1972)]

Figure 1. Summary statistics of 10 SSR markers analyzed on 17 wheat genotypes.

5). The dendrogram generated from the results showed the evaluated wheat genotypes segregate into three major clusters and six sub-clusters. The mutants clustered with their respective parental varieties as their resistance profiles were similar or related significantly all the mutants segregated with their parental varieties and hence resistance profiles of parents can be used as references to characterize resistance of the mutant lines to stem rust.

DISCUSSION

Morphological diversity existed between mutants, their parents and other commercial checks used in this study. Both qualitative and quantitative traits showed diversity. This was supported by the average Shannons-Weavers index for qualitative traits (Table 3). The genotype × season (G × S) interaction observed on number of grains per spike, maturity time period, number of tillers

Observed and expected moments of K, the number of heterozygous loci between two randomly chosen gametes in a population : where M1 to M4 are the first four observed moments and EM1 to EM4 are the respective expected values; Xi=Mi/EMi -1.

pop ID	M1	M2	M3	M4
1	4.7059	7.5017	1.4924	121.5397

pop ID	EM1	EM2	EM3	EM4
1	4.7059	2.8136	0.6427	22.7521

pop ID	X2	X3	X4
1	1.6663	1.3221	4.3419

Figure 2. Values of HO and HE for the population.

Gene Frequency :

Allele \ Locus	sr22A	sr22B	sr22C	sr26D	sr26E	sr26F	sr2G	sr2H
Allele 1								
Allele 2	0.7059	0.4706	0.6471	1.0000	0.6471	0.7647	0.8824	0.8824
Allele 3	0.2941	0.5294	0.3529		0.3529	0.2353	0.1176	0.1176

Allele \ Locus	sr2I	sr28J	sr28K	sr25bfL	sr25bfM	sr25gbN
Allele 1						
Allele 2	0.9412	0.4118	0.5294	0.7647	0.8235	0.7647
Allele 3	0.0588	0.5882	0.4706	0.2353	0.1765	0.2353

Figure 3. The gene frequency data.

and 1000 seed weight showed the influence of seasonal differences (Table 4). With the necessity for early maturing varieties, there exists a correlation between growth habits, heading time and maturity time period with most genotypes having erect growth habit. Two types of grain textures the soft white grains and the hard red grains were exhibited by the genotypes. The hard red grains are most preferred by bakers and farmers and weighed from 35 to 45 g while the soft white grains weighed 30 to 35 g/1000 grains weight with a moisture content of 13.5%.

Spike traits and number of tillers per plant were major traits that separated the mutants from their parent varieties (Table 5). The number of tillers and number of grains per spike was greatly influenced by the nutrients supplied and environmental conditions. Differences in grain texture and spike traits contributed significantly to variability between the mutants and their parent. The mutants and their parents clustered into three major clusters with mutants clustering with their respective parents an indication of closer relationships. Positive correlations were observed between seed weight and grain yields per spike. Seed weight affected grain yield per spike which influence the final yield. Correlation between number of grains per spike, seed weight and grain yield per spike have been recorded by Leilah and Al-Khateeb (2005), who observed a negative correlation between number of grains per spike and seed weight as

