Characterization and genetic analysis of maize germplasm for resistance to northern corn leaf blight disease in Tanzania

By

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Thesis abstract

The majority of farmers in Tanzania have not yet adopted modern maize varieties and still cultivate landraces and open pollinated varieties (OPVs) with low production potential and susceptible to diseases like maize streak virus (MSV), grey leaf spot (GLS) and northern corn leaf blight (NLB). The NLB disease is among the major causes of low yield and has been reported in all 21 maize growing regions in Tanzania. Breeding for host plant resistance with high yielding potential and involving the community in the breeding process is expected to address the problem of low yield, NLB disease susceptibility and low rate of F_1 hybrid adoption. Therefore, the study was conducted to obtain additional sources of resistance to NLB disease, high yielding cultivars with community acceptable traits adapted to Tanzanian conditions. The main objective was to contribute to increased maize productivity in the western zone of Tanzania. The specific objectives of this study were therefore to : 1) investigate maize production limiting factors for smallholder farmers in western Tanzania, 2) identify farmers and stockist perceptions, opinions and maize variety selection criteria in western Tanzania, 3) establish NLB disease status in farmers' fields of western Tanzania, 4) determine the genetic relationships among landraces and assess maize landraces as sources of breeding materials, 5) determine the combining ability and heterosis for NLB disease resistance of eleven maize inbred lines adapted to Tanzanian conditions, and 6) determine the gene action and inheritance of resistance to NLB disease in five maize inbred lines adapted to Tanzanian conditions. The study was conducted from 2008-2011 in three diverse environments which represent all the maize growing regions in the country

The participatory rural appraisal (PRA) was conducted in three districts to investigate farmers' and stockists preferred traits for maize selection in western Tanzania, determine maize production constraints facing farmers and assess NLB disease prevalence in the same area. A focus group of 30 farmers was selected in each of the three villages. Transect walks, wealth ranking and historical profiles were used in an informal survey. One hundred and fifty questionnaires were used in a formal survey. The recorded yield was only 1 t ha^{-1}. Thirteen major maize production constraints, 13 insect pests and vermin and, 11 diseases were recorded. The NLB disease was reported to be increasing in severity in all farmers' fields. Farmers' preferred traits included resistance to abiotic and biotic stresses, early maturity, preferred milling qualities, high storage qualities and high yielding potential. Stockists mentioned 12 preferred maize variety traits which included high yielding, disease and insect pest resistance, heavy grain, large cob size and large grain sizes. Similarity between farmers and stockist variety preference ranking were found to exist.

The occurrence and distribution of northern leaf blight (NLB) disease study was conducted to assess the incidence and severity of NLB disease in farmers' fields in seven districts. The study was conducted for two seasons. In each season, 175 fields with 5600 plants were sampled. There were sixteen varieties grown with wide NLB disease reaction variation. Gembe, a landrace, was among the three observed resistant varieties. The NLB disease has changed its distribution pattern affecting all districts of the western zone. The disease incidence in season two (2009/2010) significantly increased from season one (2008/2009) t= -3.25 (348), P= 0.001. About 30% of both means of blight incidence and severity were recorded in the area.

Characterization and screening of maize landraces for northern leaf blight disease resistance was conducted to determine the genetic relationships among landraces, assess maize landraces as sources of NLB disease resistance and assess important agronomic traits for future maize improvement. Ninety breeding materials consisting of 71 landraces and 19 commercial varieties were evaluated. The average yield of landraces under research management was 2.3 t ha^{-1}. Landrace TZA 3075 was identified as NLB disease resistant. Yield potential, dent grain texture, white endosperm and husk cover were important agronomic traits observed among landraces. There were high variations in terms of morphology and NLB disease resistance among the landraces. Five principal components contributed to 71.98 % of total variation. Clusters analysis revealed five distinct groups of landraces. Leaves/plant, infested leaves/plant, lesion number, lesion length, lesion width and NLB disease incidence traits highly contributed to variation and grouping of landraces.

Combining ability analysis for northern leaf blight disease resistance was conducted to estimate the combining ability for NLB disease resistance of 11 maize inbred lines adapted to Tanzanian conditions, determine maternal effects which are involved in NLB disease resistance in maize germplasm, and determine the heterosis in the F_1 hybrids. A full 11 x 11 diallel cross was performed. All top ten experimental hybrids in each of the three sites had negative midparent heterosis for NLB disease severity. The overall mid-parent heterosis means for yield across sites was 152%. The mean sum of squares for GCA was highly significant (P< 0.001) on disease severity indicating additive gene action effects. Mean sum of squares for SCA were highly significant for disease severity and yield implying non-additive gene action effects.

The mean squares for reciprocal effects were highly significant on yield and non-maternal sum of squares had significant effect ($P<0.05$) on yield. The GCA contribution was high for disease severity (91%) and lesion number (85%). Almost, all GCA effects for NLB disease resistance were negative implying contribution to disease resistance. Due to preponderance of the additive gene action, recurrent selection could be used to improve the resistance of inbred lines while the non-additive gene action could be exploited in breeding for disease resistant hybrids.

Generation mean analysis of northern leaf blight disease resistance was conducted to determine the mode of gene action involved in the inheritance of resistance to NLB disease in five inbred lines adapted to Tanzania at contrasting environments, estimate heterosis and heritability in five tropical inbred lines. Generation mean analysis was conducted using a six parameter model comprising P_1, P_2, F_1, F_2, BCP_1 and BCP_2 generation progenies. The mean sum of squares for environment, replication with the nested environment, generations, generations x environment interactions were highly significant ($P<0.001$). The full model of additive, dominance, additive x additive and additive x dominance epistatic effects was highly significant ($P<0.001$). Nonetheless, the additive gene effects were predominant ranging between 57% and 89% which was matched by large heritability (54%-85%). The average degree of dominance ranged between -0.52 and 0.88 supporting observations of partial dominance. The NLB disease severity showed a continuous distribution in all three sets for F_2, BCP_1 and BCP_2 populations which is an indication of quantitative nature of inheritance and additive gene effects. The mid parent heterosis ranged from -19 to 1%. Therefore, resistance to NLB disease could be improved through selection by exploiting the additive gene effects. The epistatic gene effects would cause less complications because they were negligible (<25%).

The client oriented breeding for maize northern leaf blight disease resistance was carried out to perform farmers and stockists assessment on the 110 F_1 experimental maize hybrids and compare them with breeders selection criteria. Breeders selection criteria ranked 10 top high yielding experimental hybrids. Farmers developed 14 while stockists developed 13 selection criteria. The most preferred hybrids by farmers were VL 05616 x CML 159, CML 159 x KS03-0B15-47 and EB04-0A01-304 x CML 442 while stockists preferred VL 05616 x CML 395, EB04-0A01-304 x CML 442 and VL 05616 x CML 159. Two F_1 experimental hybrids EB04-0A01-304 x CML 442 and CML 159 x CML 442 appeared in all top five ranked hybrids by breeders, farmers and stockists. Generally, findings showed that, farmers, stockists and breeders coincide in some selection criteria but also differ in other cases.

Declaration

I, Tulole Lugendo Bucheyeki, hereby declare that;

(i) The research reported in this thesis, except where otherwise indicated, and is my original research.

(ii) This thesis has not been submitted for any degree or examination at any other university.

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Tulole Lugendo Bucheyeki (Candidate)

As the candidate’s supervisors, we agree to the submission of this thesis:

Signed:……………………………………………Date…………………………………………

Prof. Pangirayi Tongoona (Principal Supervisor)

Signed:……………………………………..…..Date…………………………………………

Prof. John Derera (Co-Supervisor)

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Dedication

In memory of my late parents, Bucheyeki Lugendo and Kabula Kafula

To my children, Rhoda, Prosper and Joseph

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General introduction to the thesis

1. Importance of maize in Tanzania

Maize is one of the dependable food and cash cereal crops in Tanzania. It is estimated that 45% of the cultivated land in Tanzania is covered with maize crop (Kaliba et al., 2000). The grain can be processed to be used in different dishes and animal feeds formulation. In Tanzania, when the government announces food shortage and crises, it just means inadequate production and supply of maize grain regardless of the availability of other crops. Maize was ranked by farmers as the most important crop in the western zone followed by cassava, beans, groundnuts and oil palm (BACAS, 2000). Kaliba et al. (1998) contented that the western zone is the fourth largest producer of maize and contributes about 11% of maize produced in Tanzania and benefiting the 3 million population of Kigoma and Tabora regions.

2. Maize production in Tanzania

For the past 41 years (1969-2009), maize average harvested area in Tanzania was estimated to be 1, 667,114 ha (FAOSTAT, 2011). Figure 01 depicts the harvested area, yield and maize production. The figure shows that, from the 2003, there was a general decrease in maize production and yield which could be attributed to abiotic and biotic stresses like NLB disease. However, the harvested area was slightly increasing.

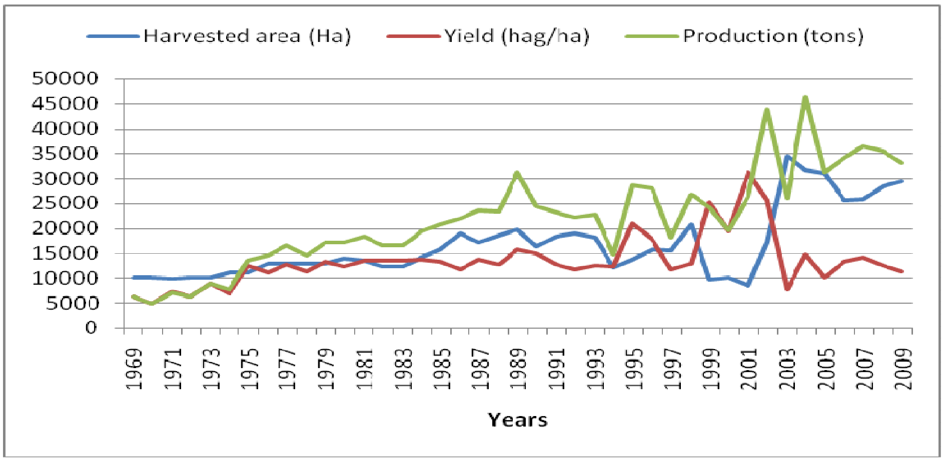

Figure 01: Maize harvested area (ha), yield (hag/ha, 1kg = 10 hag) and production (tons) in Tanzania from 1969-2011 (FAOSTAT, 2011)

3. Constraints facing maize production in western zone of Tanzania

Maize production in the western zone of Tanzania is affected by many factors. These can be grouped into biotic and abiotic factors. Abiotic factors include low soil fertility and soil acidity. The large proportion of soils in the western zone is sandy loams. The soil is eroded and degradation and desertification are evident (Nyadzi et al., 2003). On the other hand, biotic stresses found in the zone include insect pests like army worms, cutworms stalk borers, *Sitophilus* spp, and large grain borer. Others include weeds and striga especially *Striga asiatica*. The common diseases that affect maize production in the zone include fusarium and gibberella stalk and cob rots, leaf rust, maize streak virus disease (MSV) and northern leaf blight. Northern leaf blight is a common disease affecting all maize growing regions and invading the majority of maize varieties (CIMMYT, 2004).

4. The northern leaf blight (NLB) disease problem in western zone of Tanzania

Northern leaf blight (NLB) disease is a fungal disease caused by *Exserohilum turcicum* (Dunn and Namm, 1970) K. J. Leonard and Suggs [anamorph]. For the past decades, the disease had significant influence in cool and humid zones in the country. At present, NLB can be found everywhere including marginal areas. The observed symptoms in the field include water-soaked portions that elongated to necrotic lesions. In some cases, lesions join together and under some circumstances plant leaves appear as burnt by hot temperatures (CIMMYT, 2004). The reduced photosynthetic area of leaves follows which leads to low plant assimilate production and subsequent reduced maize yield.

In the western zone of Tanzania, all 10 districts have already been invaded by the disease. The ranges of severity and incidences vary from season to season, location and cultivars. There are reports of the disease in Urambo, Sikonge, Nzega, Igunga, Tabora municipality, Uyui, Kibondo, Kasulu, Kigoma rural and Kigoma ujiji districts. Farmers have approached the Agricultural Research Institute (ARI)-Tumbi on the possible ways of controlling the disease. However, the reliable control strategy is to explore maize genetic resources resistance and the release of resistant cultivars through strategic breeding.

5. Current efforts to control the NLB disease

In the world, efforts to control the disease have been conducted through fungicide application (CPC, 2001; Harlapur et al., 2007). The use of fungicides increases production costs and reduces profit margins forcing farmers not to apply it at the expense of crop yield.
Another method of controlling the disease is the use of cultural practices. Crop rotation, destruction of crop residues and closed seasons are being conducted to reduce inoculums in the soil debris and alternative hosts (CPC, 2001). Outputs from cultural practices take time and sometimes have no immediate visible means of verification. Under special conditions, bio-agents are being employed to control the disease. There are no effective bio-agents to contain the disease. In addition, bio-agents application is complex, needs special treatments, laboratories and specialized personnel to make it not feasible under small holder African farmer conditions (Harlan, 1975).

The use of host plant resistance seems to fit African conditions. This method is cheap, effective and gives high results (Dunn and Namm, 1970; Sharma and Payak, 1990). Through maize breeding procedures, it is now possible to achieve durable resistance to control the disease (Sharma and Payak, 1990). In the western zone of Tanzania, farmers cope with the disease infestation by employing land rotation, burning and burying crop residues. Neither chemical nor resistant cultivar is being used to contain the disease. This situation calls for breeding efforts to develop NLB disease resistant cultivars.

6. Justification for breeding for NLB disease resistance in Tanzania

Northern leaf blight (NLB) disease is threatening maize production in Tanzania. The recent disease occurrence and severity has increased tremendously to the level of an outbreak. There are many factors associated with this phenomenon. Factors range from the weather, climate change, emergence of new pathotypes, new races, host susceptibility, alternative hosts, pathogen by host interactions and possible resistance breakdown (Boland et al., 2004; Ogliari et al., 2005; Smale and DeGroote, 2003). The persistent problem brought by NLB disease remains a big challenge regardless of the number of years already taken by researchers in the world fighting the disease.

Studying, identification and exploitation of resistant materials remains the most viable option to curb the scourge. Resistant materials can be obtained from adapted inbred lines, landraces and through the application of genetic engineering. To achieve this, a clear understanding of the pathogen, environment, host-plant relationship and suitable parents is a prerequisite. However, Breeding for NLB disease resistance is very complicated and demanding due to the nature, weather, host-pathogen relationship and being one of the most widely distributed maize foliar disease in almost all continents of the world (CAB International, 1974).

Frequent outbreaks and epidemics of the disease bring another challenge to maize breeders. The NLB disease outbreak in the USA (Elliott and Jenkins, 1946), in Asia (Small, 1922), in Austria (Zwatz, 1988) and that in Uganda (Adipala et al., 1993) advocate the constant monitoring and control of the disease. In addition, the world enjoyed low and minimum levels of disease infestation after the USA and Asia outbreaks. However from 1980's, the disease had resurged affecting maize yield in the world again (Krasuz et al., 1993; Sharma and Mishra, 1988). There is evidence that, the discovery of Ht1 gene contributed significantly to the reduced level of NLB disease in the world (Hooker, 1963). Frequent use of Ht1 gene resulted to the emergence of races with virulence effect which could be one of the reasons of NLB disease resurgence the world is witnessing today (Pataky and Ledencan, 2006).

In Tanzania, the disease has resurged affecting all 21 regions of the country. Various factors have been thought to be associated with the disease epidemics. Mwakalobo and Kashuliza (1999) contented that, the economic adjustments strategy programme coupled with adjustment of plant regulatory system to attract agricultural investors led to introduction of susceptible cultivars brought by companies or individuals. Susceptible cultivars are thought to be fully responsible for inoculums increase and the subsequent outbreak of the disease. All cultivated maize varieties are now succumbing to NLB disease in the western zone. The demand for developing NLB disease resistant varieties in the zone is very high. Agricultural Research Institute (ARI-) Tumbi puts maize as priority one and the zone outlines the purpose of increased productivity that could be achieved through the reduction of disease like NLB incidences (ARI-Tumbi, 2008). Farmers have reported the NLB disease incidences and severity in all 10 districts of the zone and plead for assistance from agricultural research institute and agricultural department (URPT, 1998). In addition, CIMMYT has noted five stresses in Tanzania that include NLB disease on maize production and placed it as priority number one in its research agenda (Bänziger et al., 2000).

Maize yield from small holder farmers found in Tanzania and western zone in particular is very low. According to Makurira et al. (2007), maize yield under farmers conditions is 1.2 t ha^{-1} which is not enough to sustain families in terms of food supply per season. The low yield obtained by farmers could be attributed to many factors. However, factors limiting maize production in the zone are not well documented. There is superficial and more than 12 years old information of some constraints facing farmers in the zone (BACAS, 2000; Kaliba et al., 1998).

Farming systems, production condition and socio-economic conditions have changed since then and the need for more current comprehensive record of production constraints has been raised.

From the 1950's to 2011, about 100 maize varieties have been released in Tanzania. These varieties were meant to reach farmers in the country. However, farmers plant only 6- 12% of the improved varieties in the western zone of Tanzania (Mafuru et al., 1999). The majority of farmers still grow landraces and OPVs with low production potential. This has an implication on community variety preferences and community involvement in breeding processes. The farmers' persistence of growing landraces and OPVs could have breeding implications and presence of specific variety selection criteria which needs some investigation to be incorporated in the maize breeding programme. Farmer participatory plant breeding information in western Tanzania is lacking. This could be the reason of rejection of high yielding varieties in the zone. For example Kaliba et al., (1998) cited rejection and abandonment of maize varieties H6302 and H614 in the area.

Participatory plant breeding methods of variety evaluation and dissemination have managed to minimize information gaps between breeders from agricultural research centres and communities in various crops elsewhere (Harris et al., 2001; Rice and Smale, 1998). However, the majority of participatory plant breeding information is only concentrated between farmers and breeders without considering stockist who plays the bridging role between them (Gyawali et al., 2007; Nabirye et al., 2003; Sall et al., 2000). There are reports that addition of other maize beneficiary could increase the rate of adoption. For example Joshi et al. (2007) reports on the modification of participatory plant breeding by the inclusion of consumers in addition to farmers for rice variety selection in Nepal. The result from that study was the highly increased rate of adoption for the tested rice breeding materials. This approach could be adopted in western Tanzania to improve the low rate of maize cultivar adoption by involving farmers and stockists in the breeding process.

In summary, the other NLB control practices such as cultural, biological and fungicide use have not been effective in controlling the disease. These practices therefore require some complementation by host plant resistance. Breeding for NLB disease resistant varieties is therefore advocated as a sustainable strategy. Thus, the demand for searching for additional cultivars resistant to NLB disease resistance, high yielding with accepted community preferences in western Tanzania was prompted by 1) increased severity and occurrence of NLB disease in farmer's field, 2) low maize yields in the farmer's field, and 3) low adoption rate of introduced cultivars in the zone. However, cultivar adaptation to various environments and farming systems is another challenge facing plant breeders. In Tanzania, there are well adapted landraces and inbred maize breeding materials. However, maize landraces genetic relationships for NLB disease resistance and other traits are lacking. There are no characterization studies conducted on these breeding materials. For full utilization of landraces and incorporation into the breeding system, a landraces characterization study is needed. On the other hand, there is no combining ability information and gene action on adapted to Tanzania. Therefore, the study was conducted to obtain additional source of resistance to NLB disease, high yielding cultivars with community acceptable traits from breeding materials adapted to Tanzanian conditions. The main objective of the study was to contribute to maize productivity in the western zone of Tanzania.

7. Research objectives

The specific objectives of this study were therefore:

1. To investigate maize production limiting factors to small holder farmers in western Tanzania.

2. To identify farmers and stockist perceptions, opinion and maize variety selection criteria in western Tanzania.

3. To establish NLB disease status in farmers' fields of western Tanzania.

4. To determine the genetic relationships among landraces and assess maize landraces as sources of breeding materials for NLB disease resistance

5. To determine the combining ability and heterosis for NLB disease resistance of eleven maize inbred lines adapted to Tanzania condition

6. To determine the gene action and inheritance of resistance to NLB disease resistance from five maize inbred lines adapted to Tanzania condition

8. Research hypothesis

The tested hypotheses were shown below

1. Factors limiting maize production in western Tanzania are known by farmers and can be tapped for documentation. This information would be used to set priorities for the breeding programme in Tanzania
2. Farmers and stockists in western Tanzania have specific preferences and variety selection criteria to be documented, which would be useful to the maize breeders.
3. There is no genetic variation among maize landraces found in Tanzania for NLB disease reaction
4. There is high combining ability for NLB disease resistance and no epistatic gene action among eleven maize inbred lines adapted to Tanzania condition

This thesis is comprised of eight chapters presented in a composite style. You can find that, in some cases overlapping of ideas, context and references occur due to nature of thesis presentation. The thesis starts with general introduction and the following chapters follows:

1. Literature review,
2. Maize production constraints, NLB disease status, stockists and farmers' opinions on varieties selection preferences in western Tanzania
3. Occurrence and distribution of northern leaf blight disease in western Tanzania,
4. Characterization and screening of maize landraces for northern leaf blight disease resistance in the western zone of Tanzania,
5. Combining ability analysis for northern leaf blight disease (*Exserohilum turcicum*) resistance in adapted inbred maize lines (*Zea mays* L in western Tanzania,
6. Generation mean analysis of northern leaf blight (*Exserohilum turcicum*) disease resistance in five Tanzania adapted maize (*Zea mays* L.) inbred lines, and
7. Client oriented breeding for maize (*Zea mays* L.) northern leaf blight disease (*Exserohilum turcicum*) resistance in western Tanzania
8. General overview and the way forward.

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Chapter 1

1 Literature review

1.1 Introduction to literature review

This review of literature elaborates maize production constraints in Tanzania. The review also highlights the major known maize production constraints in Tanzania which includes the northern leaf blight (NLB) disease. The NLB disease has increased its importance in Tanzania. Therefore the major focus of the study was on NLB disease in maize. The causative agent, transmission, epidemiology, the resurgence of NLB disease and disease symptoms are explained in this chapter. There is also discussion on sources of resistance to NLB and disease mode of inheritance. Current efforts to control the disease, genetics of northern leaf blight disease resistance, gene action estimation from diallel cross and generation mean analysis are explained. Roles of heritability, heterosis maternal effects in NLB disease resistance are explained in detail. Finally, breeding for desired maize traits in Tanzania is also discussed.

1.2 Maize production constraints in Tanzania

Maize production in Tanzania is carried out by small-scale farmers who account for up to 85% of the total maize produced in the country (Bisanda et al., 1998). Despite maize research programme efforts in breeding for high yielding cultivars, the average yield in the country is still low. According to research, the average yield under farmers condition is still resting at 1.2 t ha^{-1} (Aquino et al., 2001; Makurira et al., 2007)$^{.}$ The low yield is attributed to socio-economical, biotic and abiotic constraints (Katinila et al., 1998; Pixley et al., 2006).

With the reduction of price subsidy, the prices of farm inputs increased beyond small farmers reach (Mwakalobo and Kashuliza, 1999). Prices of agricultural inputs like seed are 30 times what it used to be in the 1990's while the price for maize increased only three times during the same period. The situation has forced less than 35% of farmers to use purchased seeds (Morris, 2001). In addition, due to lack of seeds availability in the country, farmers are forced to use recycled seeds which further complicates the situation (Doss et al., 2003). These factors have lead to reduced maize yield which have resulted into food shortages and frequent hunger (Katinila et al., 1998).

The abiotic factors include low-N, low-K and drought while biotic factors include stalk borer and army worms. The common diseases are Leaf rust (*Puccinia maydis*), Brown spot (*Physoderma* *maydis*), Northern leaf blight (*Helminthosporium turcicum),* Phaeosphaeria leaf spot (*Phaeosphaeria maydis),* Tassel smut (*Sphacelotheca reiliana*), Gibberella stalk rot (*Gibberella zeae),* Fusarium ear rot *(Fusarium moniliforme)* and Fusarium stalk rot (*Fusarium moniliforme)* (Bisanda et al., 1998; Nkonya et al., 1998*).* Some reasonable efforts have been made to manage soil fertility problem and reducing its effects on maize production (Nyadzi et al., 2006; Nyadzi et al., 2003a; Nyadzi et al., 2003b). Therefore, the focus of this study will be on northern leaf blight disease which is currently affecting the majority of maize fields in the country.

1.2.1 Northern corn leaf blight (NLB) disease in Tanzania

Among the biotic factors, northern leaf blight (*Exserohilum turcicum)* is one of the major constraints to maize production in Tanzania. Northern leaf blight is one of the major diseases affecting cereal production (Pixley et al., 2006). The disease can be found in all 21 maize growing regions, including the marginal areas, which were previously considered unfavourable for disease development. To date, most of improved maize varieties grown in Tanzania are susceptible to NLB disease (Kanampiu et al., 2003). There is a need for breeding for NLB disease resistant varieties which will curb the current outbreaks. In order to deal with this problem, an effective breeding strategy is needed. The use of available sources of resistance from some of the inbred lines, landraces and exotic materials may kick-start the introgression of resistance genes into the currently released and new cultivars.

1.2.2 Causative agent and transmission of northern leaf blight disease

The disease is caused by the fungus *Exserohilum turcicum* (Pass.) K. J. Leonard & Suggs [anamorph]. It is one of the major diseases of maize, sorghum and pearl millet. The primary hosts are maize (*Zea mays*) and sorghum (*Sorghum bicolor*) while the secondary host is pearl millet (*Pennisetum glaucum*). Densely populated cultivars facilitates movements of spores from one plant to another and thus increases disease severity (Adipala et al., 1995). On the maize plant, the disease starts at the lower leaves and then spreads to other parts of the plant (Elliott and Jenkins, 1946). Wind and rainfall splash spreads spores from disease to healthy plants (Amusa et al., 2005; Boland et al., 2004). The disease also survives on wild hosts of the graminae family and attack maize in the next season. It survives from one season to another in the form of conidia on crop residues which acts as the source of inoculums to the new cropping season (CPC, 2001; Esele, 1995; Shang, 1980).

1.2.3 Disease epidemiology

Severity of the disease occurs when conditions are favourable. High humidity associated with low temperature and cloudy weather is conducive conditions for disease development on the host plant (Singh et al., 2004). Heavy dew on the growing plant has also being cited as one of the factors leading to NLB disease severity (Dingerdissen et al., 1996; Levy and Cohen, 1983). Conidia germination on leaves is high when the temperature ranges from 18 to 27°C (Levy and Cohen, 1983). Levy (1989) mentions that high relative humidity and presence of susceptible hosts are other factors that influence the disease epidemiology. Ceballos et al. (1991) reports that, disease severity is high for early maturing maize varieties than late maturing varieties. This implies that, late maturing cultivars are relatively more resistant than early maturing maize varieties.

1.2.4 The resurgence of NLB disease

Past decades witnessed breeders containing the NLB disease in maize production. Previous studies showed the concentration of the disease to high humidity and low temperature areas of the world (Dunn and Namm, 1970; Esele, 1995; Raymundo and Hooker, 1981). Recently, the disease has resurged and is affecting all maize growing regions in the world (CIMMYT, 2002; Mwangi, 1998). Researchers have been associating the resurgence of NLB disease with many factors.

Planting susceptible cultivars coupled with the extensive use of fungicides to control the disease is one reason towards this scourge. Small-holder farmers are forced to use cheap and susceptible genotypes due to their low purchasing power, at the same time commercial farmers may use susceptible materials and intensify spraying regimes (Adipala et al., 1993; Amusa et al., 2005; Kaliba et al., 1998a; Pataky et al., 1998). Many resistant cultivars were developed to confer gene- for- gene, monogenic and race specific type of resistance (Robinson, 1987; Robinson, 2002; Robinson, 2004). This type of resistance can easily be broken down and succumb to new emerging races (Brown, 2002; Campaña and Pataky, 2005). Susceptible genotypes act as the source of inoculums build up which in turn could result to disease resurgence.

Transhumance and tendency of farmers to exchange recycled seeds among themselves are other factors contributing to NLB severity and occurrence. A survey conducted in southern Tanzania by Nathaniels and Mwijage (2000) reported seed exchange among farmers in

Nachingwea district as one of the sources of planting materials. A similar observation has been found in Zambia where 40 % of seed exchange among farmers exists (Gwanama and Nichterlein, 1995). Recent studies have shown that land scarcity is another source of disease resurgence, land scarcity forces farmers to practice intensive farming while compromising crop rotation, improved fallow and rotational woodlots (Kimaro et al., 2008; Nyadzi et al., 2003b). This tendency has resulted in the increased number of fungal spores sufficient to cause the disease outbreak (Esele, 1995; Okori, 2004).

The effects of trade liberalization on agricultural sector could have contributed significantly to NLB resurgence. Trade liberalization has been associated with double effects on NLB disease occurrence. First, trade liberalization was accompanied with the reduction of agricultural input subsidy that resulted in the increased input prices and lower crop yields (Jean and Christina, 1991; Kaliba et al., 1998; Love, 1994; Mwakalobo and Kashuliza, 1999). Increase in input prices has forced farmers to resort to cheap and NLB disease susceptible cultivars which increases inoculums in the field. The second effect is agricultural policy regulations to attract investors in agriculture sector. Governments were obliged to reduce the strict importation and crop inspection rules and regulations. The result was the introduction of inferior and NLB susceptible genotypes in agricultural fields which lead to the increased NLB fungal inoculums (Geisler, 1992; Love, 1994).

