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Full Length Research Paper

Effect of salicylic acid (SA) seeds soaking on the NaCl salt stress induced changes in soluble sugar and protein accumulation in organs of two genotypes of okra plants

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Salt stress is a major challenge in agricultural system. In this study, okra seeds of two genotypes (47-7 and LD 88) were presoaked with 10^{-2}, 10^{-4}, and 10^{-6} mM salicylic acid and control in distilled water, then the soil was treated with 0, 50, 100, 150 and 200 mM NaCl. The experiment was conducted to study the effect on osmoregulating solutes such as proline, salt stress protein (glycine betaine and proline betaine) and soluble sugars (glucose and fructose). Results showed that proline content increased with increased in the concentrations of salinity. Also, treatment with salicylic acid (SA) improved salt stress proteins accumulation in both stressed genotypes. In contrast, decreased SA concentrations improved soluble sugar accumulation in the fruit of okra genotype 47-4. But in LD88, increased in the level of SA resulted to the increased soluble sugar accumulation in the leaf. Combined effect of SA and salinity caused a greater accumulation of protein and soluble sugar in leaf and fruit of both genotypes of stressed okra, but significant increased were seen only in the groups of LD88 treated with 10^{-4} mM SA at 50 mM NaCl in leaf and 10^{-2}mM SA at 150 mM NaCl in fruit when compared with the control group. Salinity induced a marked decreased in reducing sugar accumulation of okra plant (LD88), especially at high salinity level (200 mM NaCl). Therefore, accumulations of compatible solutes such as salt stress proteins may provide plant a storage form of nitrogen that will be re-utilized later and may play a role in osmotic adjustment.

Key words: Distilled water, fruit, leaf, okra, proline, protein, salicylic acid (SA), salinity, soluble sugar.

INTRODUCTION

Abelmoschus esculentus, which is otherwise known as okra, in many English-speaking countries they know it as lady's fingers or gumbo. Okra is a flowering plant in the mallow family (Chopra et al., 1956). It is a vegetable grown widely in Nigeria for its soft fruits and young leaves. It is distributed across the Africa, Asia, Southern

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Europe, and America (Khomsug et al., 2010). In some areas, the leaves are for human consumption, the major components of okra are vitamins, mineral salts, and calcium, which is deficient in the diet of people living in developing countries (IBPGR, 1990). The plant is used for medicinal purposes and also used to treat many diseases; it induces hypoglycemic activity in normal mice (Tomoda et al., 1989). Biotic and abiotic factors such as drought, water, and salinity stress drastically affect the growth and productivity of okra plant in the tropical and sub-tropical regions of the world. Plant growth and productivity affected by salinity as one of the major environmental factors (Misra et al., 1990). It is known that up to 20% of irrigated lands in the worlds are affected by levels of salinity (Mostafazadeh-Fard et al., 2007). High concentration of salt has adverse effect on many crop species (Zörb et al., 2004), salinity affected cell enlargement as well as photosynthesis (Misra et al., 2001; Munnus et al., 2006), it has negative effect on cell division and cell growth (Maghsoudi and Maghsoudi, 2008). Exposure of plants to salt stress increase reactive oxygen species which destroy membrane lipids (Zörb et al., 2004). Plants that exposed to salt stress produced metabolite like proline, exposure of higher plants to salt stress produced proline which is free amino acid. It is highly active, and plays an important role in membrane stability, also mitigates the effects of saline on cell membrane disruption (Parviz and Satyawat, 2008). Establishment of methods to induce stress tolerance in plants is important, and still need considerable attention. Methods used to develop stress tolerant in plants included genetic engineering, traditional breeding, *in vitro* selection, and the use of growth regulators (Baninasab and Ghobadi, 2011; Senaratna et al., 2000). Salicylic acid has been known as phytohormone that plays a vital role in the controlling of plant growth and development, seed germination, fruit yield, glycolysis, flowering, and heat production in plants (Klessig and Malamy, 1994), ions uptake and transport (Harper and Balke, 1981), values of photosynthesis, stomata conductance, and transpiration (Khan et al., 2003). Salicylic acid is a growth regulator with phenolic nature (Sakhabutdinova et al., 2003), it acts as non-enzymatic antioxidant, also plays a vital role in regulating some plant physiological processes (Noreen et al., 2009), such as stimulating adventitious organ, development, herbicidal effect and providing resistant to environmental stress (Hussein et al., 2007).

To withstand different environmental factors, plants alter their metabolic pathways to adjust to changes in environments (Rathinasabapathi, 2000), compatible solutes made up of a wide range of organic compounds, such as: Simple sugars (fructose and glucose), sugar alcohols (glycerol and methylated inositols), complex sugars (trehalose, raffinose and fructans), polyols, quaternary ammonium compounds (proline, glycine betaine, alpha-alanine betaine, proline betaine) and tertiary sulfonium compounds that are hydrophilic, they can replace water at the surface of proteins, complex protein structures and membranes which explains their action as osmoprotectants and as low molecular weight chaperones (Hasegawa et al., 2000; Nuccio et al., 1999). The metabolic pathways such as proline, glycine betaine, polyols, antioxidant components are responsible to keep the plant survive under stress conditions. Proline is the most common organic compatible solute in the cytoplasm and organelles to keep stability of osmotic pressure of ions in the vacuole, high level of proline may improve the osmotic adaptation and protect the plants against the salt or drought induced injuries. Under salt stress, proline is significantly accumulated and performs the positive role in the adaptation of cells to salt and water stress (Kaviani, 2008). Proline plays a major role in protein accumulation and in cell adaptation to salinity stress (El-Enany, 1995) thus, accumulation of proline in plant may be related to osmotic and saline stress tolerance (Watanabe et al., 2000). Therefore, this present study was designed with the objective to investigate the effect of salicylic acid on negative effects of saline, as well as on accumulations of compatible solutes, and also to determine the best concentration of salicylic acid that accumulates more of these metabolic constituents in stressed okra plant.

MATERIALS AND METHODS

Plant growth conditions

The seeds of okra plant used for this work were obtained from genetic resource laboratory of National Horticultural Research Institute (NIHORT), Ibadan, Oyo state, Nigeria. Seeds were sterilized with 1% sodium hypochlorite for 15 min and washed twice with double distilled water. The seeds were then soaked with 10^{-2}, 10^{-4} and 10^{-6} mM of salicylic acid and distilled water (control) for 12 h and then placed to germinate in polyethylene bags containing 10 kg of soil with pH 7.10, Exch. acidity 0.34, clay (%) 12.30, silt (%) 13.90, sand (%) 65.40, organic carbon (g/Kg) 47.32, nitrogen (g/Kg) 2.53, phosphorous (mg/Kg) 20.00, potassium (cmol/Kg) 1.33, sodium (cmol/Kg) 0.89, calcium (cmol/Kg) 45.65, magnesium (cmol/Kg) 13.34. Seeds were grown in the soil that contains no saline (control (0 mM)) and other seeds were grown under salinity levels of 50, 100, 150, and 200 mM. Saline solutions were added to the soil until field capacity was reached.

The experiment was done in a screen house at National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. There were 60 polyethylene bags for each genotype (3 treatments x 5 levels x 3 replicates), and they were moisture with normal tap water on weekly basis to attain soil water field capacity for the period of eight weeks. After which metabolic constituents were determined on dried leaf and fruit of the two genotypes.