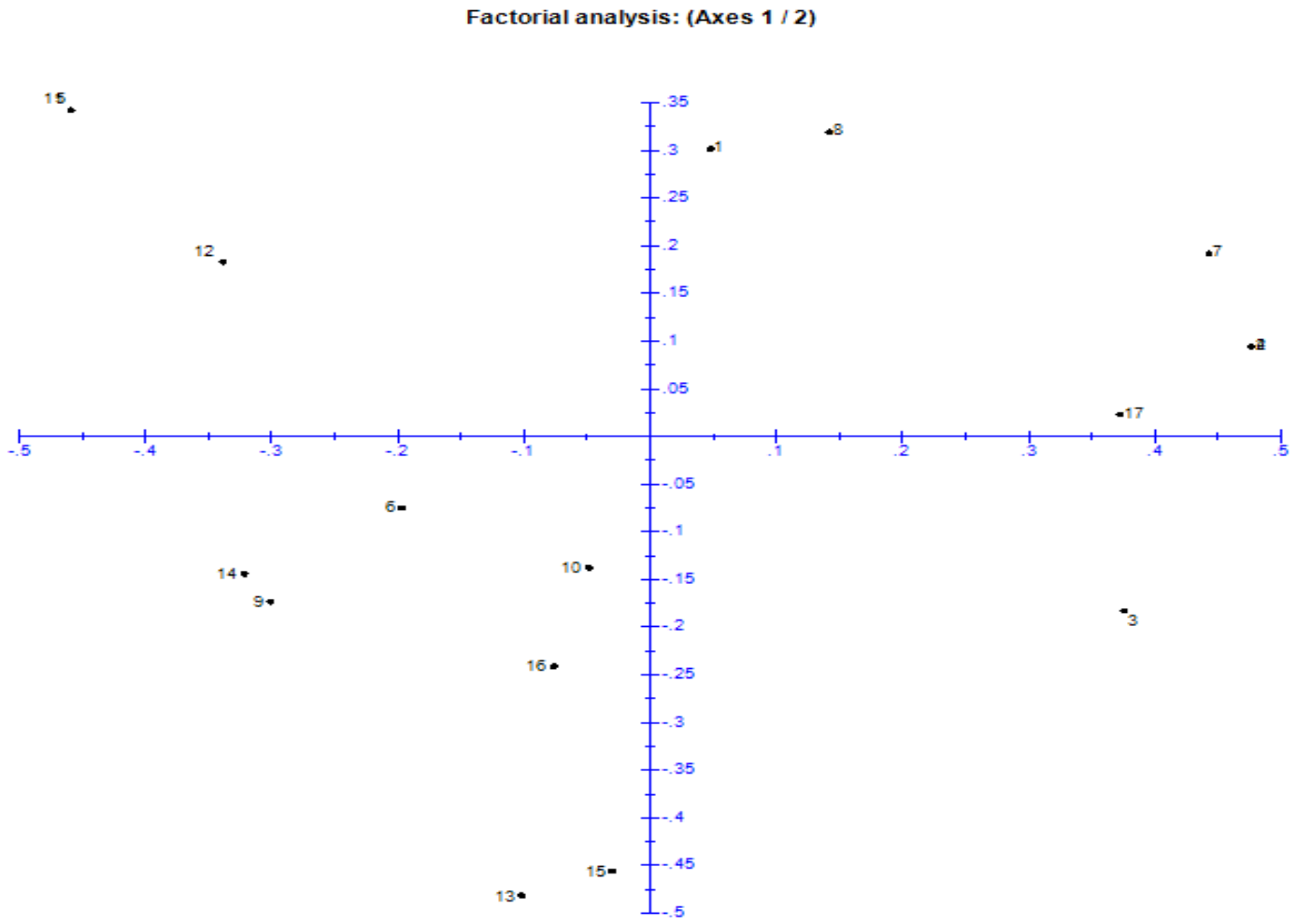

Figure 4. Factorial analysis.

more grains per spike would tend to reduce the size of grains. Negative correlation was observed between spike length and number of tillers per plant and this was attributed to reduction in food to cater for grains per spike. Negative correlation was also observed between maturity period and grain yield per spike. Genotypes with longer maturity periods had reduced seed weight due to unreliable weather conditions.

The polymorphic SSRs markers used were usefully in producing informative bands (Plate 1). Most of the SSR used were polymorphic across the 17 genotypes and a total of 13 alleles were detected with an average number of 2 alleles per locus. According to Salem et al. (2008), the number of alleles per locus ranged from 2 alleles to 7 alleles with an average of 3.2 alleles per locus while Jain et al. (2004) also reported that the number allele per locus ranged from 3 to as high as 22 with an average of 7.8 alleles per locus. Gene diversity ranged from 0 to 0.4893 for each sample, with an average of 0.3361 (Figure 3). The genetic distance analysis separated the 17 genotypes into 3 major clusters and 6 sub-clusters. The genotypes belonging to the same sub-cluster were genetically similar while those belonging to the different sub-clusters were different from each other. The SSRs used in this study demonstrate the ability of SSRs to produce unique DNA profiles and establish discrete identity. Wide range of genetic diversity was observed and it is possible to classify the genetic diversity of the elite mutant lines and select mutant lines for the highest genetic diversity. These findings demonstrated the usefulness and efficiency of SSRs markers in analyzing genomic diversity. According to Hayden et al. (2006), genotypes with the most distinct DNA profile contain the greatest number of novel genes and are likely to carry unique and potentially agronomical useful genes. The genetic diversity levels observed is potentially valuable in predicting sources for selection of genetic diversity with an objective of broadening the wheat genetic base and also have increased progeny performance for complex traits such as yield and disease resistance in