High amount of inoculums from different sources have the possibility of increasing the recombinant hybridization which results into pathogen new races development in the area (Robinson, 1987). There are reports of new NLB disease races around the world. Emergence of new races 0, 1, 23 and 23N in NLB has posed a constant threat to the efforts of controlling the disease in maize breeding programmes worldwide (Ferguson and Carson, 2007; Ogliari et al., 2005). In East Africa, Mwangi (1998) observed the presence of races 0, 1, 2, 3, 12 and three unknown races.

Other studies have cited climatic changes as a contributor of NLB disease severity (Boland et al., 2004; Chakraborty et al., 2000). According to Griefenhagen and Noland (2003), the world's temperature is escalating such that it's temperature will rise by 3^0C next century. The rise in temperature will favour diseases development including fungus sporulation which is expected to bring further NLB disease threats to the world.

Pathogen environmental competence has been cited by researchers as one of the reasons of disease resurgence in crops (Godfray et al., 1999; Jackson, 1999; Robinson, 1987). Robinson (1987) further reported on a maize landrace which was higher resistant to disease in Malawi, the same variety was highly susceptible to the same disease in Kenya. This implies that, the pathogen had high environment competence and increased pathogenicity in Kenya than in Malawi. The same scenario can be used to explain the susceptibility to NLB disease of genotypes being currently introduced in different countries without enough testing time in the target countries. The result is the build up of inoculums in maize growing areas.

Mutation can be another source of NLB resurgence in maize germplasm. Breeding advances in maize has resulted in more uniform genotypes. Genetic uniformity increases the chances of pathogen mutations, new race emergence and increased pathogenicity (Ogliari et al., 2005; Smale and DeGroote, 2003). Pataky et al. (1998) reports on epidemics of NLB in Florida due to mutation of pathogen on a super sweet hybrid maize cultivar. Mutation can also occur on plants themselves through altering the genetic structure and thus become more susceptible to diseases. Jenks et al. (1994) reports on the effect of reduced epicular wax structure in sorghum which exposed the genotype to *Exserohilum turcicum* attack.

1.2.5 Disease symptoms

The disease starts with small water-soaked spots that appear on leaves. The small water-soaked spot dry up and join in the elongated brown lesion which can reach the size of 20 x 400 mm (Mwangi, 1998). Some researchers have recorded lesion sizes of 2.5 x 150 mm (Degefu et al., 2004). However, Pataky et al. (1998) reported much lower lesions on partially resistant maize genotypes. Severity of the disease depends on weather conditions, plant growth stage (Figure 1.1), pathogenicity and genotype susceptibility (CIMMYT, 2004; Levy, 1989). Under severe conditions, the whole leaf can be covered by the disease and dry up. The accompanying NLB disease effects are reduced photosynthetic area, plant lodging, secondary infection, stalk rot, plant death and subsequent lower yields per unit area (CIMMYT, 2004).

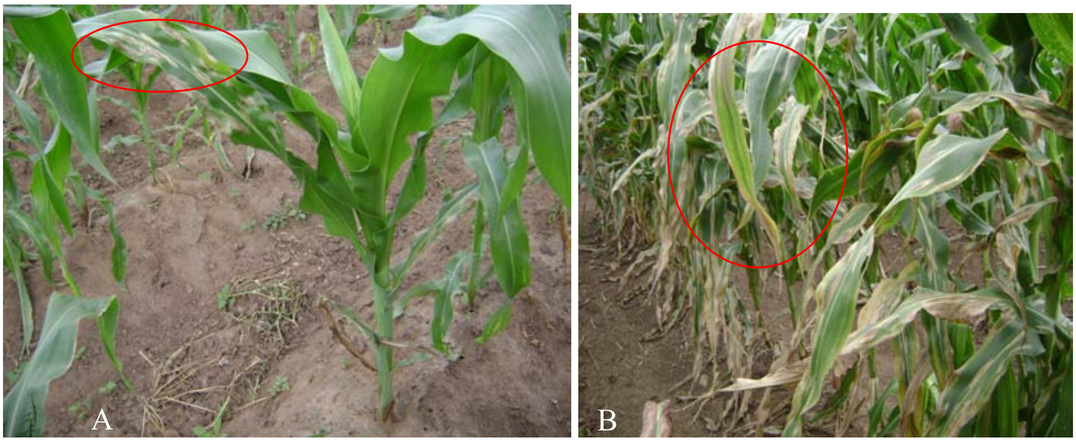

Figure 1.1 Early infestation (A) leads to loss of photosynthetic leaf area at reproductive phase (B)

1.2.6 Sources of resistance to NLB disease

Researchers have been using resistant materials to control the NLB disease effects in maize. Sharma and Payak (1990) used CM104 and CM105 NLB disease resistant inbred lines from CIMMYT to analyse the mechanisms of leaf blight disease resistance in maize. In Uganda, researchers used cultivars Babungo 3, EV8342-SR, Mo 17 and H99 as sources of resistance to NLB disease and recorded useful results (Lipps et al., 1997; Ojulong et al., 1996). According to Freymark et al. (1993) and Pratt et al. (1997), Mo17 provides polygenic NLB resistance to maize plants. Other researchers reported CML 202 as the source of horizontal resistance to NLB in tropical Africa maize (Schechert et al., 1999). In India, Inbred lines CM104 and CM105 from CIMMYT confer durable resistance to NLB disease (Levy, 1989; Sharma and Payak, 1990). According to Singh et al. (2004) early maturing, CM 145 and medium maturing lines from CIMMYT, CM 104, confer resistance to northern leaf blight disease. It was further noted that, population 31 from CIMMYT was a reliable source for NLB resistance (Singh et al., 2004). In practice, durable resistance can be achieved by population improvement through recurrent selection (Campaña and Pataky, 2005; Ceballos et al., 1991; Ogliari et al., 2005). According to Ogliari et al. (1999) L30R and L40 maize inbred lines are some sources of monogenic resistance to NLB disease.

1.2.7 Disease control

Various ways are used to contain the disease. These are cultural, chemical and biological controls. Biological control includes the use of natural enemies and resistant cultivars.

Cultural control methods aim at reducing the amount of inoculums in the field. Methods like destruction of crop residues, crop rotation, weeding and intercropping have shown some levels of controlling the disease. According to Sharma and Duveiller (2004), optimal fertilizer rates, moisture management and timely planting increase resistance and yield on maize production. Other researchers have found similar results (Reuveni and Reuveni, 1998). However, cultural control measures are sometimes limited due to land availability, labour shortages and farmers purchasing power.

Northern leaf blight disease can be controlled by using a number of fungicides. Fungicides differ in the ability of controlling the disease. The commonly used fungicides include Zinc ethylenebis (zineb), Dithane (mancozeb) M-45, O-Ethyl-S.S-diphenyl dithiophosphate (Edifenphos), Difolatan (Captafol), and benzenedicarbonitrile (chlorothalonil). The use of fungicides has managed to control the disease to a remarkable point. However, they are not sustainable, are expensive and pose environmental hazards (Chakraborty et al., 2000; Matthews et al., 2003; Reuveni and Reuveni, 1998; Shelepchikov et al., 2008).

Various biological control agents have been tested to combat the disease. The most promising is the use of *Bacillus subtilis* inoculums (Reis et al., 1994). Biological controls have the advantages that, they are environmental friendly, do not require industry processes and do not develop resistance to pathogens (Bacon et al., 2001; James-Cook, 2003). However, they have slow and unpredictable actions; they require specialized skills for rearing and ways of releasing them to the field. Furthermore, biological agents can multiply excessively and turn to be pathogens of other crops (Jutsum et al., 1988). Thus, breeding for resistance remains the reliable method.

Breeders and farmers have reported diseases as one of the major factors that limit crops production and employ some measures to reduce the effect. Previous breeding studies have significantly contributed to disease controls (Brewster et al., 1992; Shang, 1980; Sharma and Payak, 1990; Welz et al., 1999). In maize, breeding for NLB disease resistance started much earlier than 1961 (Ceballos et al., 1991). Although it seems to start earlier, more efforts are still needed as new challenges arise. Following the difficulty in controlling NLB due to high input prices, new races and unreliable biological control, more breeding for resistance is highly demanded (CPC, 2001; Mwakalobo and Kashuliza, 1999). The use of maize resistant to NLB is cheap and more reliable approach towards combating the disease (Hughes and Hooker, 1971;

Welz and Geiger, 2000). Therefore, the purpose of this study will be to seek for additional sources of resistance to maize cultivars.

1.2.8 Genetics of northern leaf blight disease resistance

Resistance to NLB disease in maize is located on chromosomes 3, 5, and 8 (Welz and Geiger, 2000; Wisser et al., 2008). Brewster et al. (1992) studied Mo17 maize line and found that, NLB disease resistance was linked to chromosome 3, the short arm of chromosome 4, and the long arm of chromosome 6. Northern leaf blight disease resistance is controlled by six dominant Ht1, Ht2, Ht3, HtN, NN and HtM and one recessive ht4 genes (Ferguson and Carson, 2004; Ferguson and Carson, 2007; Pratt, 2006; Singh et al., 2004; Wisser et al., 2006). All these provide qualitative inheritance in the form of dominance or partial dominance. According to Pataky et al. (1998) HtN gene confers partial resistance to NLB disease. Other researchers have reported on the durable resistance to NLB conferred by major genes. Ogliari et al. (2005) reports on dominant HtP genes inducing resistance to NLB pathogen and recessive rt genes inducing resistance to specific NLB pathogen races.

Several modes of gene actions are involved in controlling the inheritance of NLB disease in maize. Additive, dominance, and epistatic gene action are involved in controlling the disease (Ogliari et al., 2005). However, additive gene action was found to be more important than others (Hughes and Hooker, 1971; Ogliari et al., 2005). Maternal effects are less important for the traits associated with the inheritance of NLB disease resistance. Sigulas et al. (1988) found non significant maternal effects on 16 maize genotypes. Other researchers have reported non significant cytoplasmic and maternal effects on the inheritance of NLB disease in maize genotypes (Welz and Geiger, 2000). The maize germplasm currently in use in Tanzania has not been characterized for NLB resistance.

1.2.9 Gene action estimation from diallel cross

Gene action can be estimated by using various mating designs. Mating designs are methods used to produce progenies in breeding programmes (Dabholkar, 1992). They enable breeders to estimate genetic variances and combining abilities. Estimation of combining abilities enables the prediction of progenies performance based on the performance of parents. General combining ability measures the averages of all line crosses to a common progenitor while specific combining ability estimates the specific performance of combinations between lines (Griffing, 1956). There are various mating designs depending on the objectives (Stuber, 1980). The common mating designs include: topcross, polycross, biparental progeny, diallel and partial dialles, North Carolina I, II and III and line x tester mating designs. In the study a diallel mating design was used because it enables the estimation of GCA, SCA and other genetic effects from all possible combinations. By using diallel cross it is possible to evaluate parents, F_1 hybrids, reciprocals and maternal effects (Gupta and Kageyama, 1994; Stuber, 1980). In addition, diallel mating designs are suitable for cross pollinated crops like maize by which GCA and SCA and their interaction with environment are taken care of (Griffing, 1956; Hayman, 1954). According to Griffing (1956) estimation of genetic variances is made in terms of the combining ability by which effects are considered in terms of GCA and SCA i.e. $v_{ij}=g_i+g_j+s_{ij}$ if reciprocals are excluded, and $v_{ij}=g_i+g_j+si_j+r_{ij}$ if reciprocals are considered. Where, g_i and s_{ij} are GCA and SCA , r_{ij} is the reciprocal effect involving the ith and jth parents. The analysis conducted at one site can be modelled as:

$y_{ijkl}=\mu+ r_1 + b_{lk} + g_i +g_j+ s_{ij}+ e_{ijkl}$

where y_{ijk1} = yield (or any other trait) of the cross between lines i and j in block k; μ = overall mean; r_1 = replication effect, Σ_1 r_1 =0; b_{lk} = effect of the kth block in the 1th replication, Σ_k b_{1k} =0; g_i = the GCA of parent i, Σ_i g_i =0; g_j = the GCA of parent j, Σ_j g_j =0; s_{ij} = SCA of the cross between parents i and j, Σ_i s_{ij} =Σ_k s_{ij} =0;

e_{ijkl} =random error (assumed as normally and independently distributed i.e. $\mu=0$ and $\sigma^2=1$). The g_i +g_j+ s_{ij} is the genotypic contribution for cross i x j.

A relatively larger GCA/SCA variance ratio demonstrates the importance of additive genetic effects and the lower ratio indicates predominance of dominance and/or epistatic gene effects (Christie and Shattuck, 1992). The Significant contribution of GCA and SCA is then interpreted for breeding purpose application. If GCA is significant, it means additive gene effect is important and thus selection could improve the germplasm. If SCA is significant then, dominance gene effect is important and thus hybrid vigour could be achieved in crosses among inbred lines. If GCA and SCA are both significant, GCA/SCA ration is used for interpretation. In this case, if the ratio = 1, then both are important and if the ratio >1 then additive gene action is more important than dominance gene effects.

Depending to whether the selfed parents and or the reciprocals and F1s are included in analysis, it can be further divided into subdivision. Griffing (1956) suggested four possible experimental methods:

a) parents, one set of F1's and reciprocal included ,
b) parent and one set of F1's are included but not the reciprocals,

c) one set of F1's and reciprocals are included but not the parents and,

d) one set of F1's but neither parents nor reciprocals are included

Depending on the type of parents used for crosses, fixed or random models are used for analysis. If parents are the genotypes under consideration, this is referred to the fixed model (model I), whereas the random model (model II) is applied if the parents are random sample from the reference population (Griffing 1956).

1.2.10 Gene action estimation from generation mean analysis

Generation mean analysis (GMA) is another method used in gene action estimation. It utilizes six population means P_1, P_2, F_1, F_2, BCP_1 and BCP_2 to estimate genetic effects (Carson, 1995). The method is efficient in partitioning epistasis and non-allelic gene effects (Hettiarachchi et al., 2009). Thus, it is used to study populations which have distinct wide contrasting traits like disease resistance because it analyses one trait at a time (Frank and Hallauer, 1997).

Generation mean analysis has been employed in various crops and traits to estimate genetic effects in contrasting characteristics. In maize, GMA has been used to generate useful information. For example, it has been used for twin cobs study ((Frank and Hallauer, 1997)) and inheritance of NLB disease (Campaña and Pataky, 2005; Carson, 2001). Several studies have shown that, NLB disease inheritance is mainly controlled by additive gene action while dominance and epistasis contributions are normally non-significant (Carson, 1995; Jenkins and Robert, 1952). However, other studies observed the significant contribution of additive, dominance and non-allelic gene interaction in controlling NLB disease resistance in maize (Lingam et al., 1989). Therefore, this study employed generation means analysis because, estimation of epistasis and non–allelic gene interaction in inbred breeding materials adapted to Tanzania was required. Generation mean analysis is powerful tool for separation of additive, dominance, epistatic additive x additive, epistatic additive x dominance and epistatic dominance x dominance effects which can not be obtained in diallel cross studies. In addition, previous screening studies showed a wide reaction range on NLB disease resistance among parents which satisfied one of the requirements of GMA studies applications.

1.2.11 Germplasm characterization

A maize breeding programme depends heavily on the knowledge of breeding materials genetic relationships of the interested traits. This assists in identification of contrasting and traits and avoids work duplication. Genetic relationships studies are achieved by the application of genetic markers. These include morphological, biochemical and molecular characterization markers. In this study, morphological markers characterization was used because it is cheap and simple method used to determine the genetic relationships among species. In addition, several studies have been reported to utilize this method and find reliable and useful information in various crops. For example, Stoilova and Berova (2009) successfully evaluated 15 common beans and three accessions of cow peas in Bulgaria. Other scientists have employed morphological markers to study and identify morphological variability of 78 rice breeding materials in Benin republic (Moukoumbi et al., 2011). Morphological characterization have also employed to study 11 qualitative and 26 quantitative traits of 37 sorghum landraces collected mainly from Tanzania (Bucheyeki et al., 2008). In maize, several researchers have employed morphological markers to study genetic relationships among germplasm For example, Ruiz and Alvarez (2001) used morphological markers to study the genetic relationships of 100 landraces in Spain and Gabriel et al. (2009) used morphological markers to generate genetic relationships information among maize forage landraces in Brazil. However, morphological markers are highly influenced by environment which calls for area specific characterization (Cadee, 2000).

1.2.12 The role of heritability in NLB disease resistance

Heritability information is used by breeders in designing appropriate breeding strategies. According to Stanfield (1991) heritability value (<0.2) is classified as low, medium (0.2–0.5) and high (>0. 5). High narrow sense heritability is the indication of additive gene action involvement for controlling particular traits especially under weak dominance effects (Jawaharlal et al., 2011). In breeding for NLB disease resistance, many reports show medium to high heritability. For example Hughes and Hooker (1971) and (Chaudhar and Mani (2010) reports the heritability range of 35 to 85%. This range implies selection strategies like recurrent selection could be used to improve maize populations under those maize populations as suggested by Ceballos et al. (1991). However, heritability estimates can be influenced by parent materials and environment interactions. Thus, NLB heritability is more accurate and reliable when based on specific crosses and the target test environments where the new varieties will be deployed.

1.2.13 The role of heterosis in NLB disease resistance

Heterosis is an important trait used by breeders to evaluate the performance of offspring in relation to their parents. It estimates the enhanced performance of hybrids compared to their parents. Heterosis can be positive or negative. The interpretation of heterosis depends on the nature of trait under study. For example, a positive heterosis is preferred in yield studies because it shows inclination towards high yield (Duvick, 2011). On the other hand, a negative heterosis is preferred in disease resistance like NLB. A negative heterosis in disease resistance shows that, breeding materials leaned towards resistance direction while a positive heterosis would imply skewness towards susceptibility trend. Breeding strategies like recurrent selection accumulates gene frequencies among genotypes and are likely to fit for populations with high heterosis. However, there is limited information on NLB disease heterosis in maize found in Tanzania.

1.2.14 The role of maternal effects in NLB disease resistance

In plants maternal effects occurs due to cytoplasmic and nuclear gene interactions of female parents. If they are highly significant they could dictate which breeding materials to be used as female parent. Maternal effects are responsible for the distortion of gene effects estimation by inflating genetic variances. Traits mainly controlled additively are likely to be influenced by the presence of maternal effects and thus reduced selection response could occur. The majority of investigators report absence, low or non-significant contribution of maternal effects on the inheritance of NLB disease resistance (Sigulas et al., 1988; Welz and Geiger, 2000). Although many researchers have indicated non-significant maternal effects contribution to the inheritance of NLB disease in maize, further investigation in different genetic backgrounds may be justified. The reason could be that, maternal effects have been found to contribute significant effects on the inheritance of leaf blight (*Exserohilum turcicum)* in sorghum (Durga et al., 2008). Maize and sorghum are all cereals and the disease causative agent is the same.

1.2.15 Breeding, production and desired maize traits in Tanzania

In Tanzania, the national maize research programme has made efforts to improve the maize sector. By the late 1990s, about 15 maize varieties were released in the country (De Groote et al., 2002). However, to date from 2.0 million ha of Tanzania under maize cultivation only 25.8% is covered by open pollinated varieties, hybrids and their recycled derivatives (De Groote et al., 2002). The remaining 74.2% is still covered by maize landraces. Farmers are still using recycled seeds from 5-10 years old (Doss et al., 2003). The reported reasons for low adoption rates include undesired agronomic characteristics of introduced cultivars, biotic, abiotic susceptibility and failure to meet social acceptance in the respective areas (Adda et al., 2002; Salasya et al., 2007).   Consequently; the rate of adoption of new varieties is low (McGuire, 2008; Wubeneh and Sanders, 2006). On the other hand landraces perform well under sub optimal conditions as they are well adapted to local stresses and possess farmers' preferable traits (Bantilan et al., 2004; Setimela et al., 2004). It is therefore necessary to study the genetic relationships of these landraces and identify traits to be incorporated in maize breeding programme.

Furthermore, there is a significant yield difference between on-station and on-farm conditions (Matuschke et al., 2007). Varieties performing well on-station can yield as low as 50% under farmers conditions and management (Barron and Okwach, 2005). This could be attributed to differences in selection criteria and objectives among breeders and farmers. There is a need of combining farmers and breeders objectives to have sustainable breeding programmes. These combinations have been reported elsewhere where they brought significant contribution to agricultural developments (Ceccarelli and Grando, 2007; Huan et al., 2005; Manu-Aduening et al., 2006). Stoop and Hart (2005) elaborates on bridging gap between farmers and researchers that resulted in timely planting, soil management, intercropping and variety selection technologies developments and dissemination to sub-Saharan Africa farmers. Involvement of farmers in the breeding process is expected to increase adoption rates through selection of superior cultivars suited to farmers conditions and environments (Abang et al., 2007). Breeding approaches that involve farmers have been shown to increase farmer participation which subsequently leads to high rates of adoption of varieties (Nabirye et al., 2003). Participatory breeding ensures the incorporation of farmers' preferences. This has an added advantage of testing varieties under farmers' environmental conditions. Crop breeding that targets client preferences and their environments have shown successful results elsewhere (Gyawali et al., 2007; Joshi et al., 2007). This approach was adopted in the study.

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Chapter 2

2 Maize production constraints, NLB disease status, stockists and farmers' opinions on varieties selection preferences in western Tanzania

Abstract

Maize yield in western Tanzania is only about 1.69 t ha^{-1} which results in frequent hunger and food insecurity. Abiotic and biotic factors like fungal diseases are common in farmers' fields. High yielding hybrids are not adopted. Therefore the objectives of the study were to investigate farmers' and stockists preferred traits for maize selection, determine maize production constraints faced by farmers and assess NLB disease prevalence in western Tanzania. A participatory rural appraisal (PRA) was conducted in three districts. Both informal and formal PRA analysis tools were used to gather information. A focus group of 30 farmers was selected in each of Udongo, Kasungu and Isanzu villages. Transect walks and wealth ranking were used in the informal survey. In total, 150 questionnaires were used in the formal survey. The recorded yield was only 1.1 t ha^{-1}. The study revealed 13 major maize production constraints, 13 insect pests and vermins and, 11 diseases. The NLB disease was found to be a potential constraint in farmers' fields. Farmers' preferred traits included resistance to abiotic and biotic stresses, early maturity, preferred milling qualities, high storage qualities and high yielding potentials. The undesired maize characteristics included late maturity, low yield, pink endosperm, low storage qualities and low poundability. Stockists mentioned 12 preferred maize variety traits which included high yielding, disease and insect pest resistance, heavy grain, large cob size and large grain sizes. Six out of 10 undesired stockists maize variety preferences included low milling qualities, low germination percentage, low yield, light grains, broken seeds and dent types. There were only 12 stockist in the area and no stockists were found who were selling landrace seeds. Similarity between farmers and stockist variety preference rankings were found to exist and would be incorporated in the selection criteria in the maize breeding programme in Tanzania. Results have established that, the surveyed area has many agricultural and social constraints like variety preferences that hinder maize production.

2.1 Introduction

Maize plays a vital role to the livelihoods of human beings. It is the chief source of carbohydrates and protein for animal and human diets (Enes et al., 2006). The dual purpose nature of the crop as food and cash crop has led to its increased utilization and diverse cultivation in the world (Ramadhani et al., 2002). Low maize yield per unit area, low return on capital, farm inputs and land scarcity are common to farmers in sub-Saharan Africa (Marenya and Barrett, 2007; Matuschke et al., 2007; Sumberg, 2005). For example, in Tanzania the average yield is 1.69 t ha^{-1} (FAO, 2008).

Low maize yield in Tanzania has been associated with the use of varieties with low production potentials. Other major factors that cause low maize yields are abiotic and biotic constraints. These factors include low soil fertility, unavailability and untimely input supply, drought, insect pest attacks, and disease incidences (Lisuma et al., 2006; Ondersteijn et al., 2003). Among the diseases, northern leaf blight is one of the potential foliar disease affecting crops at varying proportion (Karnataka, 2007). The disease can be aggravated by growing of susceptible cultivars and resistance breakdown of cultivars (Brown, 2002; Pataky et al., 1998). Susceptible cultivars are responsible for the increased amount of inoculums which signifies breeding for NLB disease maize varieties (Love, 1994).

However, conventional breeding procedures have been cited to be more formal, concentrating on researchers' objectives of problem solving leaving behind farmers' preferences and opinions (Assefa et al., 2005; Khan et al., 2008). The consequences of this approach is meagre benefit to the rural people, low rate of adoption and abandonment of varieties (Adda et al., 2002; Jochinke et al., 2007; Salasya et al., 2007; Sumberg, 2005). To increase technology development and transfer, farmers and breeders linkages were considered to enhance farmers active participation in breeding process (Ceccarelli and Grando, 2007). This has lead to the emergence of participatory plant breeding approaches. Participatory plant breeding incorporates farmers preference and shortens breeding time (Abebe et al., 2005). Participatory plant breeding is one of the bottom up approaches used by researchers and breeders to develop cultivars which are easily adopted by farmers. In this approach, both farmers and breeders fully participate in sourcing for germplasm, traits selection, cultivar development and varieties evaluation (Morris and Bellon, 2004). There are reports of the use of farmers in participatory plant breeding for superior cassava breeding in Ghana (Manu-Aduening et al., 2006) and introduction of rice technologies in China (Huan et al., 2005). Reports on participatory plant breeding in maize are well documented in some areas (Abebe et al., 2005; Witcombe et al., 2003).

Factors affecting maize production, NLB disease status and favourable maize traits in the western zone are not well documented and are fragmented. Maize breeders are facing difficulty in instituting a well sound participatory plant breeding. In addition, current studies considered farmers only and left behind stockists. But farmers purchase seeds from stockists to mean that, stockists who first select seeds according to their perceptions. Therefore, stockists are key beneficiaries of plant breeding. This study employed PRA tools to study factors facing maize production, farmers' and stockists opinions on variety selection and current NLB disease rank in western Tanzania. The main objective was to establish maize production constraints, farmers and stockists criteria for maize varieties selection and importance of NLB disease.

2.2 Materials and methods

The study was conducted in two phases which are informal and formal surveys.

2.2.1 Site and farmers selection

This study was conducted in three districts namely: Sikonge, Nzega and Urambo in Tabora region, Western Tanzania. Tabora region occupies the area between 4^0 and 7^0S and between 31^0 and 34^0E with an average altitude of 1300 masl, and mean annual temperature of 23.8^0C (Nyadzi et al., 2003a; Nyadzi et al., 2003b). Tabora region has a mono-modal type of rainfall with an annual average of 928 mm which falls between November and May. According to FAO, the majority of Tabora soils are classified as Ferric Acrisol. This region is important for maize production in Tanzania hence improvement of maize productivity has serious impact on household and national food security. To obtain an overview of the NLB problem, one representative village per district was selected for a participatory rural appraisal (PRA) in the three districts. In each village, a pre-visit was paid to discuss with village leaders and setting dates for PRA exercise

2.2.2 Informal survey study

A focus group of 30 farmers was selected in each village. A focus group comprised of key informants, elders, livestock keepers, small scale farmers, progressive farmers, stockist, village leaders, religious leaders and small industry processors. Qualitative data was collected as suggested by AFN (2002). Participatory rural appraisal (PRA) analytical tools such as focus group discussions (FGD) transect walks and matrix ranking were used to solicit farmers' perceptions on maize production, and the impact of NLB resurgence on maize in western Tanzania. The following data were collected from the farmers: Crops grown and their cropping systems, maize production constraints, varieties grown, sources of varieties grown, characteristics of grown maize varieties, preferred maize traits, preferred varieties, levels of NLB disease resistance to the grown varieties, common pests, common diseases, trends and occurrence of NLB disease, factors leading to the resurgence of NLB disease and efforts to curb the disease.

The transect walks were used to assess community resources through direct observations and recording of important agricultural features, constraints and opportunities available in the community. Farmers coping strategies for solving prevailing problems in their societies were also examined. A transect walk is a strong tool used by researchers to assess communities and associated production opportunities and constraints that hinder agricultural developments (Gyawali et al., 2007; Nabirye et al., 2003; Ngugi et al., 2002).

Wealth ranking was another PRA analytical tool used in this study. A sample of households in the village was randomly selected. Each household was assigned a card. Respondents were asked to itemize wealth indicators. Wealth indicators included houses building materials, number of livestock kept, children's education levels, job or type of work description, food availability during the year, land ownerships and possession of milling machines.

Pairwise and matrix ranking tools were used to compare parameters so as to provide inferences on their importance. In pairwise ranking two parameters were considered at a time keeping other parameters constant. While in matrix ranking, more than two parameters were considered at a time. Scores were assigned to each parameter depending on farmers preferences. Parameters with the highest scores were considered as the first priority. Scientists have used these analytical tools to provide reliable information for planning and implementation of agricultural technologies development in various societies in the world (Harris et al., 2001; Sall et al., 2000).

During the informal survey, gender aspects were considered for getting perceptions across societies without one section affecting the other group. Men and women each formed separate discussion group to minimize dominance of men or women if included in the same group (Figures 2.1. and 2.2).