Proline determination

Proline levels in leaf and in the fruit were determined according to the method of Bates et al. (1973) with slight modification. Five-hundred milligrams of the dried leaf and fruit samples were dissolved in 10 mL of 3% (v/v) aqueous sulfosalicylic acid. The mixture was filtered using Whatman no 41 filter paper. After which the filtrate was acidified with glacial acetic acid and ninhydrin (1 mL each) and then heated in water bath at 100°C for 1 h. The mixture

Table 1. Effect of salinity levels on proline content (mgg^{-1}dry matter) of different organs of two okra genotypes.

NaCl (mM)	Genotype 47-4		Genotype LD88	
	Leaf	Fruit	Leaf	Fruit
Control (0)	1.12±0.02	1.08±0.01	2.00±0.02	1.15±0.01
50	2.40±1.00	1.97±0.03	2.35±0.02	2.00±0.02
100	3.50±0.03	2.85±0.04	3.65±0.01	2.86±0.02
150	4.13*±1.02	4.10*±0.01	4.14*±0.05	4.37*±0.01
200	5.21*±0.01	4.79*±0.03	4.99*±0.02	5.11*±0.02

Values are the mean of three replicates mean ± S.E; * Significant different at P ≤ 0.05 when compared with normal control group.

was extracted with 5 mL of toluene and the upper phase was decanted into a glass cuvette, and then the absorbance was taken at 520 nm.

Soluble protein determination

Proteins concentrations were analyzed according to the method of Lowery et al. (1951) using Folin-Ciocalteau reagent. Five-hundred milligrams of the dried leaf and fruit samples were weighted and digested by hot ethanol 80% two times, each on 10 mL and the extract diluted to 50 mL by double distilled water. The absorbance of blue color was read at 660 nm by spectrophotometer machine (Pharmaspec UV-1700 model). The amount of soluble protein was calculated from bovine serum albumin standard curve.

Soluble sugar determination

Soluble sugars accumulations were determined using colorimetric method described by Dubois et al. (1956). Glucose was applies as a standard.

Statistical analysis

The factorial experimental design with two varieties, two genotypes and four salinity levels were arranged in a completely randomized design (CRD) with three replications and the data were analyzed using the software package, SAS windows and the mean separation by LSD0.05.

RESULTS AND DISCUSSION

As shown in Table 1, in the two genotypes of okra, as the salinity level increased, proline accumulation also increased in leaf and in the fruit when compared with control group. This has also been reported by Amin et al. (2009) that increased in the amount of proline, protein and sugars in the plants would lead to the resistance against loosing water, protect turgor, reduce the membrane damage and accelerate the growth of plants in stress conditions. Under stress conditions, the higher proline concentration increases the activities of proline biosynthesis enzymes such as; ornithine aminotransferase and pyrroline-5-carboxylate reductase, as well as due to inhibition of proline degradation enzymes like proline oxidase and proline dehydrogenase (Kishor et al., 2005). Figure 1 showed decreased soluble protein accumulation in the leaf as concentration of salicylic acid decreased, but in fruit, SA has little or no effect on protein accumulation but nevertheless, the group treated with 10^{-4} mM SA showed highest accumulation of protein when compared with control group of genotype 47-4. But interaction of SA and salinity significantly affected protein accumulation in leaf and fruit of okra plant (genotype 47-4) in the group treated with 200 mM NaCl, whereas significant accumulation of soluble protein were seen at mild and moderate levels of salinity. On the contrary to protein accumulation in 47-4 genotype, in Figure 2, there were appreciable increased reducing sugar accumulation in the groups treated with 10^{-2} and 10^{-6}mM of SA, and the highest accumulation of reducing sugar level in the fruit of the okra genotype 47-4 was recorded in group treated with 10^{-6} mM of SA when compared with control group, but in leaf no significant effect was recorded. Combined effect of SA and salinity showed increased reducing sugar accumulation in the fruit of okra plant (genotype 47-4) at higher salinity levels (100 and 150 mM NaCl at 10^{-2} and 10^{-4} mM SA) respectively, as compared to treated control group.

Similarly, in LD88, the leaf of okra showed decreased protein accumulation level as the concentration of SA decreased, fruit exhibited highest protein accumulation in group treated with 10^{-4}mM of SA as compared to control group. Interaction of SA and salinity significantly affected the protein accumulation in both leaf and fruit of the stressed okra, but significant increase of protein accumulation were seen only in the groups (LD88) treated with 10^{-4} mM SA at 50 mM NaCl in leaf and 10^{-2} mM SA at 150 mM NaCl in fruit when compared with the treated control group (Figure 3). In LD88, reducing sugar accumulation decreased in the leaf as salicylic acid decreased, but fruit exhibited highest reducing sugar accumulation in the group treated with 10^{-4}mM SA when compared with control group, salinity induced a marked decreased reducing sugar accumulation in okra leaf (LD88) especially at salinity levels (50 and 100 mM NaCl). Fruit showed appreciable accumulation of reducing sugar in the group treated with 10^{-6} mM of SA at

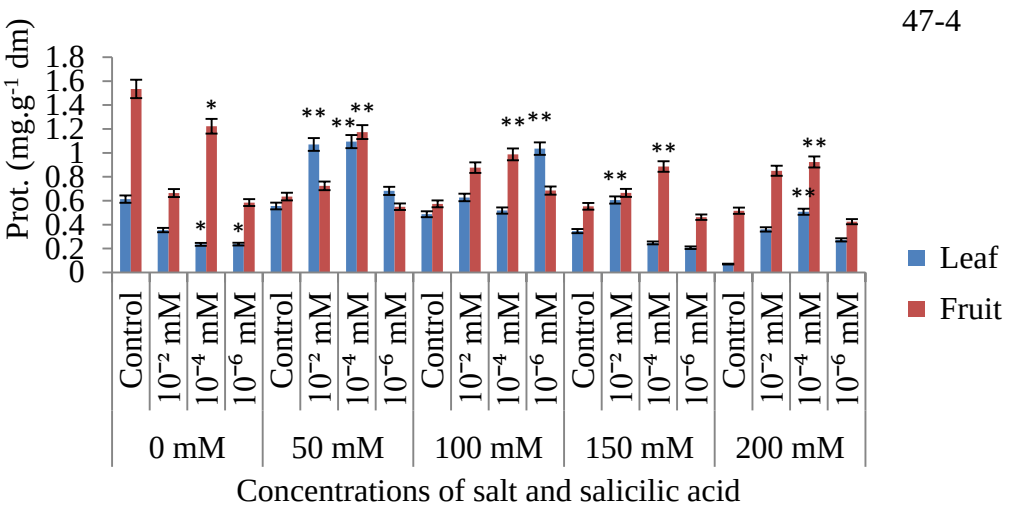

Figure 1. Effect of salicylic acid and salinity on soluble proteins accumulation in different organs of okra (Genotype 47-4). Vertical bars represent standard deviation. ** Significant different at P ≤ 0.05 when compared with treated control group; * Significant different at P ≤ 0.05 when compared with normal control group; 0, 50, 100, 150 and 200 mM are contractions of sodium chloride (salt);10^{-2}, 10^{-4} and 10^{-6} mM are concentrations of salicylic acid (SA).