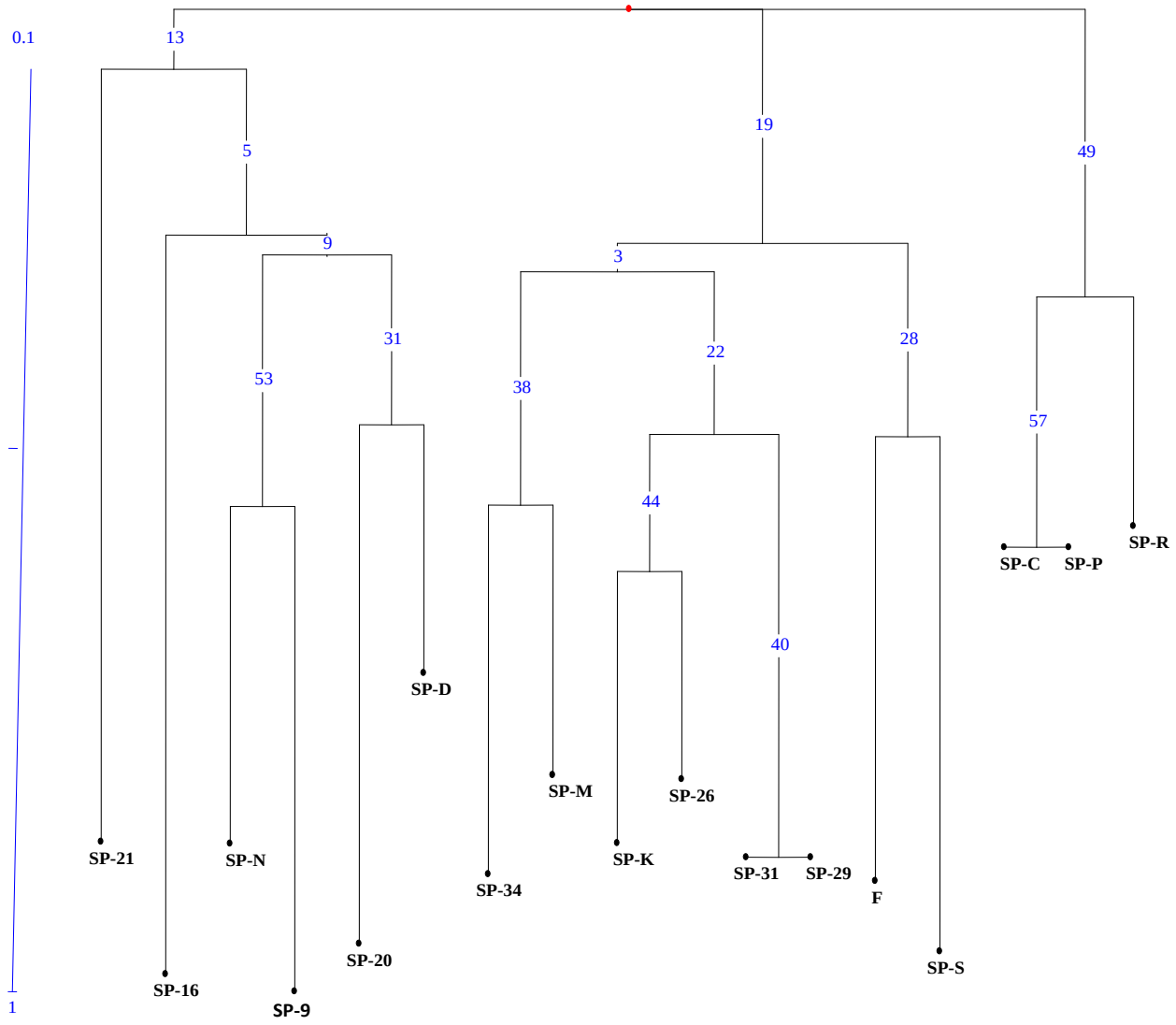

Figure 5. Dendrogram based on genetic similarities discriminated all the wheat genotypes.

wheat production.