Figure 2.1: The researcher, discussing with elders (A) and ladies group discussion (B) during PRA at Udongo village in Sikonge district, Tanzania in 2008/2009 growing season

Figure 2.2: Cross section of men group discussion during PRA at Udongo village in Sikonge district (C) and at Kasungu village (D) in Urambo district, Tanzania in 2008/2009 growing season

2.2.3 Formal survey

A checklist was developed at the beginning of the study to examine the applicability of questionnaires in the planned area. To dissect issues raised during the informal study, a formal survey was conducted. This was conducted in the places where the informal study was done. For each of the three villages, 50 structured questionnaires were distributed to collect quantitative data. In total, 150 questionnaires were used. The following data were collected from farmers: Household characteristics, crops grown and maize varieties grown. Others included:

production constraints, farmers sources of agricultural information, seed type, cultivar types (improved or not), crops utilization and maize utilization. Also the following data were recorded: maize grain yield, seed sources, extent of farmers participation on variety development (stages), factors affecting the rate of adoption of new varieties, abandoned varieties and reasons of variety abandonment. In addition, farmers preferences on maize varieties, farmers awareness on the NLB disease, importance of NLB disease on maize production, yield losses due to NLB disease infestation, varieties resistant to NLB, and control methods currently used were also recorded. In addition to farmers, structured questionnaires were developed and instituted to 12 stockists in the area. Data from PRA and stockists was subjected to non- parametric and parametric analysis in SPSS (2006) computer programme.

2.3 Results

2.3.1 Demographic factors

Table 2.1 depicts sex, marital status, and level of education and age distribution of the respondents. Most of the farmers were married who had attended primary and a few attained secondary school educations, and very few (less than 9%) without formal education. On age structure, elders (36-55) and children (< 18) years accounted for 82.69% of the total population of the surveyed villages.

Table 2.1: Demographic characteristics (%) of farmers in Udongo, Kasungu and Isanzu villages, in Tanzania 2008/2009 season

	Villages Udongo	Kasungu	Isanzu
Sex			
Male	73.33	63.33	51.67
Female	26.67	36.67	48.33
Marital status			
Married	95.00	83.33	71.67
Not married	3.33	11.67	11.67
Widow	1.67	5.00	16.67
Education			
Non educated	5.00	8.33	3.33
Adult learning	5.00	13.33	38.33
Primary education	76.67	61.67	50.00
Secondary education	10.00	6.67	5.00
College	3.33	10.00	3.33
Age distribution			
<18	29.72	27.91	32.30
18-35	20.93	23.77	12.92
36-55	43.93	42.89	50.39
>55	5.43	5.43	4.39
Farming experience (over 15 years)	80.00	85.00	68.33

2.3.2 Sources of income

The major source of income was from crop production which accounted for more than 60% in all surveyed villages (Table 2.2). These villages were representative of villages in the western zone of Tanzania because they reflected farming dependency for their livelihood earning. Other income generating activities included livestock keeping, bee keeping, lumberings, small shops, and casual labour and fishing. The majority of these were off-season activities performed by farmers to supplement income after the main rainy season activities.

Table 2.2: Farmers sources of income (%) in Udongo, Kasungu and Isanzu villages, in Tanzania 2008/2009

Activity	Villages Udongo	Kasungu	Isanzu
Crop growing	64	67	60
Livestock keeping	8	6	14
Bee keeping	13	8	-
Lumbering	9	11	-
Small shops	4	6	18
Casual labour	1	2	8
Fishing	1	-	-

2.3.3 Income expenditure

Farmers spent more than 60% of the generated income to purchase food (Figure 2.3) and uses (<10%) of money to purchase farm inputs. This denotes the typical subsistence type of agriculture and mostly found in the majority of farmers in the sub-Saharan Africa.

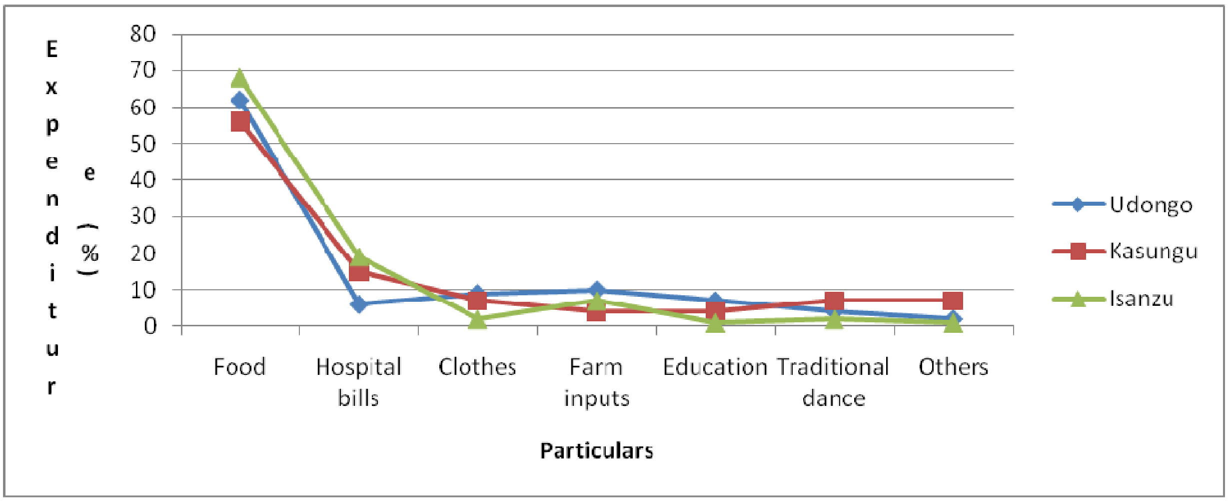

Figure 2.3: Farmers income expenditure (%) at Udongo, Kasungu and Isanzu villages, in Tanzania for 2008/2009 growing season

2.3.4 Wealth ranking

About 30% of farmers were classified as absolutely poor farmers. These were characterized by houses built of mud bricks, thatched with grass, did not or kept few goat and sheep (0-3), small fields (0.25 – 1 ha), little education to their children (normally standard seven or without formal education), were frequently used as cheap labourers and frequently experienced 6 months food shortage per year. The largest group (55%) was made up of medium income farmers. This category included farmers with houses built of burnt bricks with corrugated iron sheets, kept cattle and goat (about 6-12) animals, fields ranged from 1 – 2 ha and children with or attending secondary schools education. The lowest group (15%) was high income farmers. This group comprised of farmers with milling machines, large houses with roofed corrugated iron sheets houses, owned livestock (cattle, goats or sheep 12-20 herds) and had food availability for the whole year. However, farmers' income categories were not permanent as movements from one to another category could happen in the society.

Table 2.3 shows the perceived reasons of downward or upward wealth group changing in the community. Farmers mentioned wellbeing, high investment, monogamy, family support, favourable weather condition and high innovation ability planning as factors that led to upward movement from the poor to the rich group in the society. Farmers itemized human diseases, less investments, polygamy, extended families, unforeseen calamities and low innovation ability as prime reasons of reversed movement from rich to poor livelihood condition in villages.

Table 2.3: Reasons for wealth group change at Udongo, Kasungu and Isanzu villages, in Tanzania 2010

Upward movement	Downward movement
Wellbeing	Human disease
High investments	Less investments
Monogamy	Polygamy
Family support	Extended family
Favourable weather condition	Unforeseen events such as theft
High innovation ability	Low innovation ability

2.3.5 Maize types and varieties grown

Types of maize varieties found in three studied districts were hybrids, open pollinated (OPVs) and landraces (Figure 2.4). In all three districts, landraces accounted for more than 65% of the cultivated maize.

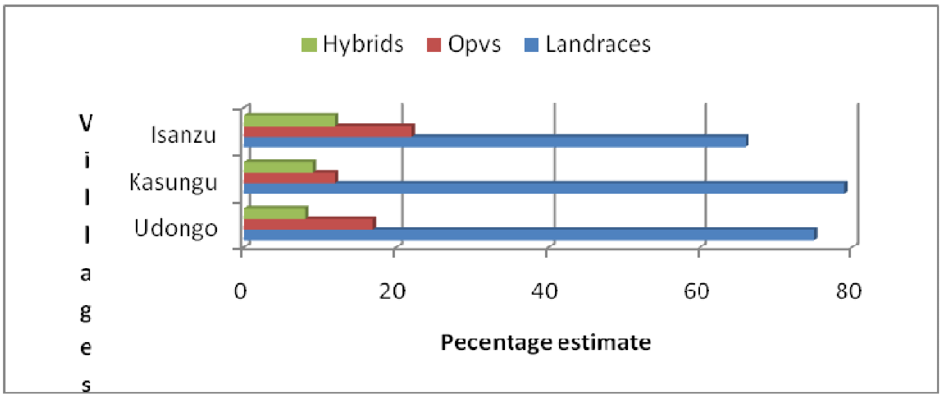

Figure 2.4: Maize types grown at Udongo, Kasungu and Isanzu villages, in Tanzania for 2008/2009 growing season

Table 2.4 summarizes farmers' perceptions on maize varieties, types and their associated desired and undesired characteristics. Favoured traits included resistance to abiotic and biotic stresses, early maturity, preferred milling qualities, high storage qualities and high yielding abilities. While undesired maize characteristics included late maturity, low yield, pink endosperm, low storage qualities and low poundability.

Table 2.4 Characteristics of common maize varieties grown in Urambo, Sikonge and Nzega districts for 2008/2009 growing season based on farmers perceptions.

Varieties	Group	Desired	Undesired
Gembe	Landrace	Drought resistant, diseases resistant and highly palatable	Late maturity and pink endosperm
Kabalagata	Landrace	White endosperm and early maturing	Low yield
Kakonkote	Landrace	White endosperm and early maturity	Low yield and diseases susceptible
Kito-ST	OPV	Early maturity	Low yield
TAN 254	OPV	White grain	Medium yield
Situka-M1	OPV	white flint and dent	Medium yield
Kilima ST-SR	OPV	white, flint, steak tolerant	Medium yield
TMV-1	OPV	white, flint, streak tolerant	Medium yield
UCA	OPV	High yield	Late maturity
Staha	OPV	High yield	Late maturity
Lishe-1	OPV	Quality protein maize	low storage qualities
Uyole hybrid 6303	Hybrid	Resistance to gray leaf spot	Late maturity and low poundability
Uyole Hybrid 615	Hybrid	Resistance to gray leaf spot	Late maturity and low poundability
PAN 6195	Hybrid	High yield and tolerance to maize streak viral disease	Late maturity and low storage qualities
SC 627	Hybrid	Early maturing and resistance to gray leaf spot	low storage qualities

The average yield of maize was found to be 1.125 t ha^{-1} which is extremely low for food security, income generation and paying other incentives to farmers in all surveyed villages. The farm size ranged from 0.5 – 3.0 ha per farmer. Farmers applied 57.5 KgNha^{-1} and 15.5 KgDAP ha^{-1} against the recommended 100 KgNha^{-1} and 40 KgDAP ha^{-1}.

2.3.6 Source of seeds

There were six sources of seeds sown in the surveyed villages. More than 50% were found to be recycled seeds. The rest of the sources which included purchasing from stockists, local market; distribution by agricultural extension department and research centres contributed the remaining half (Figure 2.5).

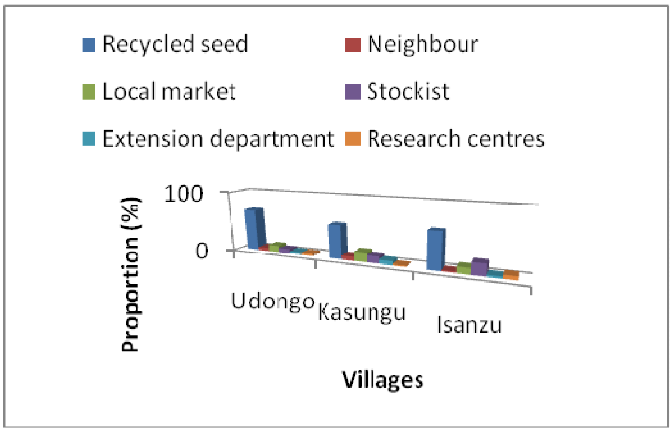

Figure 2.5: Sources of seed grown at Udongo, Kasungu and Isanzu villages, in Tanzania for 2008/2009 growing season

2.3.7 Ranked maize production constraints

In total 13 maize constraints were found to prevail which hindered maize production in the studied districts. Five top ranked problems in all three districts included Low-N, diseases incidences, lack of farm inputs, lack of improved varieties and drought prevalence (Table 2.5). Transect walks across sites confirmed these problems (Figure 2.6). Others included insect pest infestation, poor extension services, lack of implements, poor agricultural policy, weed and vermin attacks, unreliable storage facilities and variety preferences based on gender. These factors are believed to lower production, reduce crop quantity and quality.

Figure 2.6: Severe N-deficiency (A) and combined effect of stalk borer and NLB disease effects (B) at Udongo village in Sikonge district, *Striga asiatica* at Kasungu village in Urambo district (C) and maize streak virus (D) at Isanzu village in Nzega district in 2008/2009 growing season

Table 2.5: Farmers perceived common maize production constraints ranking in Udongo, Kasungu and Isanzu villages for 2008/2009 growing season

Common maize production constraints	Villages		
	Udongo	Kasungu	Isanzu
Low-N	1	1	4
Diseases incidences	3	2	5
Insect pest infestation	13	7	4
Lack of farm inputs	5	4	3
Insufficient extension services	6	10	6
Lack of improved varieties	4	3	2
Drought prevalence	2	5	1
Lack of implements	7	9	10
Insufficient agricultural policy support	11	8	-
Weeds infestation	12	-	8
Wild animal field invasion	8	6	7
Unreliable storage facilities	9	-	-
Variety preferences based on gender	10	-	11

2.3.8 Ranked common insect pests and vermin of maize

Thirteen common insect pests and vermin of maize were found to exist in the three studied districts. Stalk borer (*Busseola* spp), army worms (*Spodoptera* spp), large grain borer (*Prostephanus* spp), *Striga asiatica* and *Striga hermonthica* occupied the ranks between first and fourth as the most devastating biotic stresses of maize production in the zone (Table 2.6). Other common insect pests and vermin and their ranking order across the villages are shown on Table 2.6.

Table 2.6: Common insect pests ranking of maize in Sikonge, Urambo and Isanzu villages for 2008/2009 growing season based on farmer assessments

Common insect pests and vermin	Villages		
	Udongo	Kasungu	Isanzu
Stalk borer (*Busseola* spp)	1	2	3
Army worms (*Spodoptera* spp)	5	3	2
Large grain borer (*Prostephanus* spp)	2	1	1
Grain weevils (*Sitophilus* spp)	6	7	6
Cut worms (*Agrotis spp*)	4	5	8
White grubs (*Phyllophaga spp)*	12	11	9
Confused flour beetle (*Tribolium confusum*)	7	6	12
Angoumois grain moth (*Sitotroga cerearella)*	8	9	7
Rodent (*rattus* spp)	10	8	13
Wild pigs (*Sus scrofa*)	11	12	10
Patridge (*Alectoris spp*)	13	13	4
Termites (Coptotermes spp)	9	10	11
Striga asiatica and *Striga hermonthica*	3	4	5

2.3.9 Ranked common maize diseases

Maize streak virus (*Gemini* spp), Northern leaf blight (*Helminthosporium turcicum),* Fusarium stalk rot (*Fusarium moniliforme),* Fusarium ear rot *(Fusarium moniliforme)* and Leaf rust (*Puccinia maydis*) were ranked in the top five among 11 mentioned diseases affecting maize production in three studied districts (Table 2.7). The emergence of NLB disease in the top five most commonly found disease in the zone prompts attention as this was not common in the past decades.

Table 2.7: Common diseases ranking of maize in Udongo, Kasungu and Isanzu villages for 2008/2009 growing season based on farmer assessments

Common diseases	Villages		
	Udongo	Kasungu	Isanzu
Phaeosphaeria leaf spot (*Phaeosphaeria maydis)*	6	10	7
Gibberella stalk rot (*Gibberella zeae)*	8	6	8
Tassel smut (Sphacelotheca reiliana)	9	8	6
Fusarium ear rot *(Fusarium moniliforme)*	2	1	2
Fusarium stalk rot (*Fusarium moniliforme)*	5	3	4
Leaf rust (*Puccinia maydis*)	4	5	3
Brown spot (*Physoderma maydis*)	7	9	9
Northern leaf blight (*Helminthosporium turcicum)*	3	4	5
Maize streak virus (*Gemini* spp)	1	2	1
False head smut (*Ustilaginoidea virens*)	11	7	11
Gray leaf spot (*Cercospora zeae-maydis)*	10	11	10

2.3.10 Perceived reasons of NLB disease resurgence

Table 2.8 shows the perceived factors that lead to NLB disease increase in severity, incidences and resurgence in the western zone of Tanzania. The top ranked factors were high rainfall, land scarcity, prevalence of seed companies that sold susceptible varieties and inadequate agricultural extension service support.

Table 2.8: The perceived ranked reasons for NLB disease increase in Udongo, Kasungu and Isanzu villages based on farmer responses.

Reasons for NLB disease increase	Villages		
	Udongo	Kasungu	Isanzu
Gardening	-	8	6
Land scarcity	4	2	5
Maize trading	-	6	7
Inadequate agricultural extension service support	3	-	4
Stockists	6	7	10
High rainfall	1	1	3
Cattle shifting out	-	-	2
Neighbours	7	5	8
Seed companies	2	-	1
Famine	5	3	9
Tobacco production	8	4	-
Agricultural input voucher system	-	9	-

2.3.11 Efforts to curb the NLB disease

In all three districts, farmers tried to contain the disease by burning crop residues during land preparation. In Urambo and Sikonge districts they practised crop rotation by planting tobacco while farmers in Nzega district applied natural fallows to breakdown breeding cycle of the disease. All these are cultural practices that ensure crop sanitation in fields. There were no known biological controls like the use of resistant varieties. Similarly, farmers did not mention the use of chemicals like fungicides for controlling NLB disease in their areas.

2.3.12 Agricultural stockists attitudes and perception on maize varieties

There were only 12 stockists in the area. Numbers of stockists per district were 4, 3 and 5 for Sikonge, Urambo and Nzega districts respectively. The ranges of experience of stockists in the business were 5-30 years which reflects wide experience in agricultural input trading. All stockists sold OPVs and hybrid maize and there were no stockists who were involved in selling local cultivars. The sold OPVs were Kilima-ST, TMV-1, Katumani, Situka and Lishe from Tanzania maize breeding program. While hybrids included Seed-co 403, Seed-co 513, CG 4142, DK 8031, DK 04 and H 513 from private seed companies like Seed-co Zimbabwe, Cargil , Dekalb and Kenya seed company.

2.3.13 Maize seeds marketing problems

Stockists mentioned five major problems affecting maize seeds selling in their respective places. The outlined problems were unavailability of packaging materials, price setting by government agents, competition with recycled seeds, unstructured market channels and undesired maize varieties traits (Table 2.9). There were differential rankings of problems probably due to location and farmers seed demand. At Udongo village, unavailability of packaging materials was ranked first, while undesired maize traits and competition from recycled seeds were ranked first at Kasungu and Isanzu villages.

Table 2.9: Ranked maize marketing problems in Udongo, Kasungu and Isanzu villages in 2008/2009 growing season

Maize selling problems	Villages		
	Udongo	Kasungu	Isanzu
Unavailability of packaging materials	1	3	2
Price setting	3	2	4
Recycled seed competition	4	5	1
Unstructured market channels	5	4	3
Undesired traits	2	1	5

2.3.14 Preferred and undesired stockists maize varieties characteristics

Table 2.10 shows12 preferred maize varieties traits from stockists across the three studied districts. The top ranked five included high yielding, disease and insect pest resistance, heavy grain, high yield, large cob size and large grain sizes. Maize seeds with preferred traits were said to fetch high prices to make maize production more profitable to farmers, stockists and other stakeholders dealing in the maize industry.

Table 2.10: Ranked stockist preferred maize varieties characteristics ranking in Udongo, Kasungu and Isanzu villages in 2008/2009 growing season

Preferences	Villages		
	Udongo	Kasungu	Isanzu
High yielding	1	2	1
Disease and insect pest resistance	2	1	2
Heavy grain	3	4	1
Drought tolerance	6	5	2
Response to fertilizer uses	7	8	1
Early maturity	8	9	6
Large grain size	1	3	2
Twin cobs characteristics	-	10	7
Flour processing	4	7	3
Low-N requirements	5	6	4
High price	4	3	4
Large cob size	1	2	5

On the other hand, 10 undesired maize variety characteristics were revealed by stockists in the studied districts (Table 2.11). Six out of the 10 were ranked from 1-5 in all three villages. The highly ranked undesired maize variety characteristics were undesired milling qualities, low germination percentage, low yield, light grains, broken seeds and dent type. Any variety possessing the above mentioned characteristic was likely to fetch low price and subsequent losses in maize production.

Table 2.11: Ranked stockist undesired maize varieties characteristics in Udongo, Kasungu and Isanzu villages in 2008/2009 growing season

Undesired characteristics	Villages Udongo	Kasungu	Isanzu
	Rank	Rank	Rank
undesired milling qualities	3	4	2
Low germination percentage	2	2	1
Low yield	4	1	2
Small grains	5	6	3
Light grains	1	3	1
Coloured grains	6	5	7
Broken seeds	1	4	5
Mixed seeds	7	8	6
Dent type	2	1	4
Weed infested seeds	8	7	8

There were no stockists who were selling landraces in the area. Reasons were mainly due to low demand, low yield, lack of quality information and late maturity characteristics (Table 2.12).

Table 2.12: Ranked reasons for not selling landraces in Udongo, Kasungu and Isanzu villages in 2008/2009 growing season

Reasons for not landraces	Villages Udongo	Kasungu	Isanzu
Low demand	1	2	3
Low yield	2	3	5
Lack of quality information	5	1	4
Low germination percentage	3	5	7
Not resistant to drought	6	8	1
Late maturity	4	4	2
No established market channel	7	7	6

Maize seeds from National Research Centres showed high demand. Ten reasons for high demand are shown on Table 2.13. Stockists narrated that, they were high yielding, had white endosperm, desired husk cover and easily available. But they were ranked low on milling qualities, drought and disease resistance.

Table 2.13: Ranked reasons for selling seeds by stockist from research centres in Urambo, Sikonge and Nzega districts in 2008/2009 growing season

Selling reasons	Villages Udongo	Kasungu	Isanzu
High yield	3	3	2
White endosperm	1	2	4
Low prices	6	-	1
High demand	5	1	-
Drought resistant	7	8	6
Desired husk cover	4	4	5
Disease resistance	8	9	-
Easily available	2	5	3
High milling qualities	10	7	5
Early maturity	9	6	7

2.3.15 Comparison of farmers and stockist maize traits preferences

Table 2.14 depicts combined comparison of farmers and stockist maize traits preferences in the studied three districts. Farmers put more emphasis on food security and processing qualities. On the other hand, stockists emphasized on traits linked to high prices and profit maximization. Farmers and stockist both ranked high milling qualities and high yield traits. While farmers further emphasized on drought resistance, white endosperm and flint texture, stockists on the other hand differed with them by further high ranking heavy grains, large grain size, high fertilizer use response, large cob size, high prices, disease and insect pest resistance.

Table 2.14: Comparison of maize farmers and stockist traits preferences in Kasungu, Isanzu and Udongo villages for 2008/2009 growing season

Farmers		Stockists	
	Ranks		Ranks
High yielding	2	High yielding	1
High milling qualities	1	Disease and insect pest resistance	2
Drought resistant	3	Heavy grain	3
Diseases resistant	6	Drought tolerance	7
Large grain size	7	Response to low-N requirements	5
White grain	4	Early maturity	8
Early maturity	8	Large grain size	2
Flint texture	5	Twin cobs characteristics	6
High roasting qualities	9	High flour processing quality	5
		Low-N requirements	5
		Large cob size	3
		High price	4

2.3.16 Breeding opportunities and challenges

Figure 2.7 (A and B) shows local maize varieties with contrasting reactions to NLB disease reactions. On the other hand, Figure 2.8 (A) shows farmers coping strategies to common maize constraints like decline of soil fertility and disease control. While figure 2.8 (B) shows coping strategies to diverse varying environment and crop failure risks through complex crop intercropping.

Figure 2.7: Gembe maize local variety resistant to NLB disease at Kasungu village in Urambo district (A) and Maize variety susceptible to NLB disease at Isanzu village in Nzega district (B), Tanzania in 2008/2009 growing season

Figure 2.8: Farmers innovation of soil fertility decline and diseases control by intercropping maize with *Gliricidia sepium* at Udongo village in Sikonge district, and complex intercropping involving five crops (B) at Kasungu village in Urambo district, Tanzania in 2008/2009 growing season

2.4 Discussion

The majority of respondents were male and had a long farming experience of more than 15 years to display wealth in maize cultivation knowledge accumulation and mostly were in the productive age (36-55) or potential workers and farmers (< 18 years) in terms of labour force. The major source of income was from crop growing that accounted for more than 60%. However the average yield of maize was found to be 1.12 t ha^{-1} which is extremely low to sustain farmers for 12 months per season. The reported yield was similar to the national average yield of 1.2 (Makurira et al., 2007). Therefore there is need for intervention strategies to enhance poundability.

Most of the farmers (65%) still grew maize landraces in their fields. Reasons could be unavailability of improved varieties, community preferred traits provided by landraces and lack of information of improved cultivars. There were only six sources of seeds sown in the surveyed villages which probably contributed to 50% recycled seeds in the area. Further investigation on the levels of involvement of farmers in variety development revealed that, only a few farmers were involved in variety development through varieties demonstration plots. In addition the low percentage of OPVs and hybrids use could be probably caused by lack of focused maize breeding programme which forced farmers to recycle available landraces in their respective communities. Farmers also mentioned that, OPVs were sold at reasonably affordable prices, were palatable and stored better. These results are in accordance with other investigators who recorded the continuity of growing landraces by farmers despite their low yield potentials (Bucheyeki et al., 2009; Doss et al., 2003; Soleri et al., 2000). But in this study area, maize varieties with high yield potential like hybrids and OPVs share the remaining 35% of maize types grown in the area. According to Bänziger et al. (2006) and Mgonja et al., (2006), low hybrid utilization level could be caused by farmers preferences, abiotic and biotic stress susceptibly. The study therefore indicates that farmers preferred traits must be established to set the research agenda in order to develop varieties that are acceptable.

Farmers mentioned 13 major maize production constraints, 13 insect pests and vermins and 11common diseases that hinder maize productivity in the studied districts. Although not ranked high, disease prevalence was one of the major factors hindering maize production. Farmers normally rank low yield as the major problem and not the causes of low yields. Findings further showed that, NLB disease was found in all farmers' fields including the marginal districts like Nzega in Tabora region. The NLB disease has gained importance recently as it was not ranked high for the past decades. The significant increased importance of NLB disease was probably facilitated by heavy El-nino rainfall that occurred in 1997/1998 which increased relative humidity in that season. Researchers associate relative humidity as one of favourable condition for NLB disease development and the subsequent increased inoculums in maize fields (Levy and Cohen, 1983).

Farmers mentioned favourable conditions for NLB disease development which included poor crop rotation, high rainfall and infiltration of susceptible cultivars through stockists, agro-dealers, and food aid in the case of famine outbreak. It was also found that, some efforts were done by farmers to manage the disease by using indigenous technology knowledge (ITK). The common method was burning crop residues during land preparation and crop rotation particularly in Urambo and Sikonge districts which is dominated by tobacco-cereals farming system (Ramadhani et al., 2002). The use of resistant maize varieties was not practiced in all districts. The reasons could be the new emergence of the disease and breeders concentrating on other traits and putting aside the NLB disease. There is an indication that, NLB disease resistant varieties were not available to farmers in the area.

Farmers preferred traits included resistance to abiotic and biotic stresses, early maturity, preferred milling qualities, high storage qualities and generally high yielding abilities. While undesired maize characteristics included late maturity, low yield, pink endosperm, low storage qualities and low poundability. Breeding strategies that aim at improving these traits could lead to increased rate of adoption, yield and contribution to food security in these districts.

The study also revealed that, there were only 12 stockists in the area. The number of stockists was very low to provide adequate goods and services to farmers. However, all stockists sold OPVs and hybrids with no one selling landraces. The perceived reasons were low demand, low yield, lack of quality information, low germination percentage, not resistant to drought, late maturity and absence of established market channel for landraces. Investigation showed that landraces were kept by farmers and hence the low demand from stockists. The number of seed selling stocks should be increased in the area and these can be exploited as potential channel for deploying disease resistant seed.

Drought resistance was ranked high in Nzega district by stockists probably because, the Nzega area experiences frequent drought and is located in marginal areas that demand drought resistant and early maturing maize varieties. Although there were few maize seeds from National Research Centres sold by stockists, they showed high demand. Stockists mentioned that, they were high yielding, quality, have white endosperm, desired husk cover and easily available. However, they lacked desired milling qualities, drought and disease resistance characteristics. Maize breeding programme is required to improve these traits for increased demand and adoption rate.