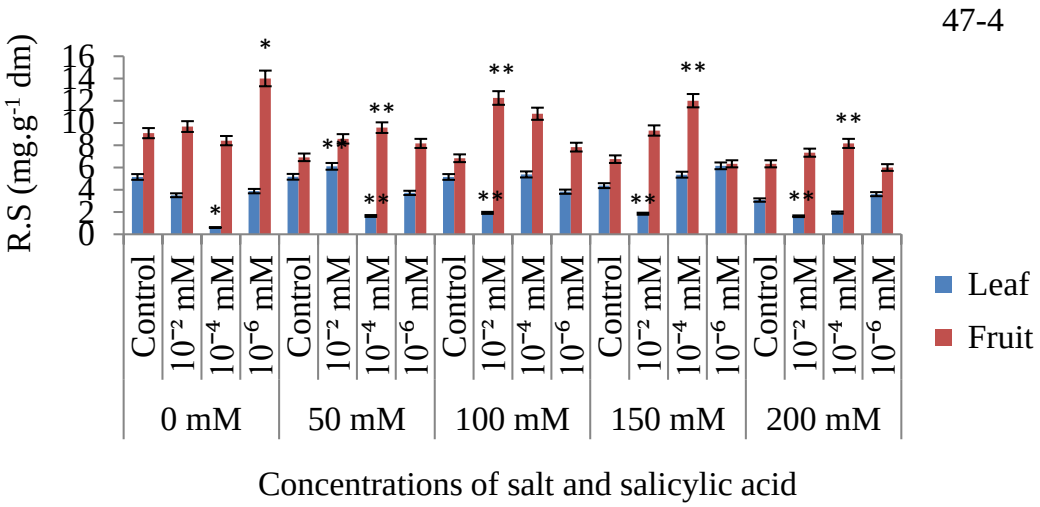

Figure 2. Effect of salicylic acid and salinity on reducing sugar accumulation in different organs of okra (Genotype 47-4). Vertical bars represent Standard deviation. ** Significant different at P ≤ 0.05 when compared with treated control group; * Significant different at P ≤ 0.05 when compared with normal control group; 0, 50, 100, 150 and 200 mM are contractions of sodium chloride; 10^{-2}, 10^{-4} and 10^{-6} mM are concentrations of salicylic acid.

50 mM NaCl, but interaction of SA and salinity in leaf showed little or no effect on reducing sugar accumulation as compared to treated control group (Figure 4).

Between the two organs of stressed okra plant, fruit accumulates more soluble sugar and soluble protein than the leaf in the two genotypes. The results were in tandem with those of Richardson and McCree (1985). They observed that increased in the concentration of solutes in plant tissues will determine its tolerance to stress conditions. Therefore, the sensitivity of both genotypes was associated with lowering soluble protein in both leaf and fruit at high salinity level, which was more severe in LD88 genotype. This result was in accordance with the findings of Marcelis and Van Hooijdank (1999), Misra et al. (1995) and Das et al. (1990). From the results obtained, pretreatment with salicylic acid or salicylic acid

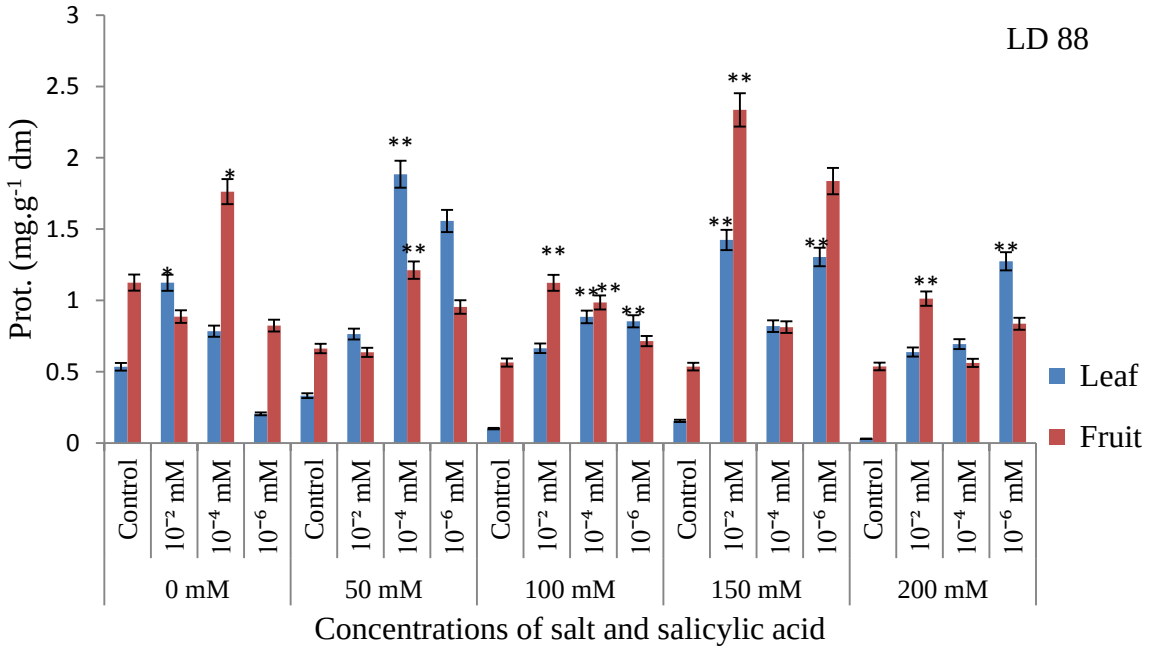

Figure 3. Effect of salicylic acid and salinity on soluble proteins accumulation in different organs of okra (Genotype LD 88). Vertical bars represent standard deviation. ** Significant different at P ≤ 0.05 when compared with treated control group; *Significant different at P ≤ 0.05 when compared with normal control group; 0, 50, 100, 150 and 200 mM are contractions of sodium chloride; 10^{-2}, 10^{-4} and 10^{-6} mM are concentrations of salicylic acid.

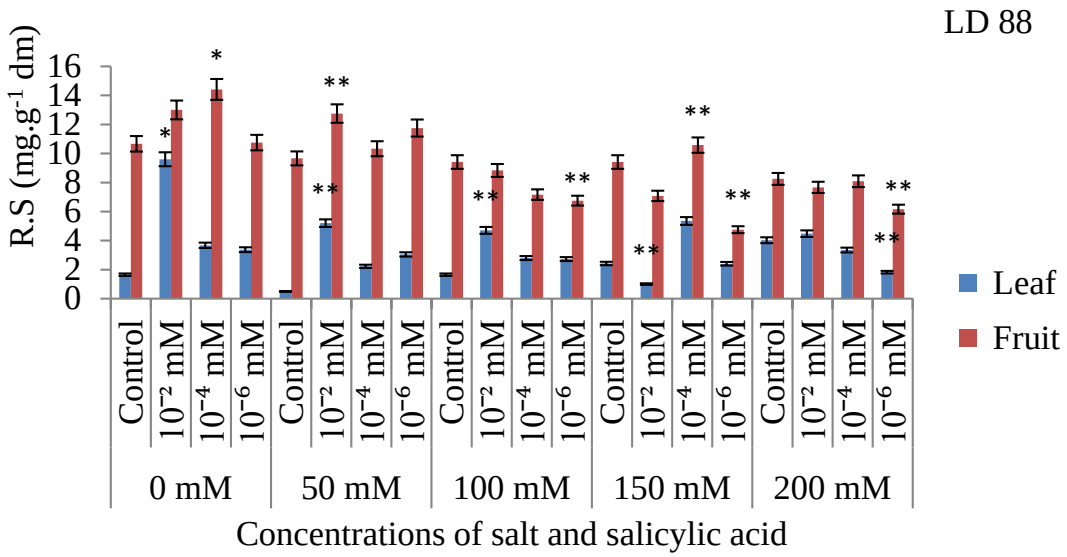

Figure 4. Effect of salicylic acid and salinity on reducing sugar accumulation of different organs of Okra (Genotype LD 88). Vertical bars represent standard deviation. **Significant different at P ≤ 0.05 when compared with treated control group; * Significant different at P ≤ 0.05 when compared with normal control group; 0, 50, 100, 150 and 200 mM are contractions of sodium chloride; 10^{-2}, 10^{-4} and 10^{-6} mM are concentrations of salicylic acid.

combined with salinity improved okra plant tolerance to salinity stressed via increasing the accumulation of non-toxic metabolites (soluble sugars, soluble proteins and proline), which reflected more in the fruit than leaf of okra plant. Therefore, exogenous application of growth regulator especially salicylic acid could be applied to

improve okra plant salt stress tolerance at 10^{-2} and 10^{-4}mM concentrations.