Conclusion

Considerable amounts of genetic diversity were observed between the mutants, parents and commercial checks varieties. There was low genetic distance between the genotypes in each sub-cluster attributed to the high genetic similarity between the mutants, their parents and the commercial checks. Observed heterozygosity was higher than expected heterozygosity due to the high genetic variations between the genotypes and within the groupings there were high similarities due to the close relationships and the effects of intense selection in search of the good quality attributes. *Sr*2 was the most polymorphic marker of the ten SSRs as it exhibited greater ability to distinguish between the different genotypes. These results confirmed the relationships between the parents and their respective mutants being placed into the same groupings on the basis of their genetic similarities. Genetic diversity studies is important in developing strategies in wheat breeding as it can be used in selecting genotypes with certain desired traits for breeding programs. The SSRs confirmed morphological traits information about wheat genetic similarities and variations mostly being separated by their grain characteristics. These results can be used in selecting diverse parents in breeding in order to utilize their genetic potential for progeny improvements. This study contributed to stable wheat production by discovering traits relationship that can be used in breeding purposes for adaptation to various desired conditions while the informative SSR markers can be used to map out traits

Table 5. Mean of different quantitative traits of 17 wheat genotypes.

ID	Quantitative traits												
	1000 SW (g)	SD (cm)	GT (no.)	M (days)	SL (cm)	L (1-9)	H (cm)	EE (day)	GY (g)	FLA (cm^2)	AL (cm)	S (no.)	GS (no.)
SP-D	29.7^{f}	0.22^{c}	3^{c}	115^{d}	9.1^{d}	4^{e}	83^{d}	65^{a}	1.15^{h}	29.5^{g}	6.9^{a}	13^{d}	29^{d}
SP-P	25.3^{g}	0.21^{d}	1^{d}	135^{b}	8.9^{e}	3^{f}	88^{a}	70^{a}	0.84^{i}	28.6^{h}	7.0^{a}	11^{e}	24^{e}
SP-S	38.5^{d}	0.25^{b}	3^{c}	115^{d}	10.7^{b}	6^{c}	82^{e}	65^{a}	1.55^{e}	36.4^{d}	6.8^{a}	15^{c}	36^{b}
SP-F	37.2^{d}	0.25^{c}	3^{c}	120^{d}	9.6^{c}	5^{d}	82^{e}	65^{a}	1.24^{g}	32.1^{f}	7.0^{a}	13^{d}	33^{c}
SP-R	33.8^{e}	0.24^{b}	3^{c}	120^{d}	9.7^{c}	6^{c}	83^{d}	65^{a}	1.32^{f}	35.3^{d}	7.2^{a}	13^{b}	34^{c}
SP-N	41.5^{c}	0.26^{a}	3^{c}	125^{c}	11.1^{b}	5^{d}	82^{e}	70^{a}	1.78^{c}	38.6^{c}	6.9^{a}	16^{c}	40^{a}
SP-M	24.6^{h}	0.20^{d}	2^{d}	125^{c}	7.1^{g}	3^{f}	76^{h}	65^{a}	0.82^{j}	27.7^{i}	6.8^{a}	9^{f}	20^{f}
SP-C	25.8^{g}	0.21^{d}	1^{e}	135^{b}	8.7^{e}	3^{f}	87^{b}	70^{a}	0.91^{i}	28.4^{h}	7.1^{a}	12^{d}	25^{d}
SP-K	35.4^{e}	0.23^{c}	2^{d}	145^{a}	9.5^{c}	5^{d}	89^{a}	70^{a}	1.37^{f}	32.8^{e}	7.3^{a}	13^{d}	32^{c}
SP-9	37.3^{d}	0.24^{b}	2^{d}	115^{d}	9.9^{c}	5^{d}	86^{c}	65^{a}	1.45^{e}	35.1^{d}	6.8^{a}	14^{d}	34^{c}
SP-16	39.7^{c}	0.25^{b}	4^{b}	115^{d}	10.8^{b}	7^{b}	86^{c}	70^{a}	1.67^{d}	38.5^{c}	7.0^{a}	16^{c}	38^{b}
SP-20	30.5^{f}	0.23^{c}	2^{d}	115^{d}	9.3^{d}	3^{f}	86^{c}	65^{a}	1.15^{h}	34.5^{e}	6.8^{a}	12^{d}	28^{d}
SP-21	47.8^{a}	0.28^{a}	4^{b}	115^{d}	12.5^{a}	7^{b}	86^{c}	70^{a}	2.12^{a}	42.8^{a}	6.8^{a}	18^{b}	44^{a}
SP-26	44.6^{b}	0.26^{a}	5^{a}	130^{c}	10.9^{b}	8^{a}	80^{f}	70^{a}	2.04^{b}	40.3^{b}	6.8^{a}	20^{a}	45^{a}
SP-29	28.9^{f}	0.21^{d}	3^{c}	130^{c}	9.5^{c}	4^{e}	79^{g}	70^{a}	1.13^{h}	32.2^{f}	6.7^{a}	13^{d}	30^{c}
SP-31	35.1^{e}	0.23^{c}	4^{b}	130^{c}	9.6^{c}	7^{b}	79^{g}	70^{a}	1.32^{f}	35.8^{d}	6.8^{a}	15^{c}	35^{b}
SP-34	27.5^{g}	0.21^{d}	4^{b}	130^{c}	9.3^{d}	4^{e}	79^{g}	70^{a}	0.97^{i}	33.1^{e}	6.8^{a}	12^{e}	28^{d}
Mean	34.3	0.234	2.9	124.5	9.8	5.0	83.0	67	1.34	34.2	6.8	13.8	32.5