Stockists constantly insisted on 12 traits for the ‘ideal’ variety for the area which included high yielding, disease and insect pest resistance, heavy grain, high price, large cob size and large grain sizes. These traits are the major factors of increased demand, price and total revenue to be collected. Other researchers revealed similar traits preferences by communities in common crops (Bucheyeki et al., 2008; Mwale et al., 2009). In contrast, 10 undesired maize variety characteristics were revealed by stockists. The highly ranked undesired maize variety characteristics were associated with food quality, field characteristics and market suitability like broken seeds. Any variety possessing the above mentioned characteristic was likely to fetch low price and subsequent losses to stockists.

The combined comparison of maize farmers and stockist traits preferences in the studied three districts raised some important issues. While farmers aims at household food security and processing qualities, stockists put more emphasize on profit maximization by selling maize varieties with high milling and processing qualities and high yield. This study further revealed the similarity of farmers and stockists objectives in some occasions. Farmers may emphasize on drought resistance, white endosperm and flint texture while stockist may prefer heavy grains, large grain size, high fertilizer use response, large cob size, high prices, disease and insect pest resistance. These traits are directly linked with household food security and processing qualities. Breeders do not generally concentrate on processing qualities during selection probably contributing to low adoption rate when released to farmers.

This study revealed some important opportunities and challenges to breeders in maize breeding programme. The presence of diverse landraces could be used as sources of breeding materials. Information gathered from farmers and stockists together with the availability of landraces creates a room of developing appropriate varieties to farmers. These include presence of landraces with contrasting resistance traits availability of committed stockists, prevalence of indigenous technical knowledge (ITK) like crop sanitation to control diseases and complex crop intercropping. These opportunities and challenges create a working condition for maize breeders in dealing with the disease and could lead to success and curbing the NLB disease in the area. To kick start this work, the gathered findings were used to select parents for 11 x 11 diallel cross reported in this thesis (see chapter 7). Thus, parents were selected based on i) farmers traits preferences ii) stockist traits preferences iii) NLB disease resistance, and iv) other favourable agronomic traits. These parents would be effectively used in the breeding programme to obtain appropriate new varieties.

2.5 Conclusions

The following conclusions were drawn from this study:

- Thirteen major maize production constraints were recorded. Five top ranked problems included Low-N, diseases incidences, lack of farm inputs, lack of improved varieties and drought prevalence. In addition 13 insect pests and vermins were identified. The highly ranked were Stalk borer (*Busseola* spp), army worms (*Spodoptera* spp), large grain borer (Prostephanus spp), Striga *asiatica* and *Striga hermonthica.* The study also recorded 11 diseases which included Maize streak virus (*Gemini* spp), Northern leaf blight (*Helminthosporium turcicum),* Fusarium stalk rot (*Fusarium moniliforme),* Fusarium ear rot *(Fusarium moniliforme)* and Leaf rust (*Puccinia maydis*). Therefore, a mechanism is needed to solve and minimize yield losses caused by these constraints.
- Farmers preferred traits included resistance to abiotic and biotic stresses, early maturity, preferred milling qualities, high storage qualities and high yielding potentials.

- Stockists identified 12 preferred maize variety traits for selection for the business. The top ranked five included high yielding, disease and insect pest resistance, heavy grain, high yield, large cob size and large grain sizes. Therefore, breeders would be encouraged to work with both farmers and stockists in developing the most appropriate varieties.
- The NLB disease was found to exist in the area as a potential maize production constraints.
- Similarity between farmers and stockist variety preference ranking were found to exist. Farmers put more emphasis on food security and processing qualities, while stockists emphasized on traits linked to high prices and profit maximization. Farmers and stockist both ranked high milling qualities and high yield traits.
- Results showed that, there are many production constraints and variety preferences which need immediate attention for increased maize production in the area.

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Chapter 3

3 Occurrence and distribution of northern leaf blight disease in western Tanzania

Abstract

The extent of the incidence and severity of NLB disease in the western zone of Tanzania has not been documented. Therefore, an objective of the study was to assess the incidence and severity of NLB disease in farmers' fields. The study was conducted in seven districts using farmers' fields as study sites in 2008/2009 and 2009/2010 seasons. During the study 175 fields with 5600 plants were sampled per season. Factors that accelerate NLB disease resurgence were investigated. Rainfall data of 51 years and 27 years for temperature and relative humidity were collected from ARI-Tumbi agro-meteolorogy station. The average rainfall was 912.90 mm per annum which is monomodal. The average relative humidity was 58.91% and the average temperature was 23.61^0C. There were sixteen varieties grown with wide NLB reaction variations. A landrace called Gembe landrace was among the three observed resistant varieties. The NLB disease which was previously concentrated in high relative humidity, cool places with extensive cloud cover has changed its distribution pattern affecting all districts of the western zone of Tanzania. The disease incidence in season two (2009/2010) significantly increased from season one (2008/2009) t= -3.25 (degree of freedom, 348), P= 0.001. Both means of blight incidence and severity approached 30%. Altitude and NLB disease severity were highly positively correlated (0.117**). Other highly significant positive correlated traits were NLB disease lesion length and number of infested leaves (0.179**), and with MSV incidence (0.546**). The study confirmed and documented the prevalence of NLB disease in Tanzania, there is need to emphasize for breeding for NLB disease resistance in maize breeding programme.

3.1 Introduction

Maize ***(**Zea mays* L.***)*** is one of the principal food crops in Africa. According to FAO (2008), maize is ranked third worldwide in importance after wheat and rice. It is the chief source of carbohydrate and protein (Bhatnagar et al., 2004). Abdulai and Aubert (2004) contend that, maize in combination with root crops and pulses supplies about 69% of the total dietary calories to the majority of people of Tanzania. In the country, the land under maize production is estimated at two million ha (FAO, 2008). However, maize yields are very low.

Low maize yield in Tanzania is caused by abiotic, biotic and socio-economic factors, (Banziger et al., 2006). Fungal, bacterial and viral diseases are common in the country. Among the fungal diseases, northern leaf blight (NLB) is the principal devastating leaf disease attacking crops at different growth stages to cause lower yield (CPC, 2001). Northern leaf blight is caused by *Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs). This disease attacks lower and upper leaves and reduces active foliar parts which results in reduced photosynthetic area. High relative humidity of around 75% associated with average temperature of around 20^0C favour the disease development (Levy and Cohen, 1983; Sharma and Mishra, 1988). Yet disease susceptible varieties are grown.

The high prevalence and severity of the NLB disease has been reported to cause a significant reduction in maize production. Yield losses with the ranges of 46 to 98% have been reported in some countries (Gowda et al., 1992; Kachapur and Hegde, 1988). The disease has been reported in all 21 regions that grow maize in the country including marginal areas where it was previously not found. For the past decades, northern leaf blight was one of the conquered diseases. However, the disease has gained importance in maize fields to threaten maize production (CIMMYT, 2002). New races resulting from sexual recombination, mutation, use of susceptible varieties that depend on fungicide applications could be possible causes of disease resurgence. Other possible causes include seed exchange among farmers, climate change, trade liberalization; intensive farming that does not allow crop rotation and resistance breakdown (Boland et al., 2004; Ogliari et al., 2005). These factors are believed to be responsible for the inoculum build up in fields and hence the resurgence of the disease.

The world has been witnessing the occurrence, spread and economic damage caused by the NLB disease. For example, in the USA, the first NLB disease report was in 1942 (Elliott and Jenkins, 1946). In East Africa, Adipala et al. (1993) reported the NLB disease affecting maize and millets in Uganda which occurred in 1988 which was aggravated by growing maize genotypes imported from Mexico, but were susceptible to NLB disease in East Africa.

Despite the fact that, northern leaf blight disease outbreak is threatening maize production in the western zone of Tanzania, there is inadequate documented information on the disease incidence and severity. Quantitative data on the occurrence, severity and disease importance is lacking. Some researchers just mentioned the presence of the disease (Kaliba et al., 1998a; Kaliba et al., 1998b; Nkonya et al., 1998). Therefore, this study was designed to assess and document the NLB disease occurrence and severity and was conducted as a prerequisite to breeding for NLB disease resistance in the area.

3.2 Materials and methods

3.2.1 Study area description

This study was conducted in Sikonge, Urambo, Nzega, Kasulu, Kibondo, Kigoma rural and Kigoma municipality districts of the western zone of Tanzania. The western zone occupies the central-western part of Tanzania.

Table 3.1 shows the description features of the studied districts. This zone has a wide range of agro-ecological zones ranging from lake shore Tanganyika to highlands to reflect different crops and farming systems.

Table 3.1: Studied districts agro-ecological, temperature, rainfall and altitudes descriptions

District	Agro-ecological zone*	Temperature (^{0}C)	Rainfall (mm)	Altitude (masl)
Sikonge	P5	23.10	Over 1000	1000-1500
Urambo	P5	23.20	700-1000	1050-1500
Nzega	P3	23.40	700-850	1000-1300
Kasulu	Highland zone	20-25	1300-1650	1750
Kibondo	Low land zone	20-25	850-1100	1200-1500
Kigoma rural	Low land zone	20-25	850-1100	1000-1200
Kigoma Municipality	Lake zone	25-30	650-1000	650-1000

* P3 = Medium altitude plains with mainly sandy soils while P5 = Medium altitude plains with mainly upland soils and clayey bottomland soils (Mowo et al., 1993; URPT, 1998).

3.2.2 Sampling and data collection

One hundred seventy five farms were randomly selected for the study. The study was conducted in two seasons (2008/2009 and 2009/2010). Efforts were made to use the same fields. Wherever there were alterations, a nearby field was sampled. Maize was examined at 8-9 leaf growth stage which corresponds to post milk dough stage of crop development. On each farm, disease incidence and severity were determined by sampling eight plants from north, south, east and west of the field starting at the centre. This resulted to a total of 32 plants per field. In total, 5600 plants were sampled per season. Lesion length and width were measured by using a ruler in mm. Number of lesions was physically counted per plot. Disease incidences were calculate as (infested plant/32) x 100.

A scale of 1 – 9 suggested by Ngugi et al. (2002) and Reid (2005) was used to rate disease severity which were converted to percentages. Assessments were done based on the percentage leaf area with their corresponding visible symptoms as follows:
1 = no visible symptoms; 2 = just a few lesions (<1% of the leaf area with symptoms) scattered on lower leaves; 3 = low scattered lesions, usually on lower leaves only, but not linked together (1- 3% infected leaf area) ; 4 = moderate number of lesions (4- 6% infected leaf area); 5 = abundant lesions on lower leaves and few on middle leaves (7- 12% infected leaf area); 6 = abundant lesions, some linked together to form a necrotic (dead) area (13- 25% infected leaf area); 7 = necrotic areas linked together and a few leaf tips are dead (26- 50% infected leaf area), lower and middle leaves showing symptoms extending to upper leaves; 8 = about 51-75% of the leaf tips are dead , abundant lesions on almost all leaves and 9 = 76-100% , most of the leaves are dead and plants are usually dead (100% infected leaf area). Figure 3.1 illustrate the scale of 1-9 for NLB disease assessment (Reid, 2005). Disease incidences were calculated as number of maize/ diseased maize x 100. In this case, 32/diseased plant x 100 for every field.

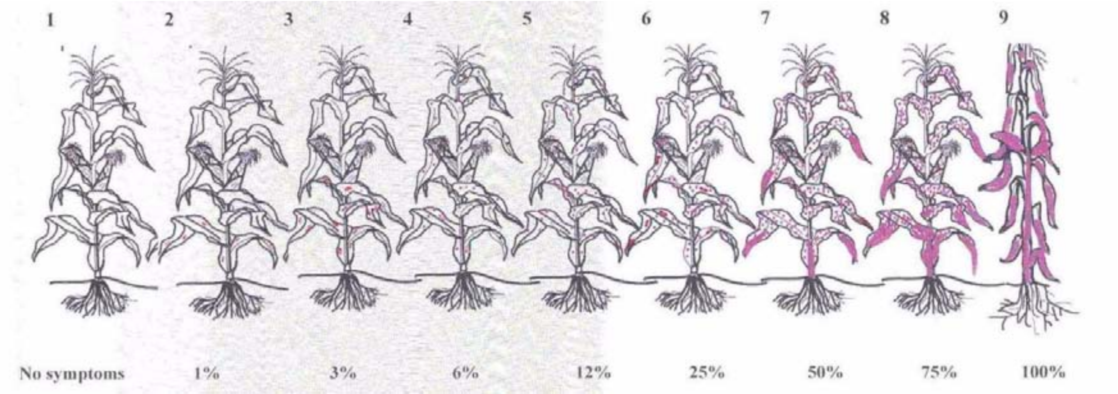


Figure 3. 1: Severity evaluation rating scale for NLB disease infestation (Reid, 2005).

Other collected data included: Number of leaves above the ear, number of infected leaves above the ear and size of lesions from five lesions randomly selected (length and width) using a ruler and recorded in mm. Disease scores and incidence data were subjected to ANOVA in Genstat computer package (Payne et al., 2007). Square root transformation on leaf lesions and arcsine transformation on percentages were done, if necessary, during data analysis. Correlations among the disease associated traits were also performed. A map showing the disease occurrence in the studied villages was also drawn. Northern leaf blight incidences were drawn by using MapInfor Professional which is the Geographical information system (GIS) package with the capacity of performing spatial analysis.

3.2.3 Weather data

Weather data from ARI-Tumbi was examined to assess its relationship with the re-occurrence of NLB disease in the western zone. Rainfall data of 51 years and 27 years for temperature and relative humidity were analysed by using descriptive statistics in Excel programme.

3.3 Results

3.3.1 Weather characteristics

Agro-meteolorogical data from 1958 – 2011 revealed that, the average rainfall was 912.90 mm per annum with monomodal type usually commencing in November and ending in April. Every 20 years there was always a peak rainfall amount (Figure 3.2.). The first peak occurred in

1960s, the second in 1980s and the third in 2000s with exception of 1998 when the area experienced Elnino rainfall type.

The average relative humidity (RH) from 1985 -2011 was 58.91% with the highest (70.89%) occurring in January and lowest (43.96%) occurring in September. On the other hand the average temperature was 23.61^{0}C. Maximum average temperature was 29.63^{0}C while the average minimum temperature was 17.88^{0}C. The highest maximum temperature was 32.63^{0}C which occurs in October while the lowest minimum temperature (15.18 ^{0}C) appears in June every year. In January which is the critical month for maize growth and development, the average temperature was 22.92^{0}C while maximum temperature was 27.93^{0}C and minimum temperature was 17.91^{0}C (ARI-Tumbi, 2011).

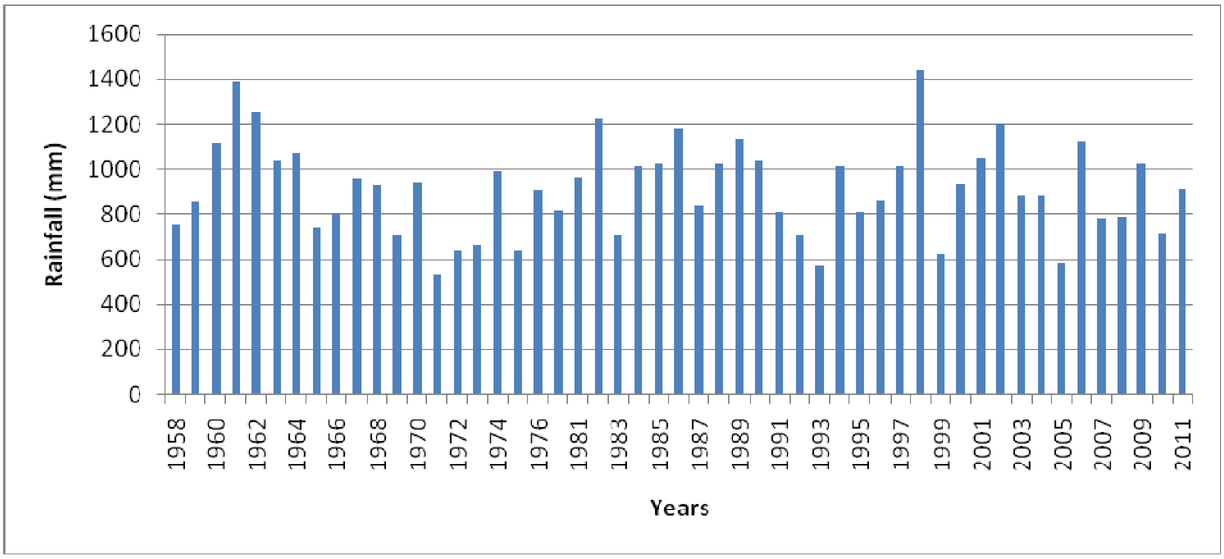


Figure 3.2: Rainfall distributions at ARI-Tumbi from 1958-2011 years (ARI-Tumbi, 2011)

Figure 3.3 shows relative humidity, maximum and minimum temperature distribution in the western zone from 1985-2011 years. These parameters seem to be constant with a little fluctuation.

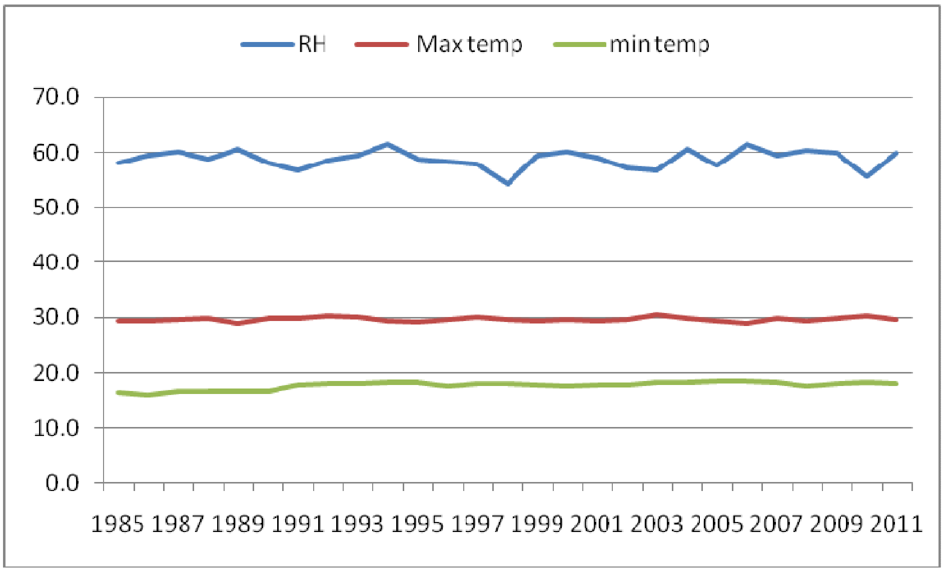
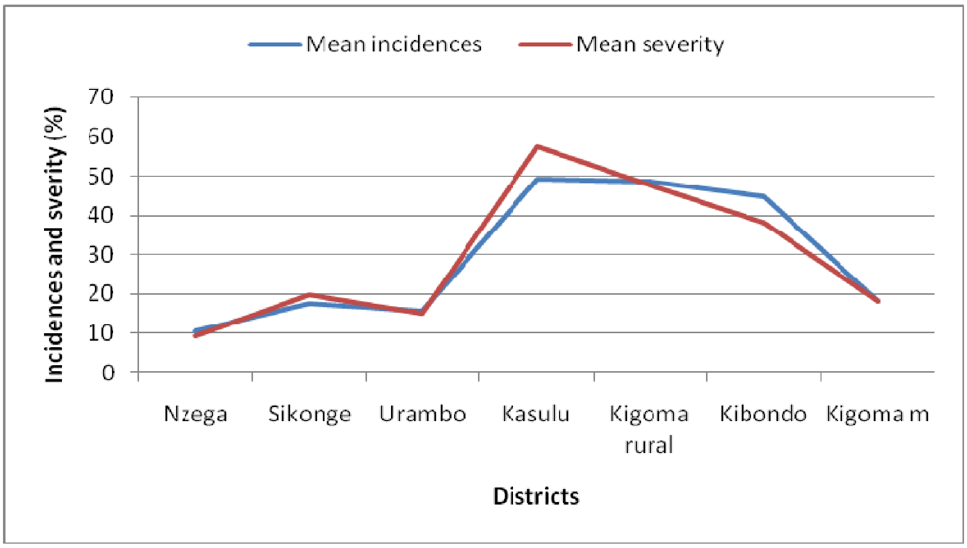


Figure 3.3: Relative humidity (%), maximum and minimum temperature (^{0}C) distributions from 1985-2011 years

3.3.2 NLB disease incidence and severity in the western zone

NLB disease incidence and severity showed similar trends (Figure 3.4). The disease was lower in Nzega, Sikonge and Urambo and highest in Kasulu districts. This could be attributed to disease favourable conditions prevailing in Kasulu district. Nzega is one of the marginal districts and Kasulu is found in the highland areas. In the past decades, there was very little disease pressure in Nzega district.



Whereby Kigoma m = Kigoma municipality

Figure 3.4: NLB incidences and severity across 7 districts of the western zone, Tanzania for 2008/2009 and 2009/2010 growing season

Figures 3.5 and 3.6 depict the relative strength of NLB disease across the studied districts. From these disease distribution maps, it is clear that, the disease is higher in highlands than lowlands invading maize cultivars and threatening its production.

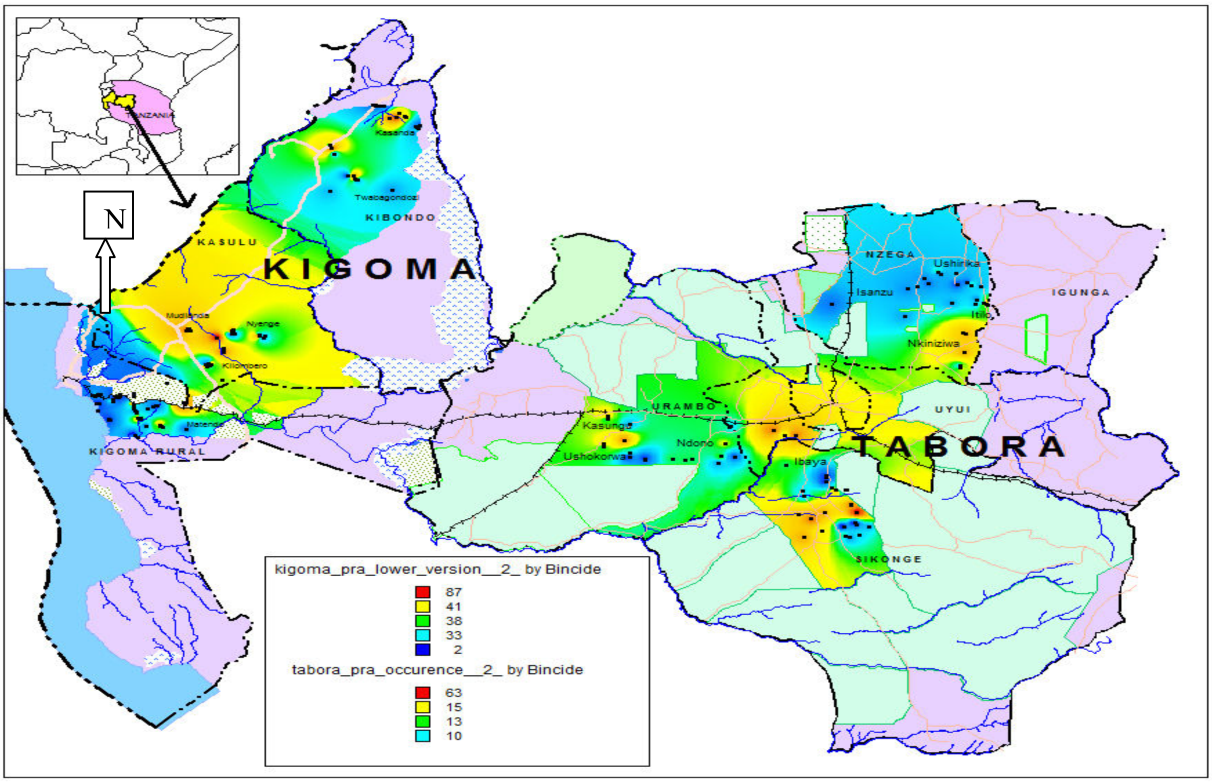


Figure 3.5: The NLB disease distribution map showing its incidences across districts of the western zone for 2008/2009 and 2009/2010 growing seasons

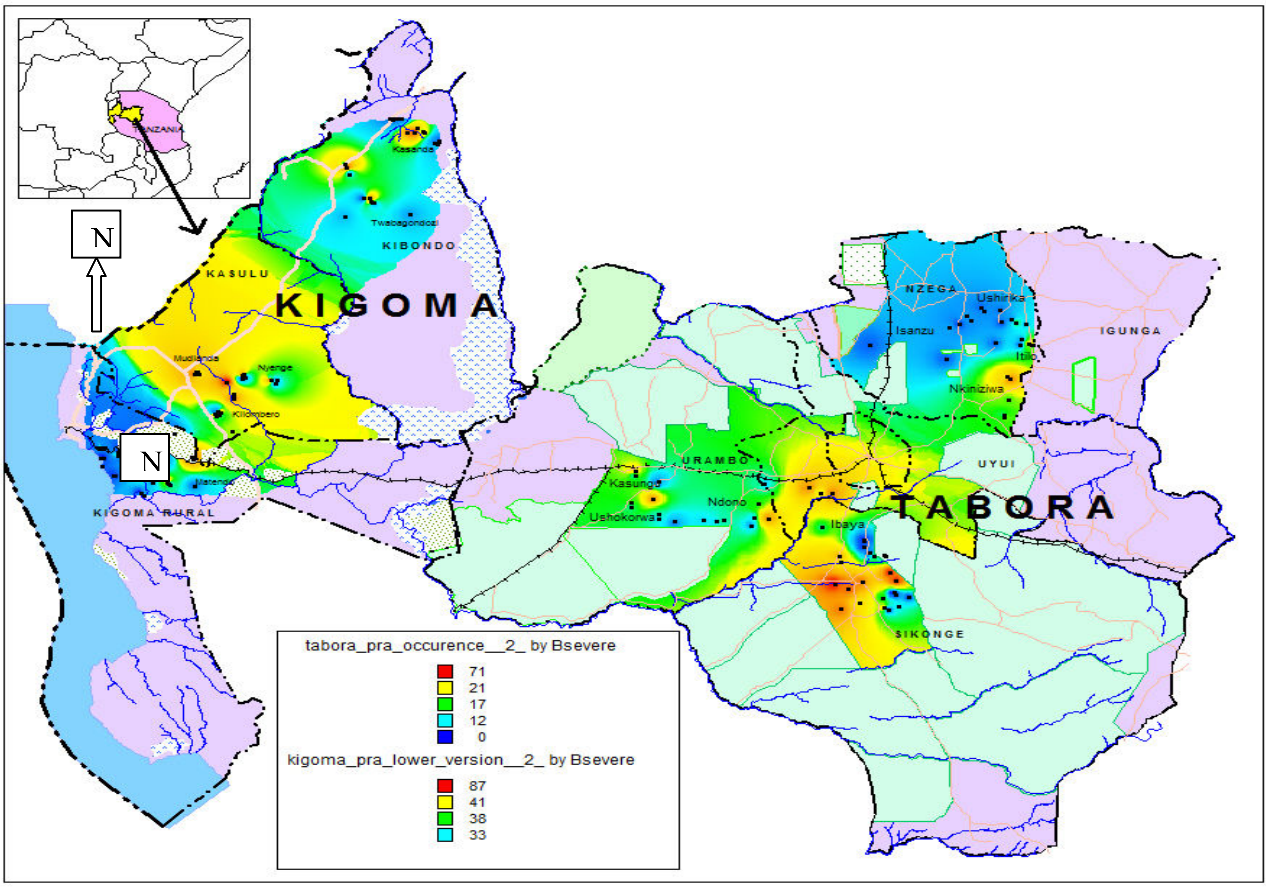


Figure 3.6: The NLB disease distribution map showing its severity across districts of the western zone for 2008/2009 and 2009/2010 growing seasons

3.3.3 NLB disease incidences and severity on maize cultivars grown in the western zone

There were sixteen varieties grown in the zone (Table 3.2). Only three were observed to be resistant to NLB disease. The resistant genotypes were Gembe (landrace), TMV-1 (composite) released in 1987 by the Tanzania maize breeding programme. The third one which had also the lowest incidence was not identified by farmers. From observation, it seems this cultivar originated from hybrids supplied during famine years.