Conflict of Interests

The authors have not declared any conflict of interests.

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Evaluation of advanced bread wheat genotypes for resistance to stem rust and yield stability

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Stem rust (*Puccinia graminis* f. sp *tritici*) poses a major threat to wheat (*Triticum aestivum* L.) production worldwide. The interaction between wheat and pathogen in presence of favorable environment can cause a complete crop failure. This study was conducted with the objective to identify wheat genotypes with resistance to stem rust with high and stable yield under four environments in Kenya. Forty wheat genotypes were tested in two consecutive growing seasons, using an alpha lattice design with three replications. Host response to stem rust was recorded based on the modified Cobb scale. The disease severity was recorded on scale of 0 to 100%. The results of the coefficient of infection indicated that about 30% of genotypes were moderately resistant. Yield and disease data were subjected to statistical analyses to estimate the stability parameters. The top three genotypes in yield performance were G25, G18 and G29 with 2.07, 1.98 and 1.97 t ha^{-1} respectively. Considering both stem rust and yield stability G5, G16, G18, G24 and G36 were the best genotypes which could undergo further testing for future release.

Key words: Wheat, stem rust, resistance, stability, genotype × environment interaction.

INTRODUCTION

Wheat (*Triticum aestivum.* L) is a staple food for 35% of the world's population (FAO, 2012) providing more than 21% of the required calories and 20% of protein. It is a central crop to achieving development in agriculture and the second most important cereal crop after maize in Kenya (Mahagayu et al., 2007). The crop is grown in approximately 150,000 ha of Kenyan land (FAO, 2012), and is basically used for domestic and commercial baking. In spite of these advantages, wheat production is still low due to unstable yield as well as incidences of diseases.

Stem rust, a fungal disease caused by *Puccinia graminis* f.sp *tritic*i, is one of the most important wheat diseases in Kenya (Mwando et al., 2012). For three decades, the world had been free from stem rust (Singh et al., 2008) until 1999 when a new race *Ug99* was identified in Uganda. This race was designated as TTKSK based on the North American rust nomenclature

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(Roelfs, 1985). Since its discovery, *Ug99* has spread to other countries of Africa, Yemen and Iran (Singh et al., 2011). Stem rust occurs frequently in warm and moist environments, which are typical of the wheat growing areas in Kenya (Wanyera et al., 2009). The growing of wheat in diverse Kenyan agro-ecological zones throughout the year (Green et al., 1970) creates a significant pool of airborne urediniospores which, coupled with favorable climatic conditions and the presence of host plants, favors rapid buildup of inoculum and occurrence of epidemics (Wanyera et al., 2009). Several resistance genes, for instance *Sr31* have been deployed over years. There is however threat due to changing nature of stem rust to more virulent races. The striking virulence combination explains the widespread susceptibility of wheat varieties (Ravi et al., 2011).

Several measures are available for stem rust management, including cultural practices and chemical control. However, they are not fully effective or applicable due to their high cost considering the poor resource wheat farmers. Breeding for resistance is still the most economical and desirable method for controlling stems rust in wheat (Shehab El-Din et al., 1991; Gamalat and El-sawi, 2015). Extensive screening of global wheat varieties for resistance to *Ug99* has been undertaken at key sites in Kenya (Singh et al., 2006). The International Centre for Maize and Wheat improvement (CIMMYT), International Centre for Agricultural Research in the Dry Areas (ICARDA) and Kenya Agricultural and Livestock Research Organizations (KALRO) are leading a global rust initiative to characterize the strain, to track its spread and to find new sources of resistance to the disease and incorporate them in new varieties (CIMMYT, 2005). The best long-term strategy to mitigate the threat from stem rust is to identify resistant sources among existing materials, or develop resistant wheat varieties that can adapt to the prevalent environments in countries under high risk, and release them after proper testing (Singh et al., 2006). Given that rainfall and other environmental factors are widely variable across locations and years, Genotype × Environment Interaction (GEI) need to be well characterized for better targeted genotype development and recommendation.

Apart from stem rust, wheat grain yield is highly influenced by production environments and breeders often determine stability of high yielding genotypes across environments before recommending stable genotypes for release (Sharma et al., 2012). Wheat breeders aim to develop new wheat varieties that are resistant to stem rust and consistently have high yield in a range of environments. In order to ensure consistent and high yields, new lines are developed, and tested for their yield performances in different environments (Mehmet and Telat, 2006). Genotype × environment interactions are of major importance, because they provide information about the effect of different environments on genotype performance and have a key role in assessment of stability of the breeding materials (Moldovan et al., 2000). It affects breeding progress because it complicates the demonstration of superiority of any genotype across environments and the selection of superior genotypes (Ebdon and Gauch, 2002). In addition, GEI reduce progress from selection due to low correlation between phenotypic and genotypic values (Alghamdi, 2004). Thus, understanding the causes of GEI would help in developing genotypes that show satisfactory performances in one to several environments. Various statistical methods have been proposed to determine the stability of new genotype. Among them are multivariate methods which include genotype main effect plus genotype by environment interaction (GGE) and biplot analysis (Yan, 2001) which have been used by Hintsa and Fetien (2013). Thus, the objective of this study was to identify wheat lines that are resistant to stem rust, with high and stable yields across environments.

MATERIALS AND METHODS

Experimental sites and genotypes

The experiment was carried out in four environments; Njoro, Kinamba, Olkalau and Eldoret. Njoro lies on 0.33 S, 35.94 E at 2185 m above sea level. It receives 939 mm of rainfall annually and has a maximum temperature of 24.0°C. Kinamba lies on 0.41 N, 36.36 E at 2303 m above sea level with 978 mm annual rainfall and an average temperature of 13.9°C. Olkalau is on 0.27 N, 36.37 E at 2404 m above sea level with 859 mm annual rainfall and average temperature of 14.2°C. Eldoret lies on 0.51 N, 35.28 E at 2073 m above sea level with annual average rainfall of 1103 mm and average temperatures of 16.6°C. These areas represent key wheat growing regions in Kenyan Rift Valley.

Thirty seven advanced lines from CIMMYT nurseries pre-selected for resistance to stem rust (*Ug99*) by KALRO wheat breeders were evaluated for reaction to stem rust and yield stability across the environments. These genotypes were selected from the lines screened by the Durable Rust Resistance Wheat project in 2012. The checks used in the trial were Robin, Eagle 10 and NJBWII, which are among the most popular commercial Kenyan varieties. The pedigree of the genotypes is presented in Table 1. The experiment was conducted in the year 2013 and 2014 growing seasons.