, * Significant relationship between the variables at *P ≤0.05*, 0.001, respectively. 1,000 seed weight= SW, Number of tillers=GT, Maturity periods (days) =M, Spike length=SL, lodging=L, plant height=H, Grain yields per spike=GY, Flag leaf area=FLA, Awn length=AL, Spikelets per spike=S, Seed diameter=SD, Ear emergence=EE, Grains per spike= gs.

Table 6. Pearson's correlation coefficient for the different quantitative traits of 17 genotypes

Correlation	SW	SD	GT	M	S	GS	PH	SL	GY
SW	1								
SD	0.772****	1							
GT	- 0.678**	-0.554****	1						
M	-0.638*	-0.510****	0.257	1					
S	0.296	0.427	0.007	0.256	1				
GS	0.767*	0.412	0.731*	0.364	0.490	1			
PH	0.273	0.381**	0.074	0.098	0.351	-0.124	1		
SL	-0.150	0.321	-0.539****	0.429	0.608	0.280	-0.116	1	
GY	0.938**	0.740**	-0.561**	-0.533*	0.880*	0.724**	0.202	0.718****	

*Significant (*P ≤ 0.05*), **Significant (*P ≤ 0.01*).

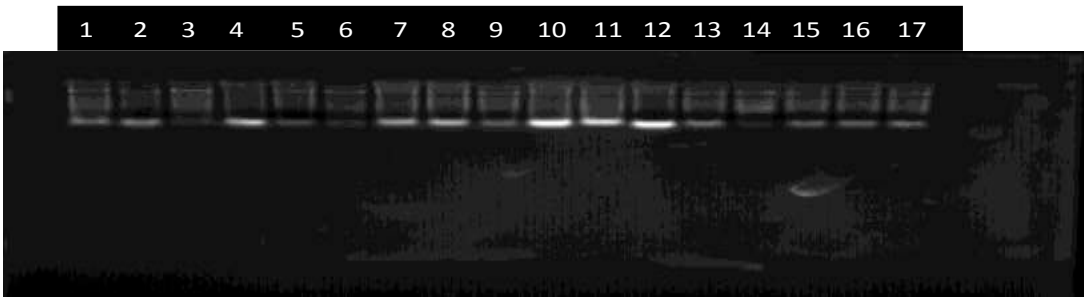

Plate 1. Quantification gel image.

and aid marker assisted selections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Bhavani S, Bansal UK, Hare RA, Bariana HS (2008) Genetic mapping of stem rust resistance in durum wheat cultivar 'Arrivato'. International Journal of Plant Breeding 2:23-26.