Table 3.2: NLB disease incidences on maize cultivars grown in the western zone for 2008/2009 and 2009/2010 growing seasons

		Blight incidences (%)		
Variety	Type	2008/2009	2009/2010	Mean
Cargil	Hybrid	29.7	31.3	30.5
Gembe	Landrace	6.3	8.9	7.6
Ilonga composite	Composite	12.3	28.0	20.2
Katumani	Composite	23.7	28.3	26.0
Katumbili	Composite	27.0	24.7	25.9
Kilima	Composite	41.2	48.1	44.7
SeedCo 513	Hybrid	58.0	62.0	60.0
Kabalagata	Landrace	85.0	35.6	60.3
Unknown	Unkown	2.0	3.0	2.5
PAN 6195	Hybrid	18.4	31.8	25.1
SeedCo 403	Hybrid	24.0	29.9	26.9
Sega	Landrace	37.0	78.0	57.5
Situka	Composite	85.0	89.0	87.0
TMV-1	Composite	4.0	2.0	3.0
UCA	Composite	30.8	45.5	38.2
	Mean	32.3	36.4	34.4

Table 3.3: NLB disease severity on maize cultivars grown in the western zone for 2008/2009 and 2009/2010 growing seasons

		Blight severity (%)		Mean
Variety	Type	2008/2009	2009/2010	
Cargil	Hybrid	16.3	17.0	16.7
Gembe	Landrace	3.4	9.6	6.5
Ilonga composite	Composite	12.3	16.3	14.3
Katumani	Composite	35.3	38.0	36.7
Katumbili	Composite	31.6	31.9	31.8
Kilima	Composite	39.0	47.4	43.2
SeedCo 513	Hybrid	49.0	32.0	40.5
Kabalagata	Landrace	29.3	34.4	31.9
Unknown	Unkown	3.0	3.0	3.0
PAN 6195	Hybrid	15.0	20.8	17.9
SeedCo 403	Hybrid	24.0	27.6	25.8
Sega	Landrace	80.0	89.0	84.5
Situka	Composite	59.0	59.0	59.0
TMV-1	Composite	2.0	2.0	2.0
UCA	Composite	43.3	43.5	43.4
	Mean	29.5	31.4	30.5

When, sixteen maize cultivars grown by farmers in the western zone were assessed on the disease reaction on the scale developed by Reid (2005). The same three genotypes found on Table 3.2 had the lowest disease severity (Table 3.3).

Figure 3.7 (A and B) and 3.8 (D and C) shows some of the assessed maize cultivars with different levels of reaction to NLB disease. Figure 3.10 shows Gembe variety left to grow in a sole groundnut field.

Figure 3. 7: NLB disease susceptible UCA maize variety (A) observed at Udongo village and susceptible maize variety Kabalagata (B) observed at Ibaya village in Sikonge district, 2008/2009 growing season

Figure 3. 8: NLB Unknown disease resistant maize variety (D) observed at Motomoto village, Urambo district and NLB disease resistant Gembe maize variety (C) observed at Isanzu village, Nzega district in 2008/2009 growing season

The disease incidence in season two (2009/2010) significantly increased from season one (2008/2009), t= -3.25 (df = 348), P= 0.001(Table 3.4). However, severity, lesion length, lesion width and infested leaves did not show significant differences across the two reasons. Leaf rust significantly increased, t= -2.27 (df = 347), P= 0.024. This parameter was assessed because there is always close association with it to NLB disease

Table 3.4: NLB disease incidence, severity, lesion length, lesion width, infested leaves and rust incidences for 2008/2009 and 2009/2010 seasons, western zone, Tanzania

						95% Confidence interval	
Parameter	t	DF	sig.(2 tailed)	Mean difference	STD	Lower	Upper
Blight incidence	-3.25	348	0.001	-8.47	2.61	-13.6	-3.35
Blight severity	-1.41	348	0.159	-4.01	2.84	-9.6	1.58
Lesion length (mm)	-1.53	348	0.127	-11.92	7.79	-27.24	3.4
Lesion width (mm)	-7.15	348	0.475	-0.57	0.79	2.12	0.99
Infested leave number	-1.13	348	0.26	-0.12	0.11	-0.33	0.09
Rust incidences	-2.27	347	0.024	-1.27	0.56	-2.37	-0.17

Both mean blight incidence and severity approached 30% (Table 3.5). There was wide variation on blight incidence, severity, lesion length and affected leaves across cultivars and districts. The high variation on the measured parameters could be probably due to genotypic differences, and environment and G X E interactions. Other diseases associated with NLB were also measured. These diseases were MSV and leaf rust which also showed wide variation in range, minimum, maximum and variances.

Table 3.5: Combined analysis of NLB disease parameters for 2008/2009 and 2009/2010 growing seasons in the western zone of Tanzania

	Min	Max	Mean	SE	SD	Variance
Blight incidence (%)	1	99	29.26	1.32	24.71	610.59
Blight severity (%)	1	99	29.53	1.40	26.21	687.22
Lesion length (mm)	8	480	138.42	3.90	73.00	5329.49
Lesion width (mm)	2	80	13.08	0.40	7.40	54.72
Leaves number	2	9	6.44	0.05	0.99	0.99
Infected leaves	0	8	2.59	0.08	1.51	2.29
MSV incidence (%)	0	98	20.09	1.35	25.17	633.30
MSV severity (%)	0	99	25.27	1.56	29.12	848.00
Rust incidence (%)	0	43	3.79	0.28	5.25	27.54

Whereby, Min = minimum and Max = maximum

3.3.4 Correlation analysis of NLB disease parameters

Table 3.6 shows correlation relationships of traits associated with NLB disease. Correlation analysis revealed that, there were significant positive correlations between season and blight incidence (0.172*), severity and lesion length (0. 295*), lesion length and width (0.308*). However, there were significant negative correlation between NLB infested leaves and streak incidences (-0.112*). Gathered data revealed that, there was a significant positive correlation coefficient between altitude and blight severity (0.117**) but highly significant negative correlated with lesion length (-0.429**), maize streak virus (MSV) incidence (-0.669**) and MSV severity (-0.615**). On blight incidence, there were high significant positive correlations with severity (0.682**), lesion length (0.272**0, lesion width (0.206**), and infested leaves (0.245**). In the case of NLB disease severity, there were high significant positive correlations with lesion length (0.295**), lesion width (0.141**) and infested leaves (0.205**). Other highly significant positive correlated traits were lesion length and infested leaves (0.179**), with streak incidence (0.546**) and with streak severity (0.530**).

Table 3.6: Correlation analysis of NLB disease parameters for 2008/2009 and 2009/2010 growing seasons in the western zone of Tanzania based on a survey in farmers' fields.

	Season	Alt	Incidence	Severity	Length	Width	Number	Infected	Streak1	Streak 2	Rust
season	1	0.000	0.172*	0.082	0.082	0.038	0.061	-0.010	0.031	0.039	0.121
Alt		1	0.098	0.117**	-0.429**	0.015	0.078	-0.070	-0.669**	-0.615**	-0.046
Incidence			1	0.682**	0.272**	0.206**	-0.053	0.245**	0.052	0.083	0.075
Severity				1	0.295*	0.141**	-0.060	0.205**	0.064	0.076	0.075
Length					1	0.308*	-0.078	0.179**	0.546**	0.530**	0.117
Width						1	0.049	0.093	0.042	0.074	-0.027
Number							1	-0.013	-0.112*	-0.067	-0.145
Infected								1	0.172**	0.180**	0.054
Streak1									1	0.889**	0.080
Streak2										1	0.098
Rust											1

**= Correlation is significant at the 0.01 level (2-tailed)
*= Correlation is significant at the 0.05 level (2-tailed)
Where
Season = 2 seasons (2008/2009 and 2009/2010
Alt = Altitude (masl)
Incidence = NLB disease incidences (%)
Severe= NLB disease severity (%)
Length= NLB disease lesion length (mm)
Width= NLB disease lesion width (mm)
Number = NLB disease infested leaf number
Streak1= MSV disease incidences (%)
Streak2 = MSV disease severity (%)
Rust = leaf rust disease incidences (%)

3.3.5 Farmers efforts to combat NLB disease spread

Farmers in the western zone of Tanzania have developed and incorporated in the farming system some basic agricultural practices to control the disease. The majority of farmers apply general crop sanitation especially during land preparation. They destroy and burn crop residues and weeds. In the process, they also interrupt the breeding cycle of the pathogen (Figure 3.9 A). In areas where a large number of cattle are kept in a free range system like in Nzega district, animals are left to graze in maize fields. They feed on maize stovers and other possible NLB disease alternative hosts. In the process, the pathogen breeding cycle is broken and thus inoculum probably is reduced. In many cases in the marginal areas like Nzega district, animal grazing is supplemented with land preparation by burning of debris (Figure 3.9 B). Stovers grazing and debris burning could be one reason of low level of NLB disease infestation in Nzega district and in other marginal areas.

Figure 3.9: Maize land preparation by burning crop residues and alternative hosts of NLB disease at Kasungu village (A), Urambo district and combination of crop residual burning and cattle grazing for breaking life cycles of NLB disease pathogen at Isanzu village (B), Nzega district in 2009/2010 growing season

3.4 Discussion

The observed average rainfall, temperature and relative humidity could be probably one of the causes of NLB disease resurgence in the western zone of Tanzania. The highest RH (70.89%) that occurs in January is coupled with the average temperature of 22.92^{0}C (maximum 27.93 and minimum 17.91) which appears in the same month. These environmental conditions are close to the suggested optimum relative humidity (75.20%) and temperature of 21. 10^0C for disease development noted by other researchers (Sharma and Mishra, 1988). At the same time maize which is normally planted in November starts to flower in January. The January synchronization of rainfall, RH and temperature subjects the plant to be vulnerable to fungal diseases like NLB infestation. However, temperature and relative humidity in this study did not show clearly the relationship between them and the NLB disease resurgence which advocates for further investigations. The occurrence of Elnino phenomena that occurred in 1998 increased the relative humidity of the area. This was because there were evenly rainfall distribution, enough rainfall amount and long duration of clouds cover. Elnino rainfall is believed to accelerate the increase of NLB disease inoculums in the soil, maize debris and pathogen alternative hosts. Farmers from Urambo district started to feel the importance of NLB disease by observing plants developing lesions and leaves appeared as burnt by hot water.

Northern leaf blight disease is becoming an important disease in the western zone of Tanzania. There are reports of the widespread distribution in all districts of the zone. This study assessed its incidence and severity of 175 farms in seven districts. The overall mean of incidence and severity was about 30%. Severity observed here was slightly higher than 25% observed by Adipala et al. (1993) in Uganda and lower than 45% recorded in Kenya (Mwangi, 1998). The disease incidence and severity in the seven studied districts indicates that, the disease is advancing from highland to lowlands. Wide distribution and severity showed by the study suggests immediate control measure to be instituted. The spread and devastation of the disease results in maize yield decline. The most recent outbreak was in Uganda that wiped tonnes of maize grains (Adipala et al., 1993).

The northern leaf blight disease incidence and severity were lower in Nzega and highest in Kasulu districts. The trend could be attributed to agro-climatic conditions differences between districts. Kasulu has high rainfall compared to Nzega districts. However for the disease to be found in Nzega district which is located in the marginal areas needs further investigation.

Sixteen varieties were observed to be grown in the zone with wide variations on NLB disease resistance. Only one composite cultivar, TMV-1 showed resistance reactions to prevailing NLB disease infestation. Gembe landrace showed high resistance to the disease. Gembe landrace could be a source of breeding material for introgresion of resistance. The use of genetic engineering could be applied to quickly map the gene of interest to hasten breeding progress.

The positive and negative significantly high correlated NLB associated traits revealed by the study suggests breeding for positive and negative associated traits to combat the disease. The implication from negative correlation traits is that, if one desired trait is highly correlated with another undesired trait, then, improvement of desired trait will lower the undesired trait. While for highly positive correlated trait, improvement of one trait will result to the increase of another trait and vice versa. In this study, altitude and blight severity were highly significant positive correlated (0.117**). The implication is that, disease incidence increases with elevation. Similar observation was reported by other researcher (Jordan et al., 1983; Ngugi et al., 2002). On the other hand, the highly significant positive correlation between NLB disease lesion length and incidence (0.546**) and streak severity (0.530**) had the implication that, one disease could have a positive influence on another disease. Breeders are required to select against both diseases to achieve, recommend and release desired varieties to be grown in the respective areas (Janick, 2010; Zheng et al., 2011). However, number of NLB disease infested leaves were negatively correlated with MSV incidence (-0.112*) to indicate that number of NLB disease infested leaves were increased by decreased MSV incidence. Because both number of NLB disease infested leaves and MSV disease incidences are both undesired traits, breeders could probably use a selection index which gives weights of different traits. The highly negative correlation between altitude and lesion length, altitude and MSV incidence and altitude and MSV severity indicates that, MSV incidence and severity was high in lowlands as compared to highlands. On the other hand altitudes decrease with increasing lesion length of NLB disease.

Farmers in the western zone of Tanzania have developed some indigenous technical knowledge (ITK) primarily and principally for general control of weeds, insect pests and diseases (Musara et al., 2010). By applying these agricultural practices, it is possible to control to certain extent some of maize diseases including the NLB disease. Practices like debris and stovers burning during land preparation and fields cattle grazing reduce number of crop residues and possible pathogen alternative hosts (CPC, 2001; Friesen and Palmer, 2004). To be more effective, an integrated diseases control is highly needed. The introduction of NLB .disease resistant cultivars could help and supplement farmers’ efforts towards effective control of the disease in the zone.

3.5 Conclusions

Therefore, the following conclusions can be drawn from this study:

- This study established that, NLB disease incidence and severity in the western zone is prevalent. The disease incidence in season two (2009/2010) significantly increased from season one (2008/2009) t= -3.25 (348), P= 0.001. The mean severity and incidences for NLB was 30%. Both modern and landraces were affected by the disease. Out of 16 varieties grown by farmers only three cultivars were resistant to NLB disease. The resistant cultivars included Gembe landrace, TMV-1 (Composite) and one unknown. This has an implication on the potential outbreak of the disease due to increased amount of inoculums.
- Result showed that, altitude and NLB disease severity were highly positively correlated (0.117**) indicating the disease increased with altitude. However the NLB disease was recorded in all seven surveyed districts to denote that, the disease has changed its distribution pattern affecting all high, medium and low altitude areas. Thus, the NLB disease prevalence recorded by this study calls for breeding for disease resistant maize varieties in the whole zone.

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Chapter 4

4 Characterization and screening of maize landraces for northern leaf blight disease resistance in the western zone of Tanzania

Abstract

Genetic relationships with regard to NLB disease resistance of maize landraces found in Tanzania is lacking. A characterization study with the objectives of determining genetic relationships among landraces, assessing maize landraces as sources of NLB disease resistance and assessing important agronomic traits for future maize improvement was conducted in the western zone of Tanzania. Ninety breeding materials consisting of 71 landraces and 19 commercial varieties were planted at Agricultural Research Institute Tumbi in Tabora, Tanzania. A 10 x 9 alpha lattice design with two replications was used. The trial was planted at a spacing of 0.75 m x 0. 30 m. The average yield of landraces under research management was 2. 3 t ha^{-1}. Landrace TZA 3075 was identified as NLB disease resistant. Yield potential, dent grain texture, white endosperm and husk cover were important agronomic traits observed among landraces. There was variation on infested leaves per plant which ranged from 1 to 7. Landraces showed high variation in lesion width with the mean of 7.84 mm and incidence (11.67%). There were significant positive correlations (0.211*) between number of leaves per plant and infested leaves above the ear. Landraces also recorded highly positive significant (<0.001) correlation between NLB disease lesion length and width (0.430**). Five principal components contributed 71.98% of total variation. Leaves plant $^{-1}$, infested leaves plant $^{-1}$, lesion number, lesion length, lesion width and NLB incidence highly contributed to variation and grouping of landraces. Principal component analysis revealed six distinct clusters composed of mixtures of TZA and ARIT series, and commercial varieties. Cluster analysis revealed five distinct groups of landraces. Agronomic traits variation and resistant landraces found in this study could be used for breeding high yielding and NLB resistant cultivars in the maize breeding programme. The identified landrace TZA 3075 NLB disease resistant could be a source of resistance for NLB disease in maize breeding programme.

4.1 Introduction

Maize was introduced in Africa by Portuguese in16 th century. From that time, environment and genotypes x environment interaction, natural selection brought by environment, genetic and some human activities has resulted in locally adapted maize landraces. Well adapted maize possesses contrasting traits to be utilized by maize breeders for developing superior cultivars. Maize breeding programmes depend heavily on the knowledge of breeding materials' genetic relationships and diversity of traits. Genetic relationships studies are achieved through application of genetic markers (Bucheyeki et al., 2007; Bucheyeki et al., 2008b). For the past number of years human beings have been busy selecting, grouping and classifying different crops to fit specific uses, purposes and utilization. Various markers have been developed, tested and employed for the purpose of studying genetic relationships, variations and grouping number of genotypes. Common markers include morphological, biochemical and molecular types (IAEA, 2002).

Although biochemical and molecular markers are more precisely and have good repeatability for some techniques than morphological markers, they need to supplement each other. For example, morphological markers are cheap; do not need laboratory equipment installation, chemicals and gives reliable results (Barnaud et al., 2007; Cadee, 2000; Zimeri and Kokini, 2003). Morphological markers are powerful tool for characterization of genotypes especially when dealing with traits of high heritability (N'Diaga and Ejeta, 2003). They can be used and utilized in the studies of genetic diversity and similarity of genotypes. Morphological markers have been used by researchers in the study of different crops, for example Fuentes et al. (2005) studied the diversity of 20 rice cultivars in Cuba, Bucheyeki et al. (2008b) used morphological traits to characterize 39 sorghum landraces from Tanzania and Zambia, Mujaju and Chakauya (2008) characterized 47 sorghum landraces from Zimbabwe in Southern Africa. In maize, Norton (1954) successfully studied the ear morphology of maize through utilization of morphological markers. Revilla and Tracy (1995) evaluated and morphologically characterized 58 sweet corn maize cultivars in North America, However, genetic relationships information in relation to NLB disease reactions of landraces found in Tanzania is missing. There is no study which attempted to characterize maize landraces, thus genetic relationship is lacking to be fully used and incorporated effectively in maize breeding programme. This current study used morphological makers to study maize landraces in western zone of Tanzania based on NLB disease reaction. The specific objectives were to determine the genetic relationships among landraces, assess maize landraces as sources of NLB disease resistance and assess important agronomic traits for future maize improvement.

4.2 Materials and methods

4.2.1 Breeding materials

The experiment was carried out at ARI- Tumbi and was located at $05^0$03.391’ S, $032^0$37.851’E and 1162 masl. The total rainfall was 715.2 mm. Ninety breeding materials were employed in this study. From this list, 60 were obtained from Genetic Resource Managements in Arusha, 11 were collected from Agricultural Research Institute (ARI) - Tumbi and 19 were commercial varieties currently on the market and farmers fields (Table 4.1).

Table 4.1: Maize genotypes used for characterization trial at ARI-Tumbi

Name	Type	Source	Name	Type	Source
TZA 2882	Landrace	PGR-Arusha	TZA 4435	Landrace	PGR-Arusha
TZA 2897	Landrace	PGR-Arusha	TZA 4445	Landrace	PGR-Arusha
TZA 2911	Landrace	PGR-Arusha	TZA 4473	Landrace	PGR-Arusha
TZA 2930	Landrace	PGR-Arusha	TZA 4476	Landrace	PGR-Arusha
TZA 2949	Landrace	PGR-Arusha	TZA 4478	Landrace	PGR-Arusha
TZA 2971	Landrace	PGR-Arusha	TZA 4484	Landrace	PGR-Arusha
TZA 3039	Landrace	PGR-Arusha	TZA 4492	Landrace	PGR-Arusha
TZA 3054	Landrace	PGR-Arusha	TZA 4496	Landrace	PGR-Arusha
TZA 3075	Landrace	PGR-Arusha	TZA 45O6	Landrace	PGR-Arusha
TZA 3114	Landrace	PGR-Arusha	TZA 4551	Landrace	PGR-Arusha
TZA 3218	Landrace	PGR-Arusha	TZA 4557	Landrace	PGR-Arusha
TZA 3272	Landrace	PGR-Arusha	TZA 4959	Landrace	PGR-Arusha
TZA 3310	Landrace	PGR-Arusha	ARIT 1	Landrace	ARI-Tumbi
TZA 3312	Landrace	PGR-Arusha	ARIT 2	Landrace	ARI-Tumbi
TZA 3343	Landrace	PGR-Arusha	ARIT 3	Landrace	ARI-Tumbi
TZA 3454	Landrace	PGR-Arusha	ARIT 4	Landrace	ARI-Tumbi
TZA 3480	Landrace	PGR-Arusha	ARIT 5	Landrace	ARI-Tumbi
TZA 3502	Landrace	ARI-Tumbi	ARIT 6	Landrace	ARI-Tumbi
TZA 3516	Landrace	PGR-Arusha	ART I7	Landrace	ARI-Tumbi
TZA 3585	Landrace	PGR-Arusha	ARTT 8	Landrace	ARI-Tumbi
TZA 3597	Landrace	PGR-Arusha	ARIT 9	Landrace	ARI-Tumbi
TZA 3605	Landrace	PGR-Arusha	ARTI 10	Landrace	ARI-Tumbi

Table 4.1 Continued

Name	Type	Source	Name	Type	Source
SITUKA-1	Landrace	C.variety♣	ARIT 11	Landrace	ARI-Tumbi
TZA 3627	Landrace	PGR-Arusha	LISHEH-2	C.variety	Stockist
TZA 3636	Landrace	PGR-Arusha	SITUKA-M1	C.variety	Stockist
TZA 3644	Landrace	PGR-Arusha	KITO-ST	C.variety	Stockist
TZA 3665	Landrace	PGR-Arusha	LISHE-K1	C.variety	Stockist
KILIMA	Landrace	C.variety	TMV -1	C.variety	Stockist
TZA 3713	Landrace	PGR-Arusha	DK 8031	C.variety	Stockist
TZA 3732	Landrace	PGR-Arusha	PAN 6549	C.variety	Stockist
TZA 3741	Landrace	PGR-Arusha	PHB 3253	C.variety	Stockist
TZA 3869	Landrace	PGR-Arusha	SC 627	C.variety	Stockist
TZA 3766	Landrace	PGR-Arusha	DKC 8053	C.variety	Stockist
TZA 3775	Landrace	PGR-Arusha	UH 615	C.variety	Stockist
TZA 3795	Landrace	PGR-Arusha	KATUMANI	C.variety	Stockist
TZ 3823	Landrace	PGR-Arusha	SC 403	C.variety	Stockist
TZA 3837	Landrace	PGR-Arusha	TZA 4505	Landrace	PGR-Arusha
LNA 3845	Landrace	PGR-Arusha	PAN 67	C.variety	Stockist
TZA 3851	Landrace	PGR-Arusha	SC 512	C.variety	Stockist
TZA 3854	Landrace	PGR-Arusha	TMV-2	C.variety	Stockist
TZA 3855	Landrace	PGR-Arusha	UH 6303	C.variety	Stockist
TZA 3873	Landrace	PGR-Arusha	TZA 3907	Landrace	PGR-Arusha
TZA 3885	Landrace	PGR-Arusha	TZA 4409	Landrace	PGR-Arusha
TZA 4427	Landrace	PGR-Arusha	TZA 17	Landrace	PGR-Arusha
TZA 4435	Landrace	PGR-Arusha	TZA 4420	Landrace	PGR-Arusha

♣ = C. variety= Commercial variety

4.2.2 Field evaluation

Ninety breeding materials were evaluated at ARI-Tumbi in 2009/ 2010 growing season under rain fed condition. Trials were planted in the first week of December which is the normal rainfall season in the western zone. There was no particular abiotic and biotic stress during the growing period. Maize was planted at a spacing of 0.75 m x 0.30 m. Fertilizer, NPK basal fertilizer was applied at a rate of 40 Kg P ha^{-1}. Murate of Potash was applied at the rate of 40 Kg $k_2$0 ha^{-1}. Top dressing was done using UREA (46%) to achieve a recommendation rate of 100 Kg N ha^{-1}. Two row plots, 5 m long each were employed to conduct the experiment.

4.2.3 Inoculation procedures

To ensure uniform disease infestation, artificial inoculation was conducted according to procedures described by Reid (2005) as follows: A sample grinder machine [Laboratory mill model-4, Thomas –Wiley, Thomas scientific U.S.A] was used to grind infected leaves into powder form. A bazooka (Sistrunk Inoculators, Starkville, MS 39759) was used to apply the powder in the whorl of plants. One dose of powder from Bazooka application amounts to 0.1 g of leaves powder. Two applications, one at 6 – 8 and the second at 11-12 leaf stage were conducted. Furthermore, two rows of a spreader local variety (Situka-1) which is highly susceptible to NLB disease was planted around the field and after every 10 rows of the test materials. The uses of spreaders have been used successfully in screening germplasm for other disease studies (Singh et al., 2004). A scale of 1-5 was used to assess husk cover by which 1 denotes poor and 5 implied good cover.

4.2.4 Experimental design, data management and analysis

A 10 x 9 alpha lattice design with two replications were used. Data were recorded according to IBPGR (1991) maize descriptors list. Data was validated in Excel Microsoft word programme. Average data of two seasons were subjected to multivariate data analysis in Genstat (Payne et al., 2007) and SPSS (2006) statistical computer programmes. Data was subjected to multivariate analysis to study and analyse genetic relationships among genotypes. Principal component analysis (PCA) and cluster analysis (CA) were employed for discrimination and grouping genotypes respectively. Principal Component Analysis was used to determine plant traits that contributed significantly to the discrimination of the landraces. Cluster analysis, based on Euclidean distances as similarity measures and the Unweighted Pair-Group Method With Arithmetic Averages (UPGMA), was used to determine the genetic relationships among genotypes. Proximity matrices of the landraces were also computed. Variables had different units, thus correlation matrix was employed. The procedure was also used to avoid dominance of variables with high variances (Payne et al., 2007).

4.3 Results

There were highly significant differences (< 0.001) on yield, plant height, ear height, and days to tasseling and silking. Landraces also showed significant differences on number of infected leaves per plant. The average grain yield was 2.20 t ha^{-1}, infected leaves per plant was 7.36 while plant height was 152.1 cm and ear height was 77.94. Days to tasseling were 58.52 and days to silking were 59.94. All landraces had flint grain texture and white endosperm. The majority of landraces achieved a five scale of husk cover (Table 4.2).

4.3.1 Landraces traits assessment

Table 4.2: Landraces agronomic characteristics 90 maize varieties evaluated at ARI-Tumbi, for 2008/2009 and 2009/2010 growing seasons

Top ten	Yield	leaves	Ht	Ear ht	Tassel	silk	Husk
TZA 3869	5.10	6.00	135.50	80.00	61.00	62.50	5.00
TZA 3312	4.65	7.00	196.50	96.00	50.50	53.00	4.00
DKC 8053	4.55	5.50	199.50	45.00	65.50	66.50	5.00
TZA 4427	4.50	9.50	187.50	83.00	87.50	69.00	5.00
TZA 4496	4.50	8.00	146.00	48.00	61.00	63.50	5.00
TZA 3851	4.30	7.00	144.50	54.00	41.50	42.50	5.00
TZA 3272	4.10	7.50	180.50	59.00	76.50	77.00	5.00
TZA 2971	3.83	5.00	99.50	113.00	40.00	43.50	5.00
TZA 3855	3.74	7.00	99.50	90.00	51.50	53.00	5.00
TZA 3644	3.57	7.50	94.00	64.50	67.00	69.00	4.00
Bottom ten							
TZA 3741	1.05	7.50	111.00	56.00	62.50	64.50	5.00
ARIT 4	1.01	10.00	136.50	45.00	65.00	67.50	5.00
ARIT 3	1.00	7.50	101.50	106.00	45.50	47.50	5.00
ARIT 11	0.95	10.00	217.50	78.00	66.50	66.50	5.00
TZA 3310	0.90	7.50	92.00	68.00	68.50	68.00	5.00
PHB 3253	0.75	5.50	84.00	66.00	69.50	71.00	5.00
TZA 3114	0.73	5.00	90.00	77.00	88.50	88.50	5.00
TZA 2882	0.65	6.50	100.00	67.00	41.00	42.50	5.00
TZA 2897	0.60	4.00	85.00	80.00	63.00	65.00	4.00
TMV-2	0.55	6.50	127.50	105.00	44.50	46.00	5.00
Statistics							
Mean	2.29	7.36	152.10	77.94	58.52	59.94	
Prob	<0.001	0.004	<0.001	<0.001	<0.001	<0.001	
Lsd	1.300	3.840	46.050	8.031	4.122	5.391	
Cv	28.600	26.300	15.200	5.200	3.500	4.500	

Where: leaves = infected leaves per plant, Ht = pant height (cm), Ear ht = ear height (cm), tassel = days to tassel, texture = grain texture, colour = grain colour, husk = husk cover.

4.3.2 Maize landraces traits description

Table 4.3 shows characteristics of maize landrace traits which are found in Tanzania. The mean leaves per plant were 5.99.

There were variations on infested leaves per plant with the range of 1-7. Landraces showed high variations on lesion number which varied from 1-8. Lesion length recorded the highest variation with the mean of 70.29 mm. landrace also showed high variations on lesion width with the mean of 7.84 mm, NLB disease incidence (11.67%), days to tasseling (76..93) and days to silking 74.30).

Table 4.3: Characteristics of 90 maize landraces traits at ARI-Tumbi, Tabora for 2008/2009 and 2009/2010 growing seasons

Descriptor	Min	Max	Mean	SE	SD	Variance
Leaves /plant	2	8	5.99	0.10	0.98	0.95
Infested leaves/plant	1	7	2.74	0.15	1.46	2.12
Lesion number	1	8	2.90	0.15	1.40	1.96
Lesion length (mm)	6	190	70.29	4.72	44.76	2003.78
Lesion width (mm)	2	20	7.84	0.42	4.00	16.02
NLB incidence (%)	0	20	11.67	0.48	4.56	20.79
MSV infested plants	0	12	2.57	0.25	2.33	5.44
Tasseling (days)	66	86	76.93	0.52	4.97	24.74
Silking (days)	65	84	74.30	0.48	4.54	20.59
Plant height (cm)	60	192	139.39	2.66	25.24	637.25
Ear height (cm)	17	94	60.30	1.62	15.33	234.86

4.3.3 Correlation analysis of maize landraces

There was a significant positive correlation (0.211*) between number of leaves per plant and number infested of leaves above the ear (Table 4.4). Infestation of above the ear leaves indicates reduction of photosynthetic area. Landraces also recorded highly positive significant (<0.001) correlation between NLB disease lesion length and width (0.430**). Other parameters which showed significant positive correlations were silking and tasseling (0.904), plant height and ear height (0.660) and MSV incidence and days to tassel (0.314). Highly positive significant correlated traits are used for minimizing studied traits as breeders can easily select for or against any of the traits.