Experimental procedure

Forty genotypes were planted using a mechanical planter in an alpha lattice design (5 blocks with 8 units within block) with three replications per environment. Each entry was planted in a plot measuring 1.4 m by 6 m with 20 cm spacing. The plots were separated by path of 30 cm while blocks separated by 2 m path. Diammonium phosphate (DAP 18:46:0) was applied during planting at a rate of 125 kg ha^{-1} to supply 22.5 kg ha^{-1} of nitrogen and 25 kg ha^{-1} of phosphorus. Timely weed control was done using Buctril® MC (Bromoxy niloctanoate and MCPA Ethyl Hexyl Ester) which is a selective herbicide, at the rate of 225 kg ha^{-1}.

Data collection and analysis

Disease (stem rust) severity and yield (t ha^{-1}) data were recorded

Table 1. The pedigree information of the genotypes evaluated in four environments in 2013 and 2014.

Entry	Pedigree
1	KACHU/KIRITATI
2	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KIRITATI//ATTILA*2/PASTOR
3	ALTAR84/AE.SQUARROSA(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/KACHU/6/KIRITATI//PBW65/2*SERI.1B
4	PFAU/SERI.1B//AMAD/3/WAXWING/4/TECUE#1/5/PFAU/SERI.1B//AMAD/3/WAXWING
5	PBW65/2*PASTOR/3/KIRITATI//ATTILA*2/PASTOR/4/DANPHE #1
6	ATTILA/3*BCN*2//BAV92/3/KIRITATI/WBLL1/4/DANPHE
7	KACHU/KINDE
8	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4/QUAIU
9	ND643/2*WBLL1//ATTILA*2/PBW65/3/MUNAL
10	KIRITATI//ATTILA*2/PASTOR/3/AKURI
11	BABAX/LR42//BABAX*2/3/TUKURU*2/4/HEILO
12	TC870344/GUI//TEMPORALERA87/AGR/3/2*WBLL1/5/CROC_1/AE.SQUAROSA (205)//BORL95/3/PRL/SARA//TSI/VEE#5/4/FRET2
13	PFAU/MILAN/3/BABAX/LR42//BABAX/4/YANG87-142//SHA4/CHIL/3/TNMU
14	PFAU/MILAN/3/BABAX/LR42//BABAX/4/ATTILA/BAV92//PASTOR
15	MILAN/DUCULA/3/PSN/BOW//MILAN/4/PASTOR/3/BJY/COC//PRL/BOW
16	NING MAI 96035/FINSI//HEILO/3/KA/NAC//TRCH
17	ATTILA/HEILO/3/KA/NAC//TRCH
18	ATTILA/HEILO//PAURAQ
19	ITP50//KAMB2/PANDION
20	KA/NAC//TRCH*2/3/ATTILA/PASTOR
21	SUNCO.6/FRAME//PASTOR/3/2*BAVIS
22	EGABONNIEROCK/6/CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA(208)/5/2*WESTONIA
23	M6SRRSN/011
24	M6SRRSN/011
25	LERKE/5/KAUZ/3/MYNA/VUL//BUC/FLK/4/MILAN/6/PROGRESO F2007/7/MUNAL
26	LERKE/5/KAUZ/3/MYNA/VUL//BUC/FLK/4/MILAN/6/PROGRESO F2007/7/MUNAL
27	PFAU/SERI.1B//AMAD*2/3/PBW343*2/KUKUNA/4/PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07
28	KRL 19/QUAIU #1
29	PBW343/HUITES/4/YAR/AE.SQUARROSA (783)//MILAN/3/BAV92/5/FRET2*2/BRAMBLING
30	TACUPETO F2001*2/BRAMBLING//ND643/2*WBLL1/3/TACUPETO F2001*2/BRAMBLING
31	WBLL4/KUKUNA//WBLL1*2/3/KBIRD
32	PRL/2*PASTOR//PBW343*2/KUKUNA/3/ROLF07/4/KINGBIRD #1
33	CROSBILL#1/DANPHE/7/CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ/6/PRL/2*PASTOR
38	CM8181-12y-06PZ-4y-5m-0y-2AL-0AL-0M
39	BABAX/LR42//BABAX*2/3/TUKURU
40	EMB16/CBRD//CBRD

on all the test environments. Host response to stem rust was recorded based on the modified Cobb scale (Peterson et al., 1948). This scale combines several infection types; resistant (R), moderately resistant (MR), moderately susceptible (MS), combination of MR and MS (M), and susceptible (S). Severity was recorded on 0-100% scale where 0% was considered immunity while 100% was completely susceptible. The severity and field response for stem rust was converted to coefficient of infection (CI) by multiplying the severity with the arbitrary constant value for field response (Stubbs et al., 1986; Roelfs et al., 1992), where R=0.2, MR=0.4, M=0.6, MS=0.8, and S=1.

The plots were harvested and threshed separately. The grain was dried to 12% moisture and the weight converted to tha^{-1} for analysis. Yield and disease data was analyzed using GenStat computer software (Genstat 15th Edition, 2012). A combined analysis of variance for CI and yield was performed using linear mixed model following restricted maximum likelihood (REML) procedure. Genotypes, replicate and environments were considered fixed while blocks nested in replicates were considered as random for CI while blocks being fixed for yield. The following model was used:

$$Y_{ijkl}=\mu+R_i+B_{(ij)}+G_k+E_l+GE_{kl}+\varepsilon_{ijkl}$$

Where Y_{ijkl} was the response, μ was the mean of the experiment, R_i was the effect of the i^{th} replicate, $B_{(ij)}$ was the effect of the j^{th} block

nested in i^{th} replicate, G_k was the effect of the k^{th} genotype, E_l was the effect of the l^{th} environment, GE_{kl} was the effect of the interaction of the k^{th} genotype with l^{th} environment, and ε_{ijkl} was the experimental error.

Genotypic variance (Si^2) was computed to determine disease stability as suggested by Lin et al. (1886). Genotypes with less than 10 CI values and those with greater than 1.8 t ha^{-1} were subjected to further analysis to determine their disease and yield stability using GGE. The lsd was calculated in by the following formula:

Lsd=average standard error of difference × t/device degree of freedom.

Where lsd is the least significant difference; the average standard error of difference was obtained from REML output while t is the value obtained from the t-table.

RESULTS

Genotypic response to stem rust

Significant (P<0.001) variation for CI was observed in genotype, environment and genotype × environment interaction (Data not shown). The CI and genotypic variance (Si^2) of the forty genotypes are presented in Table 2. The lower the Si^2 value the more stable a genotype is (Peterson, 1994). In this study, genotypes with CI values of 20 and below were considered stable for stem rust. Following this stability measure, 42% of the tested genotypes were considered stable for stem rust with genotypes G1, G3, G16 and G18 having the lowest value.

Eldoret (CI=12.61) recorded the lowest disease while Njoro (CI=17.47) had the highest disease scores. There was a slight difference between Kinamba and Olkalau with CI of 13.23 and 13.75 respectively. At 95% confidence level, Eldoret was found to be significantly different from Olkalau but not different from Kinamba. Njoro was significantly different from Eldoret, Kinamba and Olkalau at 95% level of confidence.