Börner A, Röder MS, Unger O, Meinel A (2000). The detection and molecular mapping of a major gene for non-specific adult-plant disease resistance against stripe rust (Puccinia striiformis) in wheat. Theoretical and Applied Genetics 100:1095-1099.

Blair MW, Chaves A, Tofino A, Calderon JF, Palacio JD (2010). Extensive diversity and inter-gene pool introgression in a worldwide collection of indeterminate snap bean accessions. Theoretical and Applied Genetics 120:1381-1391.

Doyle JJ (1990). Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Hayden MJ, Stephenson P, Logojan A, Khatkar D, Rogers C, Elsden J, Koebner RMD, Snape JW, Sharp PJ (2006). Development and genetic mapping of sequence-tagged microsatellites (STMs) in bread wheat (*Triticum aestivum* L.) Theoretical and Applied Genetics 113:1271-1281.

Jain S, Jain RK, McCouch SR (2004). Genetic analysis of Indian aromatic quality rice (Oryza sativa L.) germplasm using panels of fluorescently-labeled microsatellite markers. Theoretical and Applied Genetics 109:965-977.

Kenya Agricultural Livestock and Research Organization (KALRO) (2016). Kenya Wheat Production Handbook 2016. https://www.kalro.org/sites/default/files/Wheat-Handbook-2016.pdf

Kinyua MG, Ochieng DO (2005). Crop Production Handbook for Wheat, Oil crops and Horticulture. J.A.W. (Eds*.).* Gansdil Printers & Stationers, Nairobi, Kenya pp. 1-19.

Leilah AA, Al-Khateeb SA (2005). Statistical analysis of wheat yield under droughtconditions Journal of Arid Environments 61(23):483-496.

Liu K, Muse SV (2005). Power Marker: Integrated analysis environment for genetic marker data. Bioinformatics 21:2128-2129.

Liu W, Jin Y, Rouse M, Friebe B, Gill B, Pumphrey MO (2010). Development and characterization of wheat-*Ae. searsii* Robertsonian translocations and a recombinant chromosome conferring resistance to stem rust. Theoretical and Applied Genetics 122:1537-1545.

Lagudah ES, Appels R, McNeil D (2006). The Nor-D3 locus of Triticum tauschii: natural variation and linkage to chromosome 5 markers. Genome 34:3.

Mago R, Bariana HS, Dundas IS, Spielmeyer W, Lawrence GJ, Pryor AJ, Ellis G (2005). Development of PCR markers for the selection of wheat stem rust resistance genes Sr24 and Sr26 in diverse wheat germplasm. Theoretical and Applied Genetics 111:496-504.

Nei M (1973). Genetic distance between populations. American Naturalist 106:283-292

Njau PN, Jin Y, Huerta-Espino J, Singh R, Keller B (2010). Identification and Evaluation of Sources of Resistance to Stem Rust race Ug99 in Wheat. Plant Disease P 94.

Njau PN, Wanyera R, Macharia GK, Macharia J, Singh R, Keller B (2009). Resistance in Kenyan bread wheat to recent eastern African isolate of stem rust, *Puccinia graminis* f. sp.*tritici,* Ug99. Plant Breeding and Crop Science 1(2):022-027.

Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK (2000). The use of microsallites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theoretical and Applied Genetics 100:584-592.

Rouse MN, Jin Y (2011). Stem rust resistance in A-genome diploid relatives of wheat. Plant Disease 95:941-944

Salem KFM, El-Zanaty AM, Esmail RM (2008). Assessing Wheat (*Triticum aestivum* L.) Genetic Diversity Using Morphological Characters and Microsatellite Markers. World Journal of Agricultural Sciences 4(5):538-544.

Stepien I, Mohler V, Bocianowski J, Koczyk G (2007). Assessing genetic diversity of Polish wheat (*Triticum aestivum* L.) varieties using microsatellite markers. Genetic Resources and Crop Evolution 54:1499-1506

Takumi S, Nishioka E, Morihiro H, Kawahara T, Matsuoka Y (2009). Natural variation of morphological traits in wild wheat progenitor *Aegilops tauschii* Coss. Breeding Science 59:579-588.

Yeh FC, Yang RC, Boyle T (2000). POPGENE version 1.32: Microsoft Windows–based freeware for population genetic analysis. Center for International Forestry Research, University of Alberta, Edmonton, Alberta, Canada.