Table 4.4: Correlations coefficients of 11 maize landraces grown at ARI-Tumbi, Tabora for 2008/2009 and 2009/2010 growing seasons

	Leaves	Infested	lesion	Length	Width	Incidence	MSV	Silk	Tassel	Ht	Ear ht
Leaves	1	0.211*	-0.001	-0.017	0.060	0.029	0.052	0.023	-0.009	0.148	0.146
Infested		1	0.164	-0.116	-0.076	0.014	0.007	-0.029	0.037	-0.117	-0.116
lesion			1	-0.161	-0.177	0.062	-0.034	0.104	0.191	0.204	0.118
Length				1	0.430**	0.095	-0.118	0.048	0.042	0.058	-0.071
Width					1	0.184	0.028	0.069	0.051	0.012	0.118
Incidence						1	-0.153	-0.126	-0.147	-0.053	0.029
MSV							1	0.279	0.314**	0.170	0.190
Silk								1	0.904**	0.094	0.149
Tassel									1	0.056	0.207
Ht										1	0.660**
Ear ht											1

* = significant at 0.05

**= significant at 0.001

Where: silk = days to silking, tassel = days to tassel, HT = plant height (cm), and Ear ht =Ear height (cm)

4.3.4 Principal component analysis

Five principal components contributed 71.98 % of the total variation (Table 4.5). Principal component 1 contributed to 21.42% of total variability and was heavily loaded by infested leaves per plant, days to tasseling, days to silking and number of leaves per plant. Three traits were found to be highly associated with principal component 2 which accounted for 14.94% variations in landraces. Traits associated with principal component 2 were lesion length, NLB disease incidence and plant height. Principal component 3 with 14.41% of variation contribution was highly contributed by leaves per plant, infested leaves per plant and days to silking. On the other hand, lesion number and width were associated with principal component 4 of the evaluated maize landraces and contributed 11.24% of total variability. Principal component 5 was highly related with lesion width, MSV infestation, ear height and lesion number. This principal component accounted for 9.97% of the total variability and had the Eigen value of 1.096.

Table 4.5: Five principal components and variability contribution of 11 traits of maize landraces evaluated at ARI-Tumbi, Tabora for 2008/2009 and 2009/2010 growing seasons

Traits	PC1	PC2	PC3	PC4	PC5
Leaves /plant	0.3778*	-0.24402	0.46588*	0.09361	0.011873
Infested leaves/plant	0.52596*	0.06843	-0.37074*	-0.07526	0.10134
Lesion number	-0.13565	-0.26248	0.08719	-0.39578*	0.40316*
Lesion length (mm)	-0.02279	0.30075*	0.03692	-0.63393*	-0.20368
Lesion width (mm)	0.09071	-0.05559	0.24282	-0.49117*	-0.51471*
NLB incidence (%)	-0.01454	-0.54124*	-0.26868	-0.12166	0.06788
MSV infested plants	0.34421	0.07035	-0.03267	0.14807	-0.41354*
Tasseling (days)	0.54107*	0.108	-0.35332	-0.10266	0.13171
Silking (days)	0.32932*	-0.23299	0.52651*	0.12895	0.03754
Plant height (cm)	0.04905	-0.58138*	-0.19738	-0.21613	-0.07775
Ear height (cm)	0.17656	0.26738	0.25165	-0.27424	0.56569*
Eigen value	2.357	1.644	1.585	1.237	1.096
Variation contribution (%)	21.42	14.94	14.41	11.24	9.97
Cumulative variation (%)	21.42	36.36	50.77	63.01	71.98

*= important trait to explain PC
(-) denotes contrast trait

Principal component analysis revealed six distinct clusters with all clusters composed of mixtures of TZA and ARIT series, and commercial varieties (Figure 4.3). Most of the landraces occupied the area between -3 to 3 on PC1 and -2 to 3 on PC2.

Eleven outliers were observed. These included Kilima, Kito-ST, ARIT-5, TZA 4409, TZA 3272, and TZA 3873. Others were TZA 3348, TZA 3627, TZA 3854, TZA 3795 and TZA

3855. Kilima and Kito-ST are commercial composite varieties in Tanzania while ARIT 5 was collected from ARI-Tumbi in Tabora region. The remaining outlier landraces were supplied by Genetic Resource Management Centre in Arusha Tanzania. Principal component 1 with five landraces was composed of genotypes with mean NLB incidence of 6.25% while the largest PC cluster 5 had NLB incidence of 11.81%. The lowest PC 3 had the lowest number of plants (4) incidence of 11.25%. Figure 4.1 also shows the identified landrace (TZ 3075) resistant to NLB disease in PC3.This group (PC3) had the lowest number of infested leaves per plant (2.25). Lesion number (2), lesion length (32.63 cm), lesion width (3.88 cm) and NLB disease incidence (6.25%).

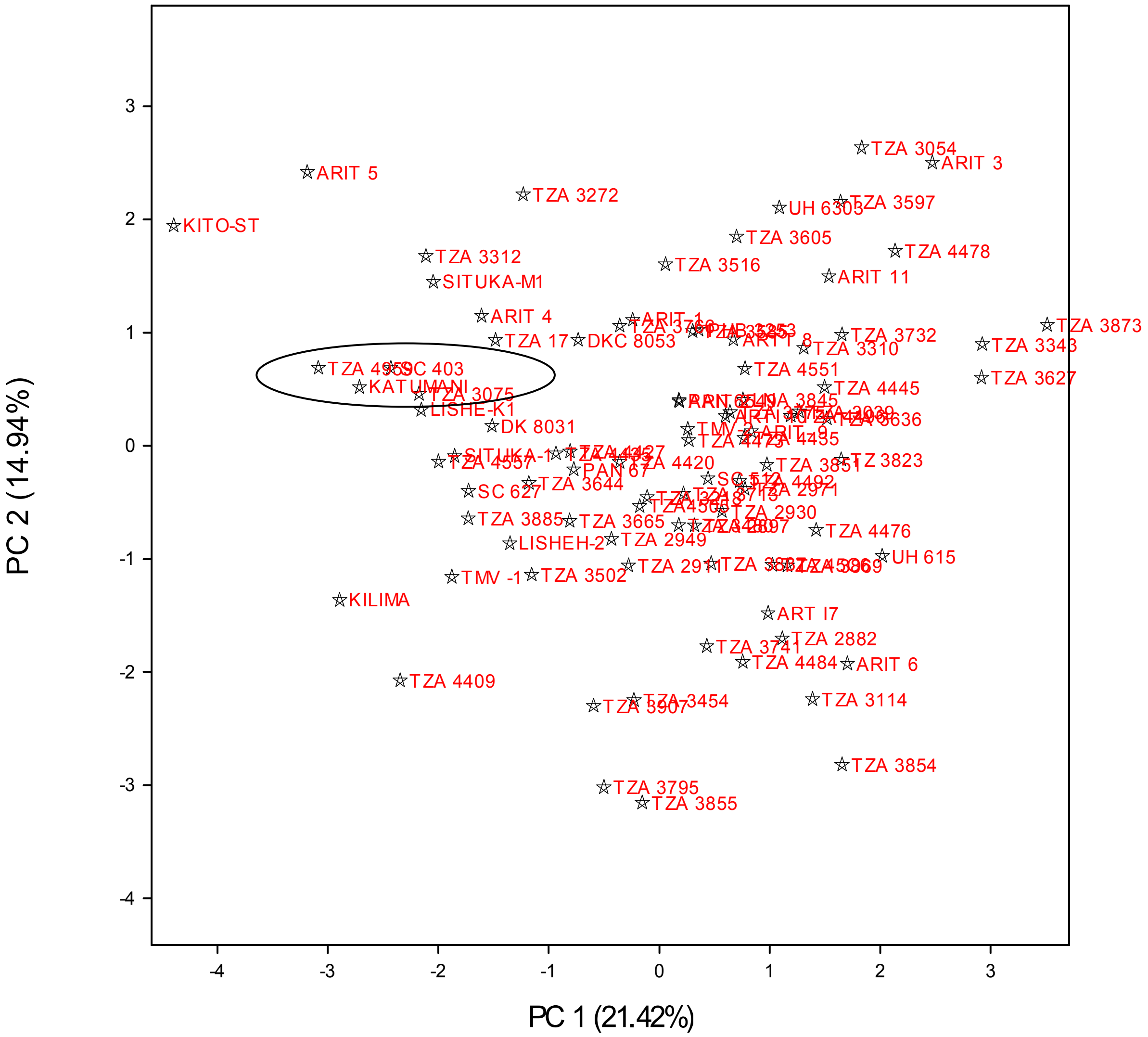

Figure 4.1: Maize landraces positions on first and second principal components based on 11 morphological traits for 2008/2009 and 2009/2010 growing seasons
Where: The cycle shows the cluster with NLB resistant TZA 3075 maize landrace

4.3.5 Relative percentages of maize landrace principal component clusters

Figure 4.2 shows principal components clusters of maize landraces. Cluster 5 had large proportion (49) while cluster 3 had the lowest (4).

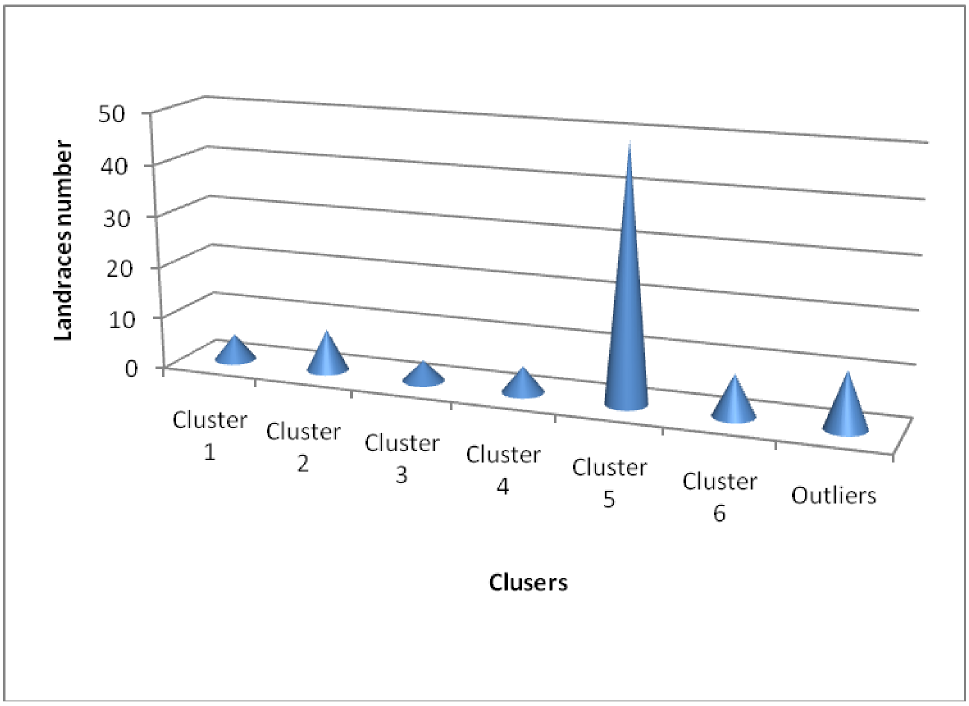

Figure 4.2: Relative numbers of maize landraces per clusters of principal component analysis based on 11 traits evaluated at ARI-Tumbi Tabora for 2008/2009 and 2009/2010 growing seasons

4.3.6 Characteristics of similarity landraces groups

Landraces showed variations among six diverse groups (Table 4.6). The mean leaves per plant in all clusters were 5.86 with the corresponded NLB disease infested leaves of 3.24. There were high variations in lesion length, width and NLB blight incidences.

Table 4.6: Similarity cluster groups of 90 maize landraces evaluated at ARI-Tumbi, Tabora for 2008/2009 and 2009/2010 growing seasons

	PC1	PC2	PC3	PC4	PC5	PC6	Mean	Min	Max	SD	CV
Leaves/plant	5.75	5.75	5.75	5.75	6.03	6.13	5.86	5.75	6.13	0.17	2.95
Infected leaves/plant	2.50	3.75	2.25	4.00	2.41	4.50	3.24	2.25	4.50	0.96	29.76
Lesion number	2.25	4.50	2.00	4.00	2.72	2.50	3.00	2.00	4.50	1.01	33.84
Lesion length (mm)	37.00	55.00	32.63	56.00	70.74	130.13	63.58	32.63	130.13	35.43	55.72
Lesion width (mm)	3.50	6.00	3.88	6.50	8.07	13.75	6.95	3.50	13.75	3.74	53.93
NLB disease incidence %	6.25	11.25	6.25	16.25	11.81	13.75	10.93	6.25	16.25	4.02	36.82
MSV number	2.25	3.50	2.75	0.50	2.36	2.88	2.37	0.50	3.50	1.02	42.93
Silking (days)	72.00	80.75	77.00	71.75	76.97	77.88	76.06	71.75	80.75	3.52	4.63
Tasseling (days)	69.25	78.75	74.75	69.75	73.98	75.00	73.58	69.25	78.75	3.57	4.83
Plant height (cm)	127.75	143.25	147.25	116.50	139.69	156.75	138.53	116.50	156.75	14.37	10.4
Ear height (cm)	47.25	61.13	53.25	45.50	60.78	73.75	56.94	45.50	73.75	10.52	18.47

4.3.7 Cluster analysis

Cluster analysis at cluster distance 10 revealed five distinct groups of landraces (Figure 4.3). The largest cluster I had 61 landraces which were further allocated into two sub clusters. This comprised of a mixture of TZA, ARIT series landraces and commercial varieties. Cluster II had only two landraces TZA 3075 and TZA 4959 all of TZA series. This cluster included the NLB disease resistant landrace TZA 3075. Cluster III was mainly composed of commercial varieties, ARIT and TZA series. Genotypes in these cluster included Situka – M1, Kito-ST, ARIT 5 and TZA 3272. Cluster IV had 6 landraces from TZA, ARIT series and commercial cultivars. This cluster was composed of TZA 3665, ARIT2, TZA 4505, TZA 3644, Kilima and TZA 3795. Cluster V had 17 landraces in two sub clusters. This cluster had a mixture of landraces from different origins. Landraces in this cluster included TZA 4476, TZA 4484, TZA 3741, TZA 2911, TZA 3869, TZA 2882, ARIT 17, TZA 3454, TZA 4409, TZA 3837, SC 627, Lishe H-2, TZA 3114, TZA 3855, ARIT 6, UH615 and TZ 3854. Table 4.7 depicts averages of 11 traits that separate the five clusters in maize genotypes found in Tanzania. Cluster I was mainly separated by lesion length (48.55 cm) and plant height (141.71 cm) while cluster II was segregated by lesion length (8.50 cm). This cluster had NLB disease resistant landrace (TZA 3075). Cluster III and IV had similar characteristics except lesion length, lesion width and plant height (cm). At the same time, cluster IV recorded the highest lesion length (157.33 cm) to imply high susceptibility of landraces in the cluster. Cluster V was mainly separated by plant height (155.73) to denote the tallest landrace group which was further amplified by long days to tasseling and silking. This group comprised of late maturing landraces which could be better for highlands that receive high rainfall, but not ideal for marginal areas like Nzega district in Tabora region.

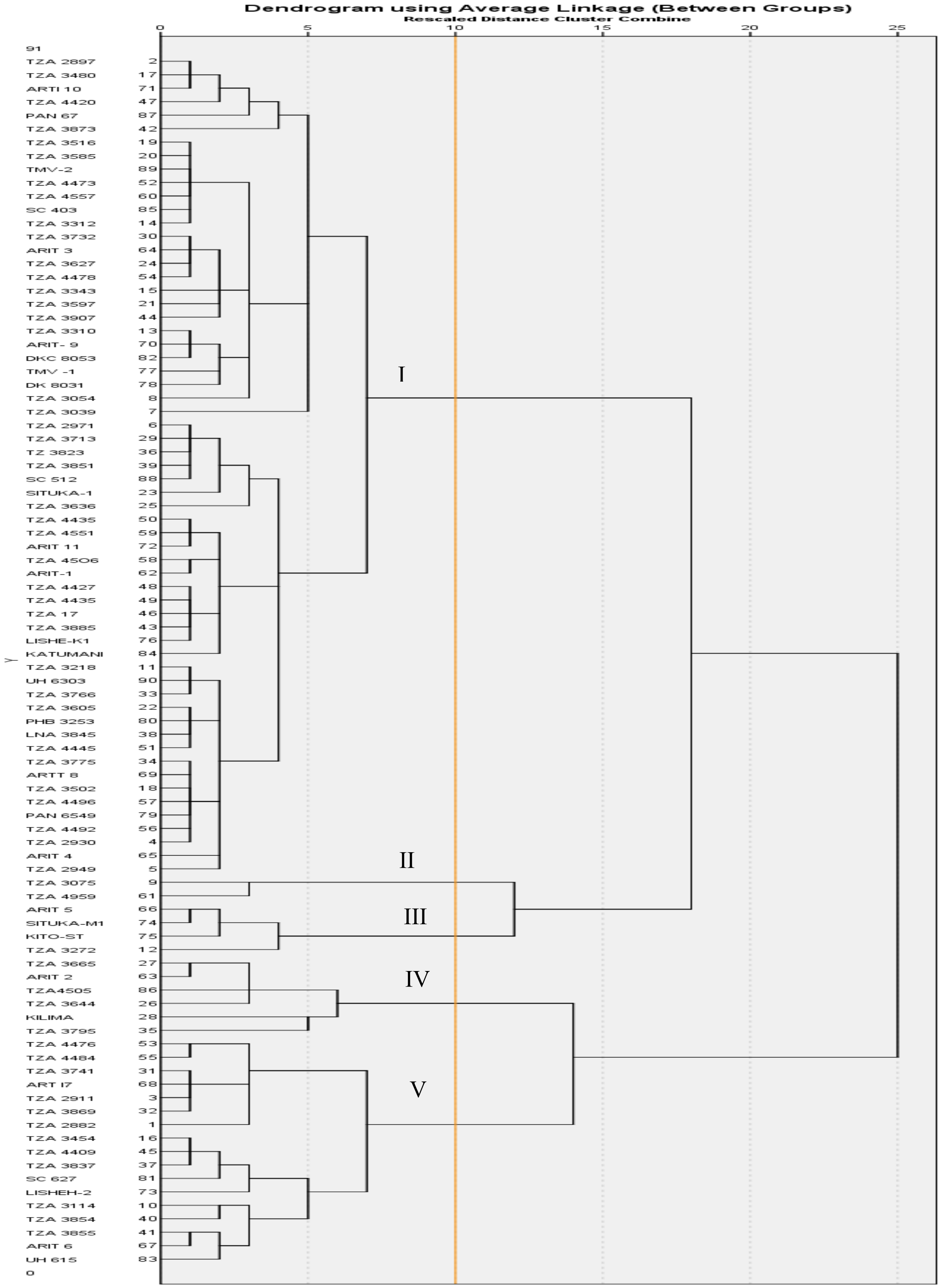

Figure 4.3: The dendrogram for maize landraces based on 11 morphological markers

Table 4.7: Averages of traits associated with 5 clusters in 90 maize landraces assessed at ARI-Tumbi, Tabora for 2008/2009 and 2009/2010 growing seasons

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Leaves/plant	6.03	5.50	6.00	5.50	6.13
NLB infested leaves/plant	2.85	2.50	3.50	3.00	2.33
Lesion number	3.08	2.00	3.00	2.67	2.13
Lesion length (cm)	48.55	8.50	18.50	157.33	131.07
Lesion width (cm)	6.98	1.40	4.50	11.00	10.47
NLB infested plants/plot	2.31	2.50	2.25	2.33	2.40
MSV infested plants/plot	2.84	1.00	1.00	1.67	2.47
Silking days	77.31	73.50	70.00	77.83	81.67
Tasseling days	74.58	71.50	71.00	74.67	80.53
Plant height (cm)	141.71	74.50	78.75	112.50	155.73
Ear height cm)	62.13	55.50	56.75	57.67	68.33

4.4 Discussion

The result showed that, landraces had flint grain texture and white endosperm. This is the implication of food preparation preference. Farmers prefer white grain for local food preparation which is white in clour 'ugali'. The majority of landraces had five scale of husk cover. This had an implication on birds attack. There is bird attack problem in the area. Farmers were obliged to select landraces which high husk covers to manage birds' damage. Husk cover also has implication for ear rot disease infection through providing barriers to maize kernels. Findings also revealed maize yield of 2.29 t ha^{-1} from landraces under research managed trial. It seems that, the low (about 1.0 t ha^{-1}) yield famers get from their fields could also be contributed largely by management practices. However, the yield of 2.29 t ha^{-1} is not the maximum maize production in Tanzania. This could be attributed to inherited low fertility and low organic matter of sandy soils found in the western zone (Nyadzi et al., 2003).

Correlation analysis revealed positive significant correlations among some landrace traits. For example, landraces recorded a highly positive significant (<0.001) correlation coefficient between NLB disease lesion length and width (0.430**). The implication to the breeder is that as lesion length increases, the lesion width also increases and vice versa. The relationship and positive association of lesion length and width could be fatal to maize plant. These findings are in accordance with Abebe and Singburaudom (2006) who reported high correlation of NLB disease severity with lesion number in maize germplasm. The tendency of increasing lesion length in association with lesion width could result to amalgamation of lesion which could end up with a large area of leaves being covered with blight laceration. However, a breeder can make progress by selecting against only one of the traits due to the positive correlation coefficient between them.

Findings also denote a typical characteristic of susceptible genotypes by which sometimes the lesions cover the whole leaf, which could lead to tremendous reduction of photosynthetic area and yield reduction of the range of 46% to 98% under severe conditions (Gowda et al., 1992; Kachapur and Hegde, 1988). Additionally, the positive highly significant correlated traits shown by this study could help breeders in removing redundant traits and concentrate on a few ones to reduce complication of the work and save breeding resources such as human and financial resources of breeders and research institutions. Lasalita-Zapico et al. (2010) found highly significant correlated traits in upland rice and suggested the reduction of redundant traits to reduce breeders work and save resources. In this study of NLB disease resistance, lesion length and lesion width were highly significant positive correlated (0.430), thus a breeder can measure either one of the two because, reduction of one leads to the reduction of the second and vice versa.

The observed lesion length of NLB disease ranged from 190-20mm which is lower than 400-25 mm reported in Kenya (Mwangi, 1998) and closer to 250 -15 mm reported by Degefu (2003). This implies that, there is potential of NLB disease resistance found in local germplasm to be tapped and utilized in maize breeding prorammes. The use of local germplasm for crop improvement has been reported elsewhere (Bertoia et al., 2006; Carpita and McCann, 2008; Ferreira et al., 2008).

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Principal components and cluster analysis further revealed variation among landraces; which was supported by 71.98 % contribution of the total variations by five principal components. Northern leaf blight disease was one of the traits that were associated with principal component two which was among the five top most principal components that contributed to variability in landraces. The high contribution of NLB disease to total variability signifies the importance of the disease in the area and thus appropriate and timely measures are needed to curb the disease.

Based on principal component and cluster analyses, genotypes were grouped according to traits and not necessarily based on origin. This could be attributed to seed exchange among farmers, farmers migration and local seed market (Bucheyeki et al., 2008b; Gwanama and Nichterlein, 1995; Nathaniels and Mwijage, 2000). On the other hand some clusters were big compared to others, for example cluster 5 in principal component analysis had 49 genotypes and cluster 1 in cluster analysis had 61 genotypes. This implies that genotypes were carefully selected by farmers to suite the environments they live in. Through generations farmers have been selecting genotypes to meet their requirements such as drought resistance, low-N, disease resistance, palatability, cooking qualities, storage properties and aroma (Abebe et al., 2005; Bucheyeki et al., 2008a; Soleri et al., 2000). The presence of outliers may be due to farmers seed exchange among farmers and introduction of new cultivars during famine seasons which are frequently supplied by extension departments and farmers retain some grains as seeds. The major objective of farmers is to sustain life through household food security and income generation. Any cultivar that meets farmers' selection criteria is likely to be adopted by the majority of farmers. Farmers select cultivars with similar traits in their farming system and recommendation domain. However, some clusters were very small for example cluster 3 in principal component analysis had 4 genotypes and cluster 2 in cluster analysis had 2 genotypes. This could be attributed to farmers selection according to specialized purposes and uses (Bucheyeki et al., 2011; Nathaniels and Mwijage, 2000). However, further investigation revealed that, those groups had NLB disease resistant landraces like TZA 3075. Because NLB disease resistant landraces were few among the 90 accessions, this could be one of the possible reasons for observation of the landraces groups with few members.

4.5 Conclusions

From this study, the following conclusions can be drawn:

- High variation among maize landraces in morphology and NLB disease reaction were observed. Landraces showed high variation in lesion width with the mean of 7.84 mm and incidence (11.67%). Five principal components contributed 71.98 % of total variations in maize landraces. Leaves /plant, infested leaves/plant, lesion number, lesion length, lesion width and NLB incidence traits highly contributed to variations and grouping of landraces. Landraces were further clustered in five distinct groups of maize landraces to denote their breeding potentials in the area.
- The important agronomic traits found in maize landraces included yield potential, flint grain texture, white endosperm and husk cover. These traits are associated to increased yield, food preparation and insect pest avoidance.
- From 90 evaluated materials, landrace TZA 3075 was identified as NLB disease resistant. This was recorded in principal component 3 which had the lowest NLB incidence of 11.25%. This implies that, TZA 3075 could be a source of resistance for NLB disease.

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Chapter 5

5 Combining ability analysis for northern leaf blight disease resistance on Tanzania adapted inbred maize lines

Abstract

Northern leaf blight disease incited by the fungus *Exserohilum turcicum* has increased in both incidence and severity due to prevalent use of susceptible varieties among other factors. Combining ability information and its interaction to the environment of locally adapted inbred lines is limited. This information is required for the development of resistance to NLB disease in new cultivars. The specific objectives were therefore to estimate the combining ability for NLB disease resistance of 11 maize inbred lines adapted to Tanzania conditions, determine maternal effects which are involved in NLB disease resistance in maize germplasm, and determine the heterosis in the F_1 hybrids. A full 11 x 11 diallel cross was performed in Tanzania. The resulting 110 F_1 hybrids with the 11 parents were evaluated together with 9 commercial varieties at three Agricultural Research Institutes: Tumbi, Uyole and Selian which represent diverse environments. All breeding materials were planted in 13 x 10 alpha lattice design with two replications per site. All top ten experimental hybrids in each site had negative mid parent heterosis for NLB disease severity. Heterosis for NLB disease severity ranged from -94 to 362 %. The overall mid parent heterosis means for yield across sites was 152%. Maternal effects had non significant ($P>0.005$) influence on the inheritance of the NLB disease severity. Mean sum of squares for GCA was highly significant ($P< 0.001$) on disease severity indicating additive gene action. Mean sum of squares for SCA were highly significant on disease severity and yield implying non-additive gene action. At the same time mean squares for reciprocals effects were highly significant for yield and non-maternal effects sums of squares had significant effect ($P<0.05$) on yield. The GCA effects contribution was high for disease severity (91%) and lesion number (85%) to further denote predominance of additive gene action on the disease expression. With the exception of CML 395 and KS03-0B15-12 parents which were susceptible, all GCA effects were negative implying the contribution to disease resistance in their progenies. Due to preponderance of the additive gene action, recurrent selection could be used to improve the resistance of inbreds while the non-additive gene action could be exploited in breeding for disease resistant high yielding hybrids.

5.1 Introduction

Northern leaf blight disease (NLB) also known as turcicum leaf blight or northern corn leaf blight incited by the fungus *Exserohilum turcicum* (syn. *Helminthosporium turcicum), Teliomorph Setophaeria turcica* is an endemic foliar maize disease in the world. The past decades witnessed the disease concentrating in the highlands of the world. However, now it affects both highlands and mid altitude maize growing areas as well causing a significant yield reduction (CPC, 2001; DeVries and Toenniessen, 2001). Maize yield losses caused by NLB disease vary depending on location, pathogen virulence, pathogenicity, plant growth stage, number and position of leaves affected, relative humidity, temperature and susceptibility or resistance of the host plant. Raymundo and Hooker (1981) narrates the grain yield loss of over 50% in the USA, while in India , the yield reductions of about 60% have been reported (Payak and Renfro, 1968). In East Africa, a maize grain yield reduction with the range of 16-24% have been reported (Adipala et al., 1993).