Figure 1 shows the GGE biplot for the forty genotypes with respect to stem rust. The two PCs explained 78.00% (PC1=52.69% and PC2=25.31%) of the total variation. According to Rubiales et al. (2014), genotypes that appear to the left of the average line are considered the best in terms of resistance. Accordingly, genotypes G18, G16, G40, G24, G5, G29, G14, G36, G25 and G37 were considered resistant to stem rust. Genotypes G18 and G16 were to the extreme left of the average line hence were leading with low infection hence most resistant. The above resistant genotypes had CI values less than the two checks G38 and G39. However, only G16 and G18 were better than the best check G40. Nonetheless, the three genotypes were not significantly different at 95% confidence interval. According to GGE biplots, the projection of the genotype to the AEC (average environment coordinate) line signifies stability. Genotypes closer to this line are more stable. Among the resistant genotypes, G18, G16, G24, G14, G36 and G40 had shorter projections hence considered stable for stem rust.

The relationship between environments is important for researcher for future selection of trial sites. The vectors connecting each environment to the origin of the biplot gives a clear comparison of the test environments. Eldoret and Olkalau lie on the same projection, an indication of similar environments (Figure 2). Then as well, Njoro was similar to Olkalau, Eldoret and Kinamba. Nonetheless, Kinamba was different from Olkalau and Eldoret which is clearly shown by the obtuse angle between them. Njoro and Kinamba had the largest projections from the biplot origin hence the best environments for testing genotypes for stem rust.

Yield performance across environments

Significant (P<0.001) variation for environment was observed in yield (Data not shown), Olkalau recording the highest yield (Table 3). When the PC analysis for yield was fitted, the two PCs explained 87.14% (PC1 = 65.59% and PC2 = 25.31%) of the total variation. According to GGE biplots, genotypes on the right side of the average line are good average performers while closeness to the AEC represents stability. Genotypes G25, G17, G40, G29, G6, G18, G23, G36, G27, G28 and G30 were on the right side of the average line hence considered above average performers (Figure 3). Genotypes G16 and G5 were near the average line and were considered average performers. Among the genotypes with average and above average performance, G5, G16, G25, G17, G40, G29, G23, and G18 were closest to the stability line hence considered stable for yield.

Figure 4 shows the relationship among test environments in respect to yield. This was visualized by the line connecting each environment to the origin of the biplot (Vector). Environments closer to each other are more similar and therefore, Njoro and Eldoret were similar, Eldoret and Olkalau were similar and, Olkalau and Kinamba were similar. The obtuse angle between Olkalau and Njoro indicate that these environments were different. Kinamba was different from Njoro and Eldoret. Olkalau had the largest projection from the biplot origin. This makes it a good environment for selection of wheat genotypes since it had the largest contribution of GEI. There was a negative relationship between yield and disease (Figure 5).

DISCUSSION

Wheat production in Kenya is constrained by stem rust which can cause 100% yield loss in susceptible genotypes (Singh et al., 2009). Whereas resistance to the disease has been reported in some genotypes, lack of genotypes that combine sufficient resistance to stem rust and yield stability across environments has posed a new challenge (Singh et al., 2006). Furthermore, the

Table 2. Coefficient of infection (CI) and genotypic variance (Si^2) of forty wheat genotypes tested in four environments.

Genotype	Eldoret	Kinamba	Njoro	Olkalau	Mean	Si^2
G1	7.96	5.96	9.46	6.12	7.38	2.76
G2	19.09	25.93	15.09	21.43	20.39	20.52
G3	10.93	14.60	13.10	12.60	12.81	2.29
G4	14.47	2.37	18.47	11.80	11.78	46.85
G5	8.40	6.24	6.07	15.24	8.99	18.50
G6	9.33	17.16	18.66	11.49	14.16	19.90
G7	26.13	4.60	29.13	10.30	17.54	142.66
G8	24.66	14.33	17.99	19.33	19.08	18.32
G9	9.90	18.23	12.23	9.56	12.48	16.10
G10	27.73	21.06	28.06	24.73	25.40	10.60
G11	23.06	32.23	21.73	30.06	26.77	26.60
G12	19.73	32.06	29.73	28.06	27.40	28.80
G13	10.19	29.35	25.52	20.35	21.35	68.98
G14	6.84	6.51	13.84	7.51	8.68	12.03
G15	2.87	27.54	24.20	15.54	17.54	121.19
G16	2.17	4.04	6.51	6.67	4.85	4.64
G17	9.41	16.91	16.24	7.24	12.45	23.55
G18	6.72	2.46	4.86	3.22	4.32	3.57
G19	32.93	31.59	24.26	31.26	30.01	15.22
G20	16.72	24.06	10.22	14.89	16.47	33.08
G21	22.86	20.19	17.53	22.53	20.78	6.10
G22	7.33	11.99	21.33	10.66	12.83	35.97
G23	9.41	16.64	14.41	11.07	12.88	10.60
G24	2.39	5.72	10.56	3.89	5.64	12.61
G25	3.85	16.01	11.18	5.85	9.22	30.05
G26	11.73	13.40	20.07	13.90	14.78	13.32
G27	4.56	17.40	23.90	15.23	15.27	64.57
G28	13.18	10.01	18.51	18.68	15.10	18.01
G29	13.98	2.15	8.82	11.65	9.15	26.23
G30	15.85	8.85	23.85	13.85	15.60	38.92
G31	20.15	4.42	18.48	9.65	13.18	55.29
G32	6.92	11.42	21.42	12.09	12.96	37.06
G33	32.62	3.78	8.95	17.95	15.83	159.65
G34	11.78	5.42	31.28	12.62	15.28	124.18
G35	5.07	15.41	13.07	14.07	11.91	21.68
G36	4.43	6.26	15.76	7.76	8.55	24.94
G37	6.36	3.19	15.76	13.69	9.23	35.39
G38*	7.10	13.10	24.43	12.76	14.35	52.75
G39*	14.62	4.28	29.28	9.45	14.41	116.13
G40*	1.08	2.48	7.08	5.42	4.02	7.45
Mean	12.61	13.23	17.47	13.75		

[a]Comparing means between genotypes, [b] Comparing means between environments, [c] Comparing means for genotype × environment interaction, * Checks. Lsd = 3.25[a], 1.08[b] and 6.75[c].

emergence of new stem rust races is a challenge in breeding and selection for the disease (Wanyera et al., 2006). Although there are recommended fungicide to reduce the disease pressure, these chemicals are not environment friendly and are not economical. Chemical control using fungicides, though an option is not sustainable due to high cost of recommended fungicides and their undesirable effect on environment (Wanyera et al., 2009). Just like yield, response of wheat to stem rust varies in different environments. This is due to the

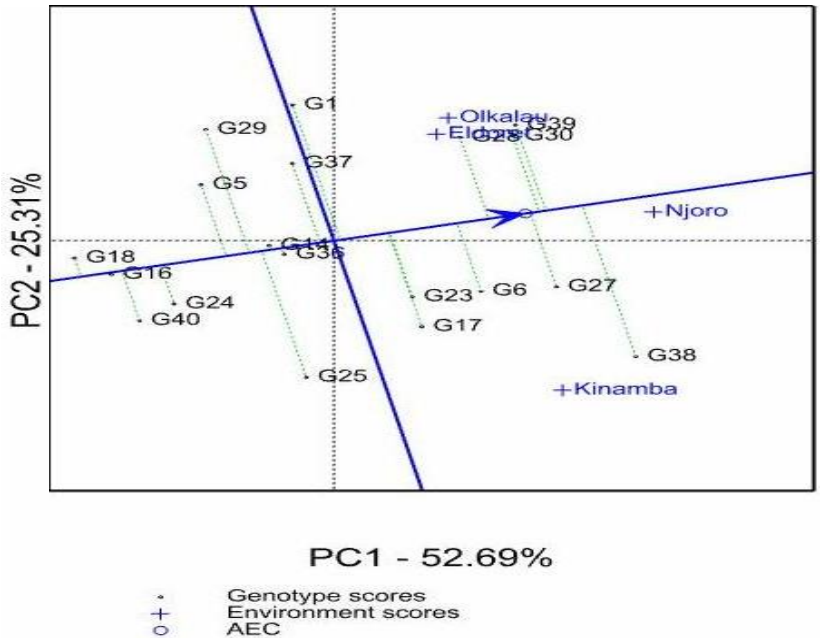

Figure 1. GGE biplot showing disease stability for selected wheat genotypes.