Breeding for host plant resistance remains the most reliable and steadfast method of controlling the disease. Hence, host plant resistant (HPR) is considered as the best option and alternative to deal with the NLB disease problem (Carson, 2006; Turner and Johnson, 1980). However, breeding for HPR largely depends on correct methods of selecting suitable parents to be candidates of breeding for disease resistance. To study the potential, suitability and applicability of breeding materials, breeders apply different mating designs to achieve this purpose. One of the common mating designs is the diallel cross which have been extensively used in evaluations of breeding materials potential in various crops (Christie and Shattuck, 1992; Griffing, 1956; Gupta and Kageyama, 1994; Karaya et al., 2009; Lim, 1975). The diallel cross design enables breeders to estimate general combining ability (GCA) and specific combining ability (SCA) which are frequently used in genetic studies. Literature survey shows that, GCA for NLB disease is normally significant to denote its importance in additive gene action contribution (Vivek et al., 2010). Vivek et al. (2010) further contend that, GCA in NLB disease is area specific and is affected by environmental effects. This means that resistance to NLB disease varies from one location to another unless it is monogenic before breaking down (Vieira et al., 2009; Bigirwa et al., 1993).

The majority of resistance of maize diseases is quantitatively inherited. In maize, NLB disease shows vertical and horizontal resistance inheritance mechanisms (Ceballos et al., 1991). Northern leaf blight disease is reported to be controlled by six dominant Ht1, Ht2, Ht3, HtN, NN and HtM and one recessive ht4 genes (Ferguson and Carson, 2004; Ferguson and Carson, 2007; Pratt, 2006; Singh et al., 2004; Wisser et al., 2006). All these provide qualitative inheritance in the form of dominance or partial dominance. According to Pataky et al. (1998) the HtN gene confers partial resistance to NLB disease. Other researchers have reported on the durable resistance to NLB conferred by major genes (Sharma and Payak, 1990). Ogliari et al. (2005) reports on dominant HtP genes inducing resistance to NLB pathogen and recessive rt genes inducing resistance to specific NLB pathogen races. Several types of gene action are involved in controlling the inheritance of NLB disease in maize. Additive, dominance, and epistatic gene action have been reported in controlling the disease expression. However, additive gene action was found as the most important (Hughes and Hooker, 1971; Ogliari et al., 2005). Maternal effects are less important for the traits associated with the inheritance of NLB. For example, Sigulas et al. (1988) found non significant maternal effect on 16 maize genotypes. Other researchers have reported non significant importance of cytoplasmic and maternal effects on the inheritance of NLB resistance in maize genotypes (Welz and Geiger, 2000).

Cultivars grown by farmers in Tanzania are potentially vulnerable to the NLB disease. Breeding for additional resistant varieties is needed. But, genetic information like GCA and SCA on the available inbred lines which is adapted to Tanzania conditions to be used as sources of NLB disease resistance and hybrid development is not known. Although various studies have been conducted in the world to estimate gene action related to NLB disease resistance and revealed useful information, these findings are limited to specific crosses and in some cases area bounded (Beyene et al., 2011). There is still potential room to widen the resistance genetic base as reports of new occurrence, distribution and resurgence of NLB disease are escalating worldwide. Another challenge facing the NLB disease struggle is the presence of pathogen races. The common known races include 0, 1, 2, 3, 4, 12, 23 and 23N. To make the matter more complex, there is emergence of new races. These create a new dimension to fight war against the pandemic. The recent studies in Kenya by Muiru et al. (2010) revealed 0, 1, 2, 3, N, 12, 13, 13N, 3N, 123, 23 and 23N races to connote the constantly and non-stopping breeding for NLB disease in the area. Therefore, the study on NLB disease resistance was initiated in Tanzania. The overall objective was to study the gene action of NLB disease resistance in inbred maize lines adapted to Tanzania conditions. The specific objectives were to: 1) estimate the combining abilities for NLB resistance of 11 maize inbred lines adapted to Tanzania condition, 2) determine maternal effects which are involved in NLB disease resistance in maize germplasm and, 3) determine the heterosis in the F_1 hybrids.

5.2 Materials and methods

5.2.1 Sources and characteristics of breeding materials

Germplasm used in this study were obtained from screening for NLB disease resistance of 70 breeding materials at ARI-Tumbi in the growing season 2008/2009. The screening study resulted to the selection of 11 parents with various reaction types to NLB disease which were selected for the study (Table 5.1). Cob sizes were assessed by the developed scale of 1-5 where 1= very small, 2= small, 3= average, 4= big, and 5 = excellent while NLB disease reactions assessment was performed according to procedures developed by Reid (2005).

Table 5.1 Characteristics of 11 parents used in a diallel mating

Name	Source	Grain colour	Cob size (1-5)	Rxn
KS03-0B15-126	SARI-Tanzania	White	4	R
EB04-0A01-304	SARI-Tanzania (QPM line)	White	4	R
CML 159	CIMMYT-Mexico	White	5	R
KS03-0B15-2	SARI-Tanzania	White	4	R
KS03-0B15-45	SARI-Tanzania	White	4	R
VL 05616	CIMMYT-Zimbabwe (Vivek)	White	5	R
KS03-0B15-47	SARI-Tanzania	White	5	R
CML 395	CIMMYT-Zimbabwe	White	5	S
KS03-0B15-12	SARI-Tanzania	White	4	S
CML 442	CIMMYT-Zimbabwe	White		R
CKL 05007-B-B	CIMMYT-Kenya (KARI)	White	4	R

Where, Trt= treatment, Rxn = reaction, Num = treatment number, cob size 1= very small, and 5 = bigger.

5.2.2 F_1 hybrid development

Breeding materials used in this study were developed from an 11 x 11 full diallel cross mating design. Crosses were performed at Selian Agricultural Research (SARI in Arusha in Tanzania. These crosses resulted in 55 F_1 and 55 reciprocal families.

5.2.3 Field evaluation

The resulting 110 F_1 progeny and 9 commercial hybrids (Kilima-ST, Kito-ST, Lishe-H1, Lishe-K1, Selian-H208, Selian-H308, Stuka-1, TMV-1 and Vumilia-k1)were planted in 13 x 10 alpha lattice design in three locations with two replications per site. The breeding materials were evaluated in three sites namely ARI-Tumbi, Selian and Uyole in the 2010/2011 growing season. One F_1 hybrid CML 159 X VL 05616 was doubled per replication to make 130 maize genotypes for evaluation. An experiment involving 11 inbred materials was set adjacent to the hybrid trial to avoid inter-plot competition and was planted on the same day to avoid biasness (David et al., 1996). Inbred lines were planted in RCBD with two replications per site. Maize was planted at a spacing of 0.75 m x0. 30 m, which gives a plant population density of 44,444 plants per hectare. Basal fertilizer was applied at a rate of 40 Kg P ha^{-1} and Murate of Potash was applied at the rate of 40 Kg K_2O ha^{-1} Top dressing was done by using UREA (46%) to make a recommended rate of 100 Kg N ha^{-1}. Disease field assessment was conducted at about three weeks after silking of each particular genotype.

5.2.4 Inoculation procedures

To ensure uniform disease infestation, artificial inoculation was conducted according to procedures described by Reid (2005) as follows: A sample grinder machine [Laboratory mill model-4, Thomas –Wiley, Thomas scientific (TM), and U.S.A] was used to grind infected leaves into powder form. Leaves were collected from Tabora, Arusha and Mbeya where trials were conducted and then mixed. A bazooka (Sistrunk Inoculators, Starkville, MS 39759) was used to apply the powder in the whorl of the plants. One dose of powder from the bazooka application amounted to 0.1 g of leaves powder. Two applications, one at 6 – 8 leaf stage and the second at 11-12 leaf stage were conducted. Furthermore, two rows of a spreader local variety (Situka-1) which is highly susceptible to NLB disease was planted around the field and after every 10 rows of the test materials. The uses of spreaders have been used successfully in screening germplasm for other disease studies (Singh et al., 2004). In addition to NLB disease resistance, maize genotypes were assessed for other agronomic characteristics. Other collected agronomic data included: Days to 50% anthesis, days to 50% silking, plant height, ear height, husk cover, kernel type, grain texture and yield. Grain yield was estimated using the following formula:

$$\frac{\text{Grain yield (t/ha)} = \text{FW } (100 - \text{MC}) \text{ X SH\% X } 10{,}000}{(85) \text{ X PS}}$$

Where, FW = field weight of unshelled cobs (kg)

MC = grain moisture content (%)
SH% = Shelling percentage (expressed as a fraction)
10,000 = 1 ha = 10,000 m^2
PS = Plot area (m^2)

85% = grain moisture content adjustment factor. Grain yield was adjusted to 15% (100-15). Data were recorded according to IBPGR (1991) maize descriptors list. Yield and disease resistance data were validated and analyzed in Genstat statistical computer software (Payne et al., 2007).

5.2.5 Data analysis

Collected data were first analysed by analysis of variance (Lindskog and Moldanova, 1994) by using Genstat computer software Payne et al. (2007) to test the existence of significant differences on the measured maize traits. Gene action genetic components were estimated by application of SAS 05 computer programme using Griffing's (1956) Method 1, Model 1 (fixed model) as follows:

$Y_{ijk} = \mu + g_i + g_j + s_{ij} + r_{ij} + \frac{1}{bc}\sum_{kl}\sum e_{ijkl}$

Y_{ijk} = value of F_1 of a cross of the i^th^ female and the j^th^ male in the k^th^ block and i plot/observation

μ = population mean; i = j = 1, 2,…,n.

g_i and g_j = GCA effects of i^{th} & j^{th} parent

s_{ij} = SCA effect ($s_{ij} = s_{ji}$)

r_{ij} = reciprocal effects ($r_{ij} = -r_{ji}$)

e_{ijkl} = error effect for ijklth observation

b = number of replications

c = number of plants/plot

The ratio of GCA/SCA was also assessed. For the ratio greater than 1 indicated additive genetic effect while the ratio lowers than one denoted dominance gene action for the particular traits. The ratio closer to one implies the possibility of prediction based on GCA component only. The use of Griffing's (1956) Method 1, Model 1 provides similar results as other approaches (Hariprasanna, 2008).

5.2.6 Heterosis estimation

Mid parent heterosis was calculated according to Falconer and Mackay (1996) and Saleh et al. (2002) as follows:

MPH (%) = $\frac{F1-MP}{MP}$ x 100 where F_1 = mean of the F_1 hybrids, MPH = mid-parent heterosis and MP = mean of two parents.

5.3 Results

5.3.1 Estimation of general combining ability (GCA) and specific combining ability (SCA)

The model of analysis explained more than 60% of the variations (R^2), R^2 was 85, 64 and 73% for disease severity, yield and lesion numbers respectively to indicate that, the model was adequate (Table 5.2). The environment mean square was highly significant (P<0.001) for severity, yield and lesion number to imply the significant impact of environment on genotypes. The same trend was recorded on genotypes and on replications with the nested environment. The interaction of Environment and hybrids were highly (P< 0.001) significant on disease severity only.

Table 5.2: Mean squares partial analysis of variance on NLB disease severity (%), yield and lesion number of maize hybrids

	Severity (%)		Yield		Lesion number	
Source	DF	MS		MS	DF	MS
Env.	2	8846.89***		92.37***		1241.12***
Rep (Env.)	3	1675.42***		86.82***		765.43***
Genotypes	120	770.3***		11.72***		74.28***
Env. * Genotypes	240	110.26***		2.19		63.9
Error	360	68.89		3.75		68.89
Corrected total	725					
R^2	0.85		0.64		0.73	

*, **, *** indicates significance level at 0.05, 0.01 and 0.001, respectively

Mean sum of squares of GCA was highly significant (P< 0.001) for disease severity and lesion numbers and significant on yield (P<0.05) while SCA mean squares were highly significant for disease severity and yield only (Table 5.3). Highly significant maternal effects (P<0.001) was recorded on yield only. At the same time mean squares for reciprocals were highly significant for yield and non-maternal sum of squares had significant effect (P<0.05) on yield. In addition, % GCA contribution much higher for disease severity (90.81%) and lesion number (85.41%). However, % GCA contribution for yield was (8.71%) to indicate non-additive gene action effects

Table 5.3: Diallel analyses of hybrids for disease reaction and yield over three sites

		Severity (%)	Yield	Lesion number
Observation	Source	MS	MS	MS
1	GCA	8078.13***	8.72*	672.41***
2	SCA	148.67***	16.62***	20.89
3	Reciprocal	63.23	7.37***	18.93
4	Maternal	109.58	15.59***	40.22
5	Non-maternal	52.93	5.55*	14.2
6	GCA x Env.	553.23***	2.12	474.92***
7	SCA x Env.	77.75	2.45	30.57
8	Reciprocal x Env.	62.23	1.93	22.49
9	Maternal x Env.	86.67	1.29	41.97
10	Non-maternal x Env.	56.79	2.08	18.16
%GCA contribution (ss)		90.81	8.71	85.41
%SCA contribution (ss)		9.19	91.29	14.59

*, **, *** indicates significance level at 0.05, 0.01 and 0.001 respectively

Table 5.4 shows the GCA effects of 11 parents used in NLB disease resistance study. All GCA effects had highly significant (P<0.001) effects on disease severity. With the exception of CML 395 and KS03-0B15-12, all CGA effects were negative. Parent EB04-0A01-304 had significant positive GCA effects on yield while VL 05616 and CKL 05007-B-B had significant negative GCA effects on yield (P<0.05). On lesion number, two parents, CML 395 and KS03-0B15-12 had highly significant positive GCA effects on lesion number (P<0.001), while parent CML 442 had negative significant (P<0.05) effects on the same trait.

Table 5.4 Combined general combining ability (GCA) effects for disease reaction and yield of parents over three sites

Parent	Severity (%)	GCA	Yield	GCA	Lesion number	GCA
KS03-0B15-126	9.92	-3.66***	2.07	0.1	4.67	-1.15
EB04-0A01-304	12.03	-3.74***	2.84	0.44**	4.67	-0.89
CML 159	13.82	-4.05***	2.46	0.24	5.33	-1.12
KS03-0B15-2	11.78	-2.95**	2.07	-0.09	4.67	-0.79
KS03-0B15-45	15.88	-3.27***	2.75	-0.13	5.17	-0.87
VL 05616	11.6	-2.40**	2.48	-0.35*	5.83	-0.60
KS03-0B15-47	14.72	-3.46***	2.29	0.25	4.33	-1.43
CML 395	29.2	14.61***	2.19	0.05	7.00	3.87***
KS03-0B15-12	46.93	16.88***	3.39	-0.22	6.50	5.14***
CML 442	11.9	-3.24***	2.37	0.04	5.00	-1.27*
CKL 05007-B-B	10.35	-4.71***	2.56	-0.36*	3.83	-0.87

*, **, *** indicates significance level at 0.05, 0.01 and 0.001 respectively

In all three sites, All GCA had highly significant ($P<0.001$) effects on disease severity. With the exception to CML 395 and KS03-0B15-12, all CGA effects were negative. The same parents had significant positive GCA ($P<0.001$) effects on yield (Table 5.5).

Table 5.5: Site combining ability effects for NLB disease severity and yield

	Tumbi	Uyole	Selian	Tumbi	Uyole	Selian
		Severity			Yield	
Parents						
KS03-0B15-126	-4.16***	-4.40***	-4.99***	-4.41	-1.59	-1.18
EB04-0A01-304	-4.90***	-4.77***	-5.01***	-2.29	-1.44	-0.93
CML 159	-4.31***	-4.65***	-4.56***	-2.27	-1.28	-0.96
KS03-0B15-2	-4.19***	-3.26***	-3.17***	-1.57	-1.16	-0.86
KS03-0B15-45	-4.15***	-2.76***	-2.67***	-1.39	-0.97	-0.76
VL 05616	-3.02***	-4.15***	-4.06***	-2.19	-1.57	-1.27
KS03-0B15-47	-5.12***	-4.95***	-4.85***	-2.24	-1.51	-1.02
CML 395	18.81***	19.26***	19.34***	9.71***	6.47***	4.88***
KS03-0B15-12	20.59***	19.66***	19.76***	9.67***	6.53***	4.78***
CML 442	-4.14***	-4.83***	-4.74***	-2.34	-1.55	-1.15
CKL 05007-B-B	-4.17***	-3.19***	-3.01***	-2.27	-1.32	-0.98

Three hybrids CML 159 x CML 395, VL 05616 x KS03-0B15-12 and KS03-0B15-2 x CML 395 had positive significant (P<0.05) SCA effects on disease severity while hybrid CML 395 x CKL 05007-B-B possessed positive highly significant SCA effects on yields (Table 5.6). Parents KS03-0B15-12 and CML 395 were susceptible to NLB disease. On yield, hybrid VL 05616 x KS03-0B15-12 had positive significant SCA effects (P<0.05) while hybrid CML 395 x CKL 05007-B-B expressed positive highly significant SCA (P<0.001) effects. Positive significant SCA is desired in yield of maize trait. All hybrids had non-significant SCA effects on lesion numbers.

Table 5.6: Means and specific combining ability (SCA) effects of crosses for NLB disease reaction and yield over three sites

Cross	Severity (%)	Yield tha^{-1}	Height (cm)	Ear height (Cm)	Anthesis days	Silking days	SCA (severity)	SCA (yield)	SCA (lesion)
3 x 8	33.42	5.93	206.17	103.33	71.00	72.83	7.89**	0.11	0.02
6 x 9	35.67	6.403	161.67	74.5	69.67	71.33	6.40*	1.45**	1.15
8 x 11	35.8	5.323	190	86.67	70.00	71.67	20.08***	3.39***	0.83
4 x 8	38.3	6.23	164.17	74.83	68.67	70.67	7.71**	0.53	3.2

*, **, * ** indicates significance level at 0.05, 0.01 and 0.001 respectively,, Where, 3= CML 159, 4 = KS03-0B15-2, 6 = VL 05616, 8 = CML 395 and 9 = KS03-0B15-12,

5.3.2 Reciprocal (maternal and non-maternal) effects on yield

Mean squares for SCA effects for yield were highly significant and positive (P< 0.001) while GCA showed positive significant (P<0.005) effects for yield (Table 5.7). Maternal effects (component of reciprocal effects) were also highly significant (P<0.001) for yield. The non-maternal effects were also siginificant but contributed less than the pure maternal effects. The hybrids with positive highly significant effects were KS03-0B15-126 x KS03-0B15-12 and CML 159 x KS03-0B15-12 indicating the contribution of cytoplasmic gene effects. Five parents showed positive significant maternal effects but the expression differed among them. For example parent KS03-0B15-126 showed positive highly significant (P<0.001) while KS03-0B15-47 showed positive significant (P<0.05) maternal effects. Non-maternal positive significant effects were observed in four hybrids. With the exception of KS03-0B15-126 x KS03-0B15-12 which showed positive highly significant effects (P<0.001), the remaining EB04-0A01-304 x KS03-0B15-45, EB04-0A01-304 x KS03-0B15-45 and CML 159 x KS03-0B15-12 showed positive significant (P<0.05) maternal effects.

Table 5.7: Reciprocal, maternal, non maternal effects on yield of parents and hybrids over three sites

Observation	Source	DF	MS
1	GCA	10	8.72*
2	SCA	55	16.62***
3	Reciprocal	55	7.37***
4	Maternal	10	15.59***
5	Non-maternal	45	5.54*
6	GCA x Environment	20	2.12
7	SCA x Environment	110	2.45
8	Reciprocal x Environment	110	1.93
9	Maternal x Environment	20	1.29
10	Non-maternal x Environment	90	2.07
	Cross/parent	**Yield (tha^{-1})**	**Effects**
Reciprocal	1 x 9	3.56	-2.08***
	3 x 6	5.17	-1.22*
	3 x 9	7.06	2.09***
	9 x 7	7.19	1.12*
	5 x 8	2.88	-1.32*
	6 x 10	6.28	1.13*
	8 x 10	7.61	1.61**
Maternal	1	2.07	-0.55***
	2	2.84	0.4*
	7	2.29	-0.32*
	10	2.37	-0.38*
	11	2.56	0.4*
Non- maternal	1 x 9	3.56	-1.7***
	2 x 5	5.71	-1.23*
	3 x 6	5.17	-1.3**
	3 x 9	7.06	1.66*

*, **, *** indicates significance level at 0.05, 0.01 and 0.001 respectively

5.3.3 Heterosis

The studied F_1 hybrids showed variations in the enhanced expression of mid parent heterosis (MPH) for NLB disease severity. At Tumbi, the mean was -12.97% and ranged from -93.46% to 361.99% while at Uyole, the mean was 13.22% and ranged from -92.80 % to 178.81%. At Selian, the mean was 11.14% and the range was from -76.60% to 144.19%. Each site ranked different F_1 hybrids to indicate that, The F_1 hybrids performed differently across sites (Table 5.8).

Table 5.8: Mid parent NLB disease severity heterosis across three sites

Tumbi		Uyole		Selian	
Top ten					
7X5	-93.46	10X5	-92.80	2X10	-76.60
11X2	-91.06	6X4	-91.45	3X5	-76.43
11X4	-90.96	11X4	-90.96	7X3	-75.47
11X8	-89.89	4X5	-85.54	3X2	-72.92
7X4	-84.91	5X4	-85.54	1X 3	-70.51
8X1	-84.66	6X3	-84.26	10X3	-68.90
10X6	-82.98	6X10	-82.98	11X10	-68.54
11X6	-81.78	11X6	-81.78	11X7	-68.09
6X1	-81.41	5X3	-79.80	11X1	-65.47
7X3	-78.98	3X10	-76.67	4X11	-62.04
Middle ten					
5X6	-41.78	4X10	1.35	5X10	7.99
4X10	-40.88	2X6	1.57	5X1	8.53
2X1	-36.22	9X11	4.75	2X1	9.34
11X7	-36.18	11X9	4.75	2X7	9.91
9X10	-35.41	9X8	5.08	9X1	10.82
10X9	-35.41	1X9	5.54	5X9	11.45
3X9	-34.16	7X6	6.38	6X1	11.52
10X7	-32.38	10X11	7.87	8X7	11.57
7X9	-31.87	5X10	7.99	8X2	11.57
11X1	-30.93	3X6	10.15	7X9	13.54
Bottom ten					
8X7	95.81	10X6	104.26	11X8	87.10
10X8	118.98	1X 3	110.61	2X8	89.18
4X7	126.42	6X5	118.34	1X 4	93.55
6X8	145.10	1X11	126.94	4X8	104.98
3X11	148.24	1X8	130.06	8X4	104.98
5X7	161.44	3X8	132.45	3X8	111.53
4X5	189.23	8X3	132.45	8X11	112.39
1X11	196.00	8X6	145.10	5X8	121.83
7X10	200.53	8X11	152.84	10X8	128.71
2X4	361.99	1X6	178.81	1X5	144.19
Statistics					
Mean	-12.97		13.22		11.14
SD	76.49		63.74		50.67
Minimum	-93.46		-92.80		-76.60
Maximum	361.99		178.81		144.19
Grand mean					3.80

Where, 1 = KS03-0B15-126, 2 = EB04-0A01-304, 3= CML 159, 4 = KS03-0B15-2, 5 = KS03-0B15-45, 6 = VL 05616, 7 = KS03-0B15-47, 8 = CML 395, 9 = KS03-0B15-12, 10= CML 442 and 11 = CKL 05007-B-B

The maximum mid parent heterosis for yield was higher than 350% in all sites.

At Tumbi, the mean was 133.60 and ranged from -27.13 to 367.37 while at Uyole, the mean was 141.62. and ranged from -45.30 to 352.98. At Selian, the mean was 180.32. and the range was from -12.55 to 460.84. The F_1 hybrids behaved differently in terms of yield heterosis among sites (Table 5.9).

Table 5.9: Mid parent NLB disease yield heterosis across three sites

Top ten	Tumbi		Uyole		Selian
7X3	367.37	3X5	352.98	2X7	460.84
5X1	310.79	4X10	350.45	4X8	392.95
8X10	268.42	3X10	337.27	8X4	342.11
3X1	257.62	3X1	332.67	4X7	329.14
8X7	248.21	6X3	304.86	6X1	317.86
2X1	242.16	8X7	301.79	3X5	310.59
2X7	239.18	4X5	295.02	10X1	307.02
4X8	238.03	8X1	294.37	10X4	305.63
8X4	238.03	5X10	275.00	3X8	305.63
11X7	234.02	4X1	274.88	8X10	300.82
Middle ten					
10X8	150.00	9X7	153.52	1X 4	200.00
1X7	147.71	6X7	151.57	4X9	197.25
4X11	146.22	2X5	150.45	5X3	195.46
11X1	146.22	2X9	147.83	1X10	195.46
4X2	144.40	8X9	143.73	11X5	193.28
8X5	142.92	4X11	141.90	3X9	191.50
2X10	141.84	1X5	140.66	2X6	190.21
7X2	133.92	8X2	138.57	4X3	180.70
1X 4	131.88	7X1	138.53	1X8	178.26
4X9	130.77	7X4	138.53	7X9	176.92
Bottom ten					
5X11	24.29	8X11	9.47	10X5	49.15
9X3	23.08	7X11	7.22	6X5	47.69
6X4	18.68	9X1	6.96	4X6	42.42
10X9	14.58	9X4	2.56	10X11	37.50
11X3	7.57	9X2	-3.69	11X10	29.08
9X6	2.21	3X11	-20.32	9X5	22.66
7X11	-1.03	6X4	-20.88	9X3	18.76
5X10	-6.25	5X11	-32.20	6X4	12.50
3X11	-16.33	5X8	-35.22	10X9	0.40
5X8	-27.13	9X3	-45.30	9X6	-12.55
Statistics					
Mean	133.60		141.62		180.32
SD	76.96		87.74		92.35
Minimum	-27.13		-45.30		-12.55
Maximum	367.37		352.98		460.84
Grand mean					151.84

Where, 1 = KS03-0B15-126, 2 = EB04-0A01-304, 3= CML 159, 4 = KS03-0B15-2, 5 = KS03-0B15-45, 6 = VL 05616, 7 = KS03-0B15-47, 8 = CML 395, 9 = KS03-0B15-12, 10= CML 442 and 11 = CKL 05007-B-B

5.3.4 Yield performance and NLB disease reactions of parent materials

There were no significant differences (P> 0.05) in yield performance across test sites implying stability of inbred lines across environments (Table 5.10). On severity, testing environments differed significantly (P<0.001) with ARI Selian site recording the highest severity (22.9%) followed by ARI-Uyole 17.95%. Genotype performance across sites showed significant difference (P<0.001) on NLB disease lesion number with ARI-Uyole recording the highest (8.68) while ARI-Tumbi showed the lowest (1.55). On lesion length, there were also highly significant differences (P<0.001) across sites with ARI-Selian recording the highest (156.5 mm) followed by ARI-Uyole 26.1mm).

Table 5.10: Yield and disease reaction of 11 inbred lines over three sites

		Yield tha-^{1}			Severity (%)			Lesion number			Lesion length(mm)	
Genotype	Tumbi	Uyole	Selian	Tumbi	Uyole	Selian	Tumbi	Uyole	Selian	Tumbi	Uyole	Selian
KS03-0B15-126	1.8	2.8	1.6	10.5	8.5	10.75	2.5	9	2.5	11	17.5	57.5
EB04-0A01-304	3.6	1.31	3.6	0	13	23.1	0	10	4	0	27	100
CML 159	2.55	2.28	2.55	14.5	10.5	16.45	1.5	9	5.5	8.5	22.5	143
KS03-0B15-2	2.55	1.1	2.55	7.5	10	17.85	1	7.5	5.5	7.3	19.5	275.5
KS03-0B15-45	2.62	3	2.62	5	18.5	24.15	1	10	4.5	2.5	40.5	159
VL 05616	2.1	2.64	2.7	6	12	16.8	1.5	10	6	2.4	27	192
KS03-0B15-47	2.1	2.68	2.1	16	14.5	13.65	1	8	4	2.5	27	60
CML 395	2.55	2.42	1.6	15	32	40.6	4	10	7	12.8	40.5	135
KS03-0B15-12	2.7	4.78	2.7	38	51	51.8	4	8.5	7	12	19.5	193
CML 442	3.15	2.12	1.85	0	17.5	18.2	0	8.5	6.5	0	35.5	180
CKL 05007-B-B	3.3	1.44	2.95	2.5	10	18.55	0.5	5	6	3.5	10.5	226
Mean	2.64	2.42	2.44	10.45	17.95	22.9	1.55	8.68	5.32	5.7	26.1	156.5
Lsd		0.756			4.014			1.074			33.4	
Cv		49.3			38.2			33.8			86.7	
Prob		0.805			<.001			<.001			<.001	

5.3.5 Yield performance of hybrids

The yield performance of experimental F_1 hybrids and commercial hybrids did not differ ($P>0.05$) significantly across sites (Table 5.11). However on NLB disease severity, breeding materials showed highly significant ($P<0.001$) differences across sites. All commercial hybrids showed susceptibility to NLB disease with the scale of more than five. At ARI-Tumbi, the range was 4.85 – 6.18, while at Uyole the range was 5.90 – 7.19. At the same time the range at Selian was 5.08 – 6.93. Based on NLB resistance ranking performance of hybrids, there were no hybrids which performed consistently across sites (Table 12). However, on NLB disease resistance mean, ARI-Tumbi had the lowest (3.03) followed by Selian (4.38) and Uyole (4.39). For experimental hybrids, the majority showed resistance to NLB disease reactions. For example the range at Tumbi was 1.57 – 2.55, while at Uyole, the range was 1.37 – 7.24 and at Selian, the range was 1.83 – 6.97 of the square root transformed NLB disease severity percentage.