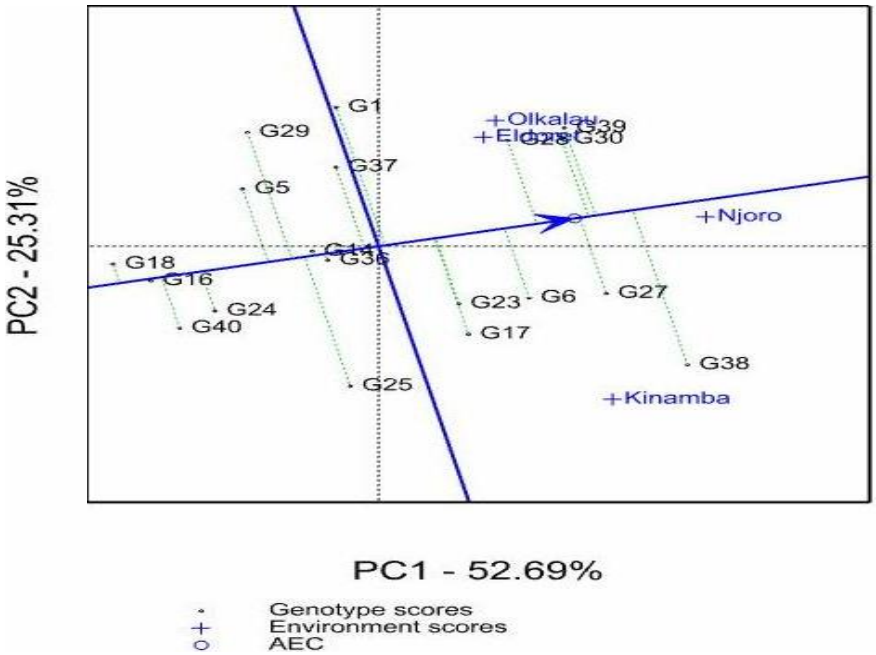

Figure 2. GGE biplot showing the relationship among the test environments in relation to stem rust.

variation in weather conditions which influence the interaction between stem rust and wheat. In the Kenyan context, beside stem rust, variation in yield performance of wheat is known to result from variation in climate conditions.

Forty genotypes including three checks were screened for stem rust. Genotypes with low Si^2 values and low mean CI are considered stable (Peterson, 1994). Although several genotypes had low mean infection, their Si^2 values were high hence not considered stable. According to Letta and Tilahun (2007), means are not efficient measure of disease stability. As such, in addition

Table 3. Yield performance (t ha^{-1}) of forty wheat genotypes tested in four environments.

Genotype	Eldoret	Kinamba	Njoro	Olkalau	Mean
G1	1.02	2.50	1.18	2.22	1.73
G2	1.19	1.84	1.07	2.10	1.55
G3	1.60	1.87	1.46	2.21	1.78
G4	1.24	2.12	1.39	2.14	1.72
G5	1.43	1.98	1.75	2.13	1.83
G6	1.33	1.82	1.80	2.63	1.89
G7	1.24	1.71	1.51	2.35	1.70
G8	1.53	2.05	1.59	1.86	1.76
G9	1.45	1.90	1.48	2.07	1.73
G10	1.23	1.47	1.34	2.22	1.56
G11	1.64	1.61	1.33	1.67	1.56
G12	1.38	1.30	1.04	1.78	1.38
G13	1.32	1.99	1.53	2.08	1.73
G14	1.54	1.92	1.57	1.87	1.72
G15	1.51	1.45	1.49	2.29	1.69
G16	1.42	1.90	1.65	2.01	1.75
G17	1.43	1.83	1.55	2.77	1.89
G18	1.61	2.07	1.96	2.27	1.98
G19	1.54	1.64	1.26	1.93	1.59
G20	1.53	1.94	1.45	1.90	1.70
G21	1.53	1.63	1.42	2.48	1.77
G22	1.46	1.68	1.27	1.97	1.59
G23	1.33	1.87	1.82	2.26	1.82
G24	1.32	2.10	1.76	1.91	1.77
G25	1.68	1.99	1.77	2.82	2.07
G26	1.39	1.74	1.58	2.00	1.68
G27	1.43	2.17	1.57	2.21	1.85
G28	1.54	2.21	1.43	2.34	1.88
G29	1.49	2.18	1.75	2.48	1.97
G30	1.65	2.19	1.32	2.36	1.88
G31	1.45	1.64	1.45	2.00	1.64
G32	1.36	1.99	1.31	2.28	1.74
G33	1.39	1.52	1.75	2.18	1.71
G34	1.47	1.78	1.39	2.44	1.77
G35	1.48	1.84	1.71	1.38	1.60
G36	1.31	2.04	1.59	1.38	1.78
G37	1.43	1.36	1.88	1.71	1.60
G38*	1.42	1.40	1.53	1.02	1.34
G39*	1.21	1.62	1.63	1.90	1.59
G40*	1.47	2.01	1.56	2.62	1.92
Mean	1.42	1.85	1.52	2.13	

[a]Comparing means between genotypes, [b]Comparing means between environments, [c]Comparing means for genotype × environment interaction, * Checks. Lsd = 0.73[a], 0.21[b], 1.36[c].

to Si^2, GGE biplots were used to determine resistance and disease stability. None of the genotypes was completely resistant or immune to stem rust. This is an indication of the challenge in breeding for resistance to the disease. However, several genotypes were found to have some level of resistance. Results from the current study showed that genotypes with moderate resistance had low infection. Probably these could be having partial

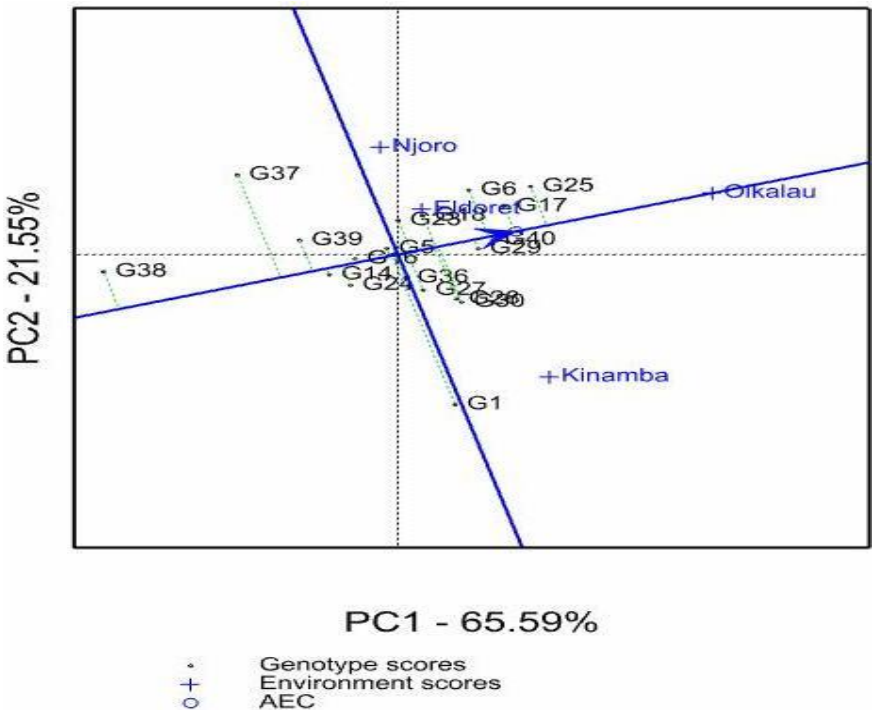

Figure 3. GGE biplot showing yield stability of selected wheat genotypes.

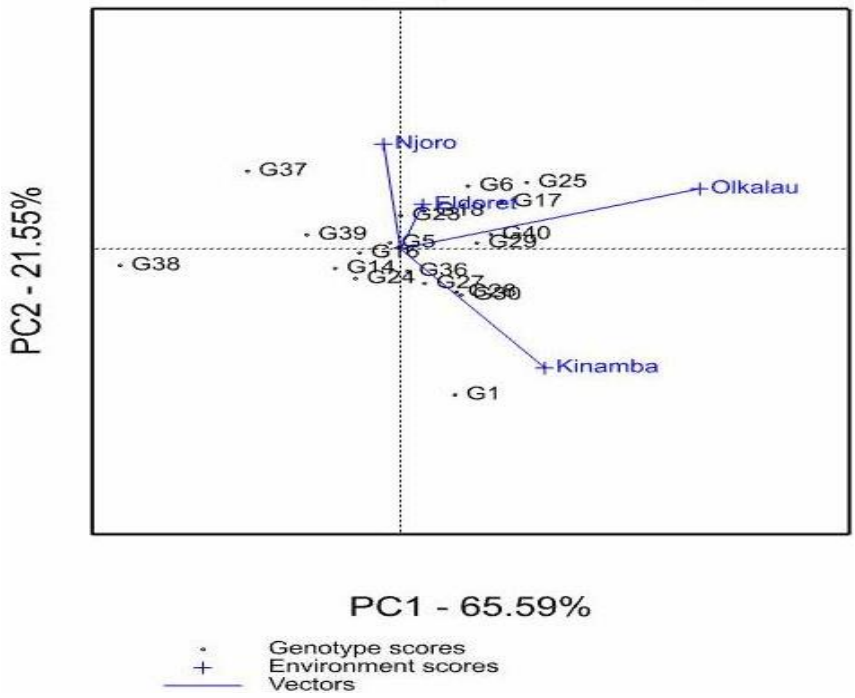

Figure 4. GGE biplot showing the relationship among the test environments in relation to yield.

resistance which is attributed with additive or epistatic genes (Nzuve et al., 2013). Partial resistance is known to be non-race specific, highly inheritable and durable (Singh et al., 2009).

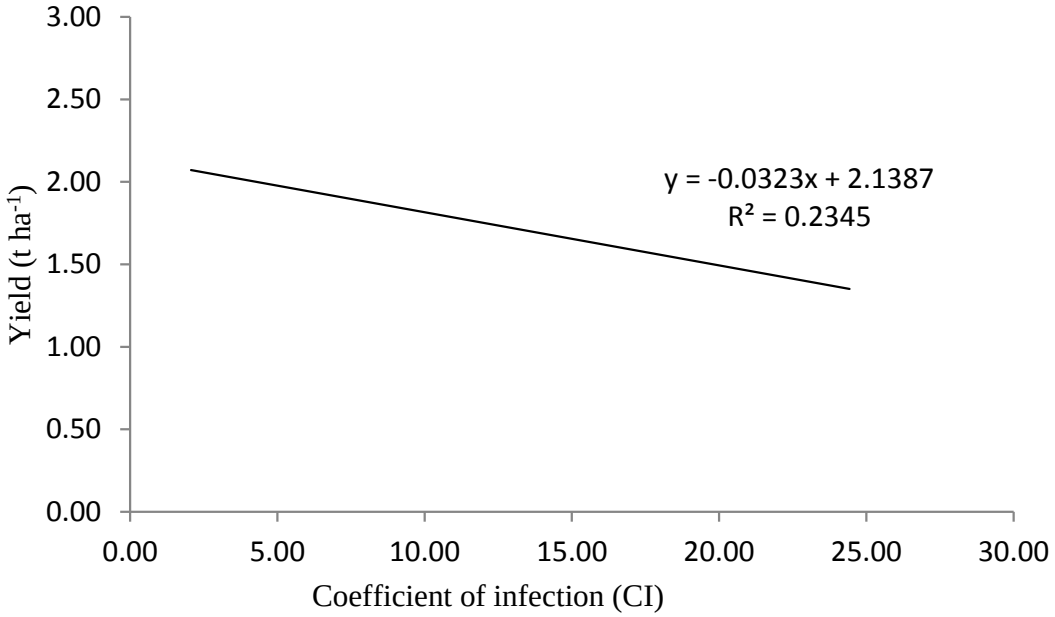

Figure 5. The correlation between yield and CI for the selected genotypes in the four environments.

GGE biplots was found to be adequate in determining disease stability according to Rubiales et al. (2014). The results of this study revealed that resistant genotypes had relatively high yield performance which explains the effect of the disease on yield. Stem rust causes shriveled grains thereby reducing wheat yield. It was also found that disease stability and yield stability are independent. Some of the genotypes highly stable for yield, for instance G29, were unstable for disease. Although some of the genotypes were stable for both disease and yield, stability should be done for one trait independently even when studying several traits. The weak correlation between yield and CI indicated that apart from stem rust, there were other factors influencing the wheat yield. Considering stem rust resistance, yield and their stability, G16, G18 and G36 were the best genotypes which could be tested further for future release. Although genotypes F6, F17, F23, F27, F28 and F30 had high yield, their CI was below the average line hence moderately susceptible. These genotypes could probably be tolerant to stem rust and could be considered for further tests for future release.

The disease pressure in each environment influenced the average performance of individual genotypes as well as that of individual environment. There was a slight difference in disease pressure across the four test environments. The high disease pressure at Njoro could be attributed to build up of stem rust inoculum during the international screening of wheat materials which is carried out yearly by Durable Rust Resistant Wheat (DRRW) project. In this study, significance in genotype × environment in CI was an indication of inconsistency in genotype in response to the changing environment as a result of GEI. Similar results were observed by Mohammed (2009).

Stem rust is favored by warm and moist environment which is the characteristic of the test environments (Wanyera et al., 2006). Eldoret and Olkalau were similar for both stem rust and yield. This similarity shows that, while carrying out a multi-location trial, breeders can select one environment to be representative in the case of limited resources. Njoro and Kinamba had large projection from the biplot origin and would therefore be good sites for evaluation of wheat response to stem rust. These results were in agreement with Hintsa and Fatien (2013), in their study on wheat. Olkalau had the largest projection for yield hence highest contribution to GEI.

Conclusion

The field experiments allowed assessment of the response of genotypes to stem rust and their yield performance across environments. The differences observed on genotypes across various environments were an indication of the presence of GEI. The results of this experiment showed some promising genotypes that were stable for stem rust resistance, with high and stable yield. These genotypes, G5, G16, G18, G24 and G36 are potential lines which could be advanced for future release.

Conflict of Interest

There is no conflict of interest between the authors or anybody else.

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