Table 5.11: Yield (tha^{-1}) performance of hybrids across three sites

Tumbi		Uyole		Selian	
Top ten	Yield		Yield		Yield
7X11	4.65	6X10	5.30	7X3	9.54
11X4	5.85	1X7	6.70	3X7	9.18
6X5	3.75	8X5	7.32	2X10	8.82
7X5	6.30	11X6	4.76	8X10	8.82
9X11	4.35	7X10	4.16	8X7	8.82
4X5	5.70	2X10	8.84	10X4	8.46
8X2	5.40	3X2	7.20	2X4	8.28
3X2	4.80	5X4	3.90	9X1	8.28
5X9	5.10	4X5	8.16	11X6	8.10
3X10	6.45	11X10	7.02	2X7	7.92
Middle five					
9X4	3.00	9X5	7.20	2X5	6.12
10X4	7.05	10X4	6.70	3X6	6.12
8X1	6.00	1X5	5.90	5X4	6.12
9X5	4.50	7X1	5.72	5X9	6.12
4X3	3.75	8X4	5.50	6X8	6.12
Bottom five					
5X10	3.90	8X11	3.76	9X3	3.60
3X7	7.65	10X1	3.70	9X4	3.60
7X6	4.20	7X8	7.40	1X9	3.43
4X8	6.30	11X4	6.80	3X11	3.24
4X6	4.50	6X8	5.84	5X8	2.70
Commercial checks					
Kilima-ST	6.90		5.40		5.16
Kito-ST	2.25		2.15		3.15
Lishe-H1	3.75		6.80		5.25
Lishe-K1	3.30		4.92		5.62
Selian-H208	5.85		7.70		6.19
Selian-H308	4.20		6.30		5.88
Stuka-1	3.40		5.32		4.76
TMV-1	4.20		5.32		5.81
Vumilia-k1	5.70		5.90		5.48
Statistics					
Mean	5.04		6.31		6.05
Lsd	3.348		4.377		3.895ns
Cv	33.60		35		32.5
Prob	0.542ns		0.293ns		0.581ns

Where, 1 = KS03-0B15-126, 2 = EB04-0A01-304, 3= CML 159, 4 = KS03-0B15-2, 5 = KS03-0B15-45, 6 = VL 05616, 7 = KS03-0B15-47, 8 = CML 395, 9 = KS03-0B15-12, 10= CML 442 and 11 = CKL 05007-B-B

Table 5.12: NLB disease severity of hybrids across three sites for 2010/2011 growing season

Tumbi		Uyole		Selian	
Top ten					
7X1	1.57	11X4	1.37	11X3	1.83
11X6	1.41	4X5	1.57	1X 3	2.05
5X4	2.09	10X5	1.62	4X11	2.14
11X7	2.41	5X7	1.71	11X1	2.16
10X2	2.85	6X10	1.83	3X10	2.28
3X7	1.62	11X7	1.98	11X7	2.32
3X1	1.5	3X7	1.98	11X10	2.5
1X7	1.5	4X11	2.09	11X4	2.51
8X7	5.34	4X2	2.12	7X3	2.52
1X 4	2.3	1X7	2.16	3X5	2.56
Middle four					
4X9	2.03	3X1	3.74	4X2	3.65
2X7	3.57	3X6	3.74	4X3	3.65
10X6	1.71	10X6	3.77	10X7	3.65
3X5	2.16	2X11	3.81	1X7	3.67
Bottom four					
10X1	1.98	8X9	6.98	8X11	6.84
5X3	1.87	8X6	7.07	5X8	6.86
10X7	2.21	6X9	7.1	3X8	6.87
2X1	2.55	8X3	7.24	9X11	6.97
Commercial hybrids					
Kilima-ST	5.09		6.51		6.48
Kito-ST	5.56		6.04		5.60
Lishe-H1	5.14		6.12		6.74
Lishe-K1	5.24		6.40		5.08
Selian-H208	5.57		6.85		6.70
Selian-H308	5.72		5.90		6.93
Stuka-1	4.85		7.19		5.66
TMV-1	6.18		6.17		5.53
Vumilia-K1	5.43		6.20		5.29
Statistics					
Grand mean	3.03		4.39		4.38
Lsd	2.251		2.2048		1.4464
Cv	37.50		25.40		16.70
Prob	<0.001		<0.001		<0.001

Where, 1 = KS03-0B15-126, 2 = EB04-0A01-304, 3= CML 159, 4 = KS03-0B15-2, 5 = KS03-0B15-45, 6 = VL 05616, 7 = KS03-0B15-47, 8 = CML 395, 9 = KS03-0B15-12, 10= CML 442 and 11 = CKL 05007-B-B

5. 4 Discussion

5.4.1 Estimation of general combining ability (GCA) and specific combining ability (SCA)

The analysis model of (R^2) explained 85, 64 and 73% effects for disease severity, yield and lesion numbers respectively to indicate that, the model was adequate. Level of significant differences among genotypes indicated by mean squares were also tested to justify the use of GCA and SCA and found to be highly significant ($P<0.001$) which fulfilled conditions suggested by Griffing (1956). Results revealed highly significant ($P<0.001$) environment mean squares for disease severity, yield and lesion number implying the significant impact of environment on genotypes. This could be caused by weather, edaphic factors and initial inoculum concentrations in the test sites which call for area specific cultivar recommendations as suggested by Macharia et al. (2009).

Mean sum of squares of GCA was highly significant ($P< 0.001$) on disease severity and lesion numbers and significant on yield ($P<0.05$) to denote the additive gene action expression. At the same time SCA mean squares were highly significant on disease severity and yield only to imply non-additive gene expression on those traits. These findings suggest that, both additive and non-additive gene effects are involved in the expression of NLB disease resistance in maize. Other researchers have reported on the additive and non-additive gene actions to express NLB disease resistance in maize (Dingerdissen et al., 1996; Ogliari et al., 2005; Okello et al., 2006; Vieira et al., 2009). The percentage of GCA contribution was 90.81% and 85.41% for disease severity and lesion numbers respectively. By considering levels of significant through mean squares and percentage of GCA contribution, it was obvious that additive gene action was more important than non-additive gene action for the expression of NLB disease resistance in maize germplasm which is in accordance to the findings of Sigulas et al. (1988).

5.4.2 Parents combining ability on disease severity and yield

In disease resistance studies, negative GCA is desired while positive GCA effects are not desired. The GCA of nine parents had negative highly significant ($P<0.001$) effects on disease severity indicating additive gene effects for resistance. On yield, parent EB04-0A01-304 had significant positive GCA effects. Parents with positive GCA have the potential to impart high yielding characteristics to the next generation. Therefore, the combination of parents with negative GCA for disease severity and positive GCA for yield are likely to produce high yielding F_1 hybrid.

Individual sites analysis also showed similar results for NLB disease severity inheritance. All parents had highly negative significant effect for NLB disease severity with the exception to CML 395 and KS03-0B15-12. However, on yield the same CML 395 and KS03-0B15-12 parents had significant positive GCA ($P<0.001$) effects for yield. This means that these parents contributed to NLB disease susceptibility at the same time contributed to high yield potential. Based on individual site analysis, parents like CML 395 and KS03-0B15-12 need to be improved for NLB disease resistance so as to have parents with both high yielding and resistant potentials.

5.4.3 Specific combining ability (SCA) on NLB disease severity on F_1 hybrids

The positive significant ($P<0.005$) SCA effects on disease severity showed by F_1 hybrids indicates the non-additive gene action as expressed in CML 159 x CML 395 and VL 05616 x KS03-0B15-12 hybrids which could be probably due to masked susceptibility or maternal cytoplasmic effects of VL 05616 and CML 159 parents . However, the clear reason was not established by this study.

5.4.4 Reciprocal, maternal and non- maternal effects on yield

This study revealed that reciprocal effect which is associated with maternal and non-maternal effects were involved in yield expression. These gene actions have the tendency of reducing breeding efficiency through masking genetic variance (Durga et al., 2008). The significant contribution of reciprocal, maternal and non-maternal effects on yield indicates the contribution of cytoplasmic genes and nuclear gene effects to bring an impact on maize yield. Some environmental factors like drought can enhance maternal effects as recorded by Derera et al. (2008). Thus, appropriate mating designs such as diallel cross and North Carolina design II can be employed to improve maize breeding procedures which encompass estimation of reciprocal effects depending on the number of parents to be used in the study (Hallauer and Miranda, 1988).

5.4.5 Heterosis

Based on mid parent heterosis (MPH), F_1 hybrids showed variations in the enhanced expression of disease severity and yield. The MPH was significantly higher for disease severity across sites to imply dominance gene action effects on the studied germplasm. Negative heterosis is desired in the studies of NLB disease resistance. All top ten experimental hybrids in each site had negative heterosis. On yield, a positive heterosis is desired. High positive heterosis on yield indicates the superiority performance for NLB disease reactions of F_1 hybrids. The overall mid parent heterosis mean across sites was 151.84%. These results agree with Saleh et al. (2002) who recorded much higher heterosis in maize.

5.4.6 Parents, commercial cultivars and F_1 hybrid breeding potentials

The high variations in terms of NLB disease reactions and yield associated traits showed by parents implied high potential for breeding progress and yield increase in maize production. The selected inbred lines clearly expressed their traits across three diverse agro-ecological zones to show genetic potential for NLB disease reactions. The mean severity was higher at ARI Selian (22.9%) and ARI-Tumbi had the lowest (10.45%). The higher disease prevalence on parents and hybrids at ARI-Uyole and ARI-Selian could be attributed to weather conditions, land use and increased pathogen pathogenicity. Uyole is located in the highlands with favourable rainfall and temperature for disease development while Selian records more rainfall than Tumbi site. Uyole and Selian sites are characterized by land shortage which results in intensive land use with limited crop rotation flexibility. These practices result in accumulation of inoculums in maize stovers and alternative hosts. On the other hand ARI-Tumbi has less rainfall compared to the other two sites. At the same time, Tumbi site is characterized by abundant land to allow crop rotation, natural fallow, improved fallow and improved woodlots (Nyadzi et al., 2006). Frequent bush fire and free range animal grazing system which are common practices at Tumbi area could be other factors leading to relatively low level of NLB disease infestation in that area.

All commercial hybrids showed susceptibility to NLB disease with the scale of more than five. According to Ngugi et al. (2002) and Reid (2005), the detected commercial cultivar susceptibility can be classified as medium to high. Thus, all those commercial cultivars succumbed to NLB disease indicating their susceptibility. This implies that, farmers in Tanzania are growing NLB disease susceptible cultivars which could justify the disease resurgence and outbreaks in the near future if appropriate measures are not put in place. However, there was differential performance of individual hybrids to signify the importance of interactions which calls for area specific breeding procedures.

5.5 Conclusions

The following conclusions were drawn from this study:
The following were the research highlights from the study:

- This study showed the predominance of additive gene action for controlling the NLB disease resistance in maize. In the study the mean sum of squares for GCA was highly significant (P< 0.001) on disease severity indicating the predominance of additive gene action. This was further emphasized by the high GCA contribution for disease severity (91%) and lesion number (85%). The majority of parents had negative CGA to imply contribution to disease resistance on their progenies.
- The mid parent heterosis for NLB disease severity ranged from -93.46 to 361.99%. Genotypes with negative heterosis to NLB disease is desired because they imply superiority performance of progenies towards resistance direction.
- In this study reciprocal and maternal effects had non significant (P>0.05) effects on the inheritance of the NLB disease severity.
- Due to preponderance of the additive gene action, recurrent selection could be used to improve the resistance of inbreds while the non-additive gene action could be exploited for breeding resistant hybrids in maize breeding programme.

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Chapter 6

6 Generation mean analysis of northern leaf blight disease resistance in five tropical maize inbred lines

Abstract

Information on additive, dominance and epistatic effects of the highly adapted to Tanzania condition inbred maize lines for NLB disease resistance is not available. The main focus of the study was to estimate in detail the non-additive gene action. Therefore the study was conducted to determine the mode of gene action involved in the inheritance of resistance to NLB disease in five inbred lines adapted to Tanzania at contrasting environments, estimate heterosis and heritability in five tropical inbred lines. Generation mean analysis was conducted using five inbred lines in a six parameter model comprising P_1, P_2, F_1, F_2, BCP_1 and BCP_2 generation progenies. The trial was executed at three sites with three replications and three sets per site. In total, 1293 plants were individually evaluated per set in each site. The mean sum of squares for environment, replication with the nested environment, generations, generations x environment interactions were highly significant ($P<0.001$). The full model of additive, dominance, additive x additive, additive x dominance epistatic effects and dominance x dominance epistatic effects was highly significant ($P<0.001$). Nonetheless, the additive gene effects were predominant ranging between 57% - 89% which was confirmed by large heritability (54-85%). The average degree of dominance ranged between -0.52 - 0.88 supporting observations of partial dominance. The NLB disease severity showed the continuous distribution in all sets for F_2, BCP_1 and BCP_2 populations which is the indication of quantitative nature of inheritance and additive gene effects. The mid parent heterosis ranged from -19 - 1%. Resistance to NLB disease could be improved through selection by exploiting the additive gene effects. The epistatic gene effects would cause less complications because they are negligible (<25%).

6.1 Introduction

Northern leaf blight disease caused by fungus *Exserohilum turcicum* remains endemic problem in maize production since was discovered in Italy by Passerini (1876). There are several studies that reports on the inheritance of NLB disease in maize. For example, on gene actions, many reports contend that both additive and dominance gene actions are involved in the expression of disease resistance in maize; however additive is more important than dominance (Dingerdissen et al., 1996; Ogliari et al., 2005).

Studies on the minimum number for NLB disease development, heritability and heterosis of the disease reveal important information that leads to designing appropriate breeding strategies. Hakiza et al. (2004) crossed H99 (resistant) X A619 (susceptible) to estimated minimum number of genes for lesion development. The study revealed the range of 2-3 for lesion development and 8 when assessed on family basis. Earlier, Hooker (1961) reported 3-6 minimum number of genes for partial resistance in maize. However, Dingerdissen et al. (1996) reported 4-8 QTLs which are linked to NLB disease resistance in Kenya. On heritability, a range of 0.40 – 0.70 heritability have been reported by using GMA approach in maize germplasm (Hughes and Hooker, 1971). Recently, Chaudhar and Mani (2010) used GMA analysis on resistant inbred line V335 x V13 (susceptible) cross to study the heritability of NLB disease and found that it is in the range of 35.42-84.44%. Another parameter frequently used by plant breeders is heterosis effects (Beck et al., 1990; Mungoma and Pollak, 1988). Heterosis is used to estimate the enhanced performance of hybrids compared to their parents. A negative heterosis in disease resistance is preferred.

Generation mean analysis is frequently used for inheritance of disease studies. It is a robust tool that partitions additives, dominance gene action and epistatic non-allelic interactions effects. This method has the ability to partition gene effects as additive, dominance, additive x additive, additive x dominance and dominance x dominance. Several studies have studies the inheritance of the NLB disease in maize by using generation mean analysis (Hakiza et al., 2004; Okori et al., 1999). In addition, progeny studied in generation mean analysis can be incorporated directly in the breeding programme. Generation mean analysis also uses population means to reduce the confounding errors brought by variances for genetic effects estimation (Mather and Jinks, 1982). However, many GMA studies have reported on a single cross. Generation mean analysis that involves several crosses under diverse environments are minimal. In addition results obtained sometimes were confined to specific cross and environment.

There are maize inbred lines locally adapted to Tanzania condition developed by maize breeding programme. However additive, dominance and epistatic effects information for NLB disease resistance on these breeding materials under different environments is limited. Therefore, it could be imperative to investigate gene action that confers NLB disease resistance in the well adapted to Tanzania environment inbred lines.

This study used generation mean analysis approach in five inbred lines P145-95, CML 395, K530-106-1, P86-95-1 and E29 which are well adapted and frequently used to develop hybrids in the maize breeding programme of Tanzania. However, these inbred lines were not previously studied on NLB disease reactions In addition; these inbred lines are high yielding, resistant to other common diseases like gray leaf spot, maize streak virus disease and have farmers preferred traits. The objectives of the study were therefore to the determine mode of gene action involved in the inheritance of resistance to NLB disease in five inbred lines adapted to Tanzania at contrasting environments and estimate heterosis and heritability among inbred lines adapted to Tanzania.

6.2 Materials and methods

6.2.1 Sites description and soil analysis

The trial was conducted at three sites namely: Agricultural Research Institutes Tumbi, Uyole and Selian. Tumbi is located at (1121masl, 05^{0}64.850 S, 032^{0}66.701 E) found in the lower and midaltitude maize growing agro-ecological zones with relatively moderate rainfall. Soils are sandy loams with low organic carbon percent compared to the other two sites. These characteristics have an implication on NLB disease occurrence and maize yield. The two other sites, Uyole (1804 masl, 08^{0}55.105 S, 033^{0}31.463 E) and Selian (1519 masl, 03^{0}18.553 S, 036^{0}38.051) receive relatively higher rainfall with somehow fertile soil to denote more favourable condition for NLB disease prevalence.

6.2.2 Sources of breeding materials

Four breeding materials P145-95, K530-106-1, P86-95-1 and E29 used in this study were supplied by Dr Nick Lyimo from Agricultural Research Institute-Uyole in Tanzania who prior successfully screened them against NLB disease resistance for several years. One parent CML 395 was sourced from CIMMYT-Zimbabwe and it is widely used inbred tester in Africa. Parents P145-95, K530-106-1 and E29 were used as resistant while P86-95-1 and CML 395 were susceptible to the NLB disease.

6.2.3 Maize population development

Five parents were planted at Uyole Agricultural Institute in Mbeya during 2008/2009 growing season. Three NLB disease resistant inbred lines P145-95, K530-106-1 and E29 were crossed to two susceptible lines to get F_1 breeding materials. Resistant lines were used as male while the susceptible lines were used as female parents. Three sets of crossing were used as follows: Set A (P145-95 X CML 395), set B (K530-106-1 X P86-95-1) and set C (E29 X P86-95-1). The F_1 materials were advanced to F_2s by selfing while BCP_1 were obtained by backcrossing to resistant parent (P_1) and BCP_2 were obtained by backcrossing to susceptible parent (P_2) during 2009/2010 rain season. During back crossing, F_1s were crossed to their parents which were used as females.

6.2.4 Field experimentation

The six P_1, P_2. F_1, F_2, BCP_1 and BCP_2 populations per cross (set) were evaluated in 2010/2011 rain season at three sites. Evaluation sites included Agricultural Research Institutes Tumbi in the western zone, Uyole in the southern highland and Selian in the northern zone of Tanzania. These zones represent the bulk of the maize growing agro-ecological zones in the country. Populations were planted in three replications per site at spacing of 0.75 x 0. 30 m, this gives a plant population of 44,444 maize plants per hectare. Fertilizer, NPK basal fertilizer was applied at a rate of 40 Kg P ha^{-1} and Murate of Potash was applied at 40 Kg K_20 ha^{-1}. Top dressing was applied using UREA (46%N) to make a recommended rate of 100 Kg N ha^{-1}. Two rows of P_1, P_2 and F_1 were used while for F_2, BCP_1 and BCP_2 seven rows of 5.1 m were employed per set to conduct the experiment.

6.2.5 Inoculation procedures

Artificial inoculation was conducted as described by Reid (2005) as follows: A sample grinder machine [Laboratory mill model-4, Thomas –Wiley, Thomas scientific (TM) U.S.A] was used to grind infected leaves into powder form. An instrument called Bazooka G was used to apply the powder in the whorl of plants. One dose of powder from Bazooka application amounts to 0.1 g of leaves powder. Two applications, one at 6 – 8 and the second at 11-12 leaf stage were conducted. In addition, two rows of a spreader local variety (Situka-1) which is highly susceptible to NLB disease was planted around the field and after every 10 rows of the test materials. The uses of spreaders have been used successfully in screening germplasm in other disease studies (Singh et al., 2004).

6.2.6 Data analysis

Data was validated in Microsoft Excel while frequency distribution, mean and variances were computed in Genstat statistical computer programme (Payne et al., 2007). Square root transformation was performed on NLB disease severity to normalize data before analysis.

6.2.7 Narrow sense heritability

Narrow sense heritability was estimated according to Warner (1952) by using the following equation :

$h^2 = 100*(2\ \sigma^2F_2 - (\sigma^2BCP_1 + \sigma^2BCP_2)/\ \sigma^2F_2$

Whereby: h^2 = narrow sense heritability, BC = backcross, P_1 = parent 1, P_2 = parent 2, σ^2F_2 = total variance of F_2 and σ^2_g = genetic variance.

Genetic variance was computed as:

Genetic variance = $\sigma^2g = \sigma^2F_2 - \sigma^2_e$

$\sigma^2_e = (nP_1s^2P_1 + nP_2s^2P_2 + NF_1s^2F_1/\ (Ne)$

Whereby σ^2_e = environment variance, $Ne = nP_1 + nP_2 + nF_1$, n = number of plants per generation, s^2P_1, s^2P_2, and s^2F_1 = variance for parent 1, parent 2 and F_1 respectively.

6.2.8 Estimation of heterosis

Only mid parent heterosis was estimated. The estimation was based on F1s against their corresponding parents performance and was computed using the following equation:

Mid parent heterosis (MPH) % = $100*([M_{F1} - (MP1 + M_{P2})/2]/\ (MP1 + M_{P2}/2$

Whereby M_{F1} = mean performance of F_1, M_{p1} = mean performance of parent 1 and M_{p2} = mean performance of parent 2

6.2.9 Estimation of average degree of dominance

Variances were variable and thus weighted was necessary. These were calculated as the inverse of mean for each generation. Estimation of average degree of dominance was estimated to assess the importance of dominance gene actions in the studied germplasm. Estimation was based on the following equation:

Average degree of dominance (ADD) = M_{F1}-(M_{P1} + M_{P2})/2]/ (M_{P1} - M_{P2}/2 (Mather and Jinks, 1982).

Whereby M_{F1} = mean performance of F_1, M_{p1} = mean performance of parent 1 and M_{p2} = mean performance of parent 2

6.2.10 Estimation of genetic effects

Genetic effects were estimated according to procedures developed by Kang (1994) whereby a full mean generation model was employed in SAS05 computer statistical programme. The following full model was used:

$Y = m + \alpha a + \beta d + \alpha^2 aa + 2\alpha\beta ad + \beta^2 dd$

Where:

Y= Genetic effects

m = mean of parental homozygotes

α and β= coefficients determined by degree of population relationships

a = additive genetic effects

d = dominant genetic effects

aa = epistatic additive x additive effects

ad = epistatic additive x dominant effects

dd = epistatic dominant x dominant effects.

Other researchers used six generations and six parameters model in generation mean analysis (Shashikumar et al., 2010).

6.3 Results

6.3.1 Generation means analysis and environmental effects

In all sets, mean sum of squares for environment, replication with the nested environment, populations, population x environment interactions were highly significant (P<0.001) to denote their important contribution to variation (Table 6.1).

Table 6.1: Generations means of NLB disease severity analysis for six maize populations evaluated in Tanzania for 2010/2011 growing season

	Set A		Set B		Set C	
	P145-95 X CML 395		K530-106-1 X P86-95-1		E29 X P86-95-1	
Cross	R x S		R x S		R x S	
Source	DF	Mean squares	DF	Mean squares	DF	Mean squares
Env.	2	483.41***	2	47.23***	2	190.37***
Rep (Env.)	6	42.16***	6	41.72***	6	53.91***
Gen.	5	326.35***	5	209.15***	5	350.76***
Gen. x Env.	10	84.56***	10	14.6	10	16.44***
Pooled error	3864	1.42	3864	2.29	3864	2.8
Mean♣	3.34		4.03		3.49	
Cv	35.7		37.53		48.02	

Where, *, **, *** indicates significance level at 0.05, 0.01 and 0.001 respectively, Env. = Environment, Gen. = Genotypes (populations), Rep = replications and CV = Coefficient of variations %. ♣ = transformed data.

6.3.2 Gene actions for NLB disease resistance

Genetic effects analysis model revealed all additive, dominance, additive x additive and additive x dominance epistatic effects being negative and highly significant (P<0.001) with the exception of set B where additive x dominance was negative significant (P<0.05) for all sites. Epistatic effect dominance x dominance was positive and highly significant (P<0.001) for all sets and sites (Tables 6.2 a, b and c). Tables 6.2 a, b and c also shows the relative contribution of gene action and non allelic gene interactions.

Table 6.2a: Set A estimation of genetic effects and their relative contribution to total sum of squares of NLB disease severity

		P145-95 X CML 395							
	Combined	Tumbi	Uyole	Selian		Combined	Tumbi	Uyole	Selian
Model					Sov	% SS contribution	% SS contribution	% SS contribution	%SS contribution
m	7.03±0.36***	7.17±0.35***	6.99±0.30***	7.5±0.61***					
a	-0.7±0.11***	-0.78±0.11***	-0.66±0.13***	-1.27±0.14***	a	72.88	77.26	75.98	69.94
d	-7.35±0.89***	-7.53±0.86***	-7.23±0.79***	-9.05±1.49***	d	12.72	4.74	8.47	21.69
aa	-2.91±0.34***	-2.9±0.33***	-2.9±0.31***	-4.05±0.59***	aa	0.42	0.08	0.28	0.56
ad	-4.09±0.30***	-3.91±0.29***	-4.16±0.27***	-1.79±0.44***	ad	11.77	14.50	11.97	1.24
dd	5.07±0.60***	5.2±0.57***	5.01±0.52***	6.56±0.97***	dd	2.21	3.42	3.29	6.55

Where Sov = source of variations

Table 6.2b: Set B estimation of genetic effects and their relative contribution to total sum of squares of NLB disease severity

		K530-106-1 X P86-95-1							
	Combined	Tumbi	Uyole	Selian		Combined	Tumbi	Uyole	Selian
Model					Sov	% SS contribution	% SS contribution	% SS contribution	%SS contribution
m	6.05±0.09***	7.0±3.30***	6.66±0.56***	6.97±0.79***					
a	-0.83±0.02***	-1.35±1.03***	-1.27±0.20***	-1.29±0.25***	a	75.64	66.49	65.94	89.12
d	-5.65±0.22***	-9.46±8.10***	-8.88±1.19***	-9.31±1.96***	d	0.01	0.12	0.50	2.23
aa	-2.12±0.09***	-3.37±3.12***	-3.00±0.53***	-3.31±0.75***	aa	0.24	1.11	0.72	1.80
ad	-0.88±0.07*	-1.35±2.73*	-1.18±0.50*	-1.46±0.66*	ad	2.56	7.90	8.99	4.35
dd	3.65±0.15***	6.17±5.35***	5.98±0.92***	6.06±1.29***	dd	21.55	24.38	23.84	2.50

Where Sov = source of variations

Table 6.2c: Set C estimation of genetic effects and their relative contribution to total sum of squares of NLB disease severity

		E29 X P86-95- 1							
	Combined	Tumbi	Uyole	Selian		Combined	Tumbi	Uyole	Selian
Model					Sov	% SS contribution	% SS contribution	% SS contribution	%SS contribution
m	5.36±0.51***	6.91±0.79***	8.10±1.08***	6.88±0.88***					
a	-0.69±0.16***	-0.78±0.25***	-1.04±0.28***	-0.78±0.28***	a	74.05	56.80	73.92	69.36
d	-4.73±1.26***	-9.65±1.94***	-11.07±2.62***	-9.67±2.17***	d	9.31	15.20	10.86	11.90
aa	-0.9±0.48ns	-2.41±0.75***	-3.62±1.03***	-2.41±0.83***	aa	6.48	4.92	2.87	5.25
ad	-0.95±0.42*	-2.33±0.65***	-3.54±0.82***	-2.33±0.73***	ad	0.13	4.77	0.80	0.02
dd	3.64±0.83***	6.88±1.28***	7.09±1.73***	6.90±1.43***	dd	10.04	18.31	11.56	13.47

Where Sov = source of variations

Although there were variations for relative genetic contributions of additive, dominance and non allelic effects, the relative genetic contribution of additive effects was high in all sets and sites compared to dominance and non-allelic gene interactions (Tables 6.2 a, b and c). For example at set A, it ranged from 69.94 -77.26 with Tumbi site recorded 77.26 followed by Uyole 75.98 and Selian 69.94%. At set B, the additive effects relative contribution ranged of 65.94 -89.12 with Selian site recorded 89.12 followed by Tumbi.66.49 and Uyole 65.94%. At set C, the additive effects relative contribution ranged from 56.8 – 73.92 with Uyole site recorded the highest 73.92 followed by Selian 69.36 and Tumbi 56.80%.

6.3.3 Generations frequency distribution

Figure 6.1 shows the rate of NLB disease severity based on individual plant assessment in three sets. Graphically illustrations of F_2, BCP_1, BCP_2 populations revealed the continuous distribution characteristics. At set A, BCP_1 population showed that, majority of plants occupied the scale of 3-5 moderate resistance while the distribution of BCP_2 was skewed to the left in the susceptible direction. The F_2 population at set B also showed continuous distribution with majority of genotypes occupying the scale range of 2-5. The backcross to resistant parent (K530-106-1) of this set showed the pattern of majority of plants occupying the scale of 3-6 with negligible highly susceptible plants (scale 8-9). The backcross to susceptible (P86-95-1) population of this set showed distribution skewed towards susceptible direction with a reasonable number of highly susceptible plants at a scale of 8-9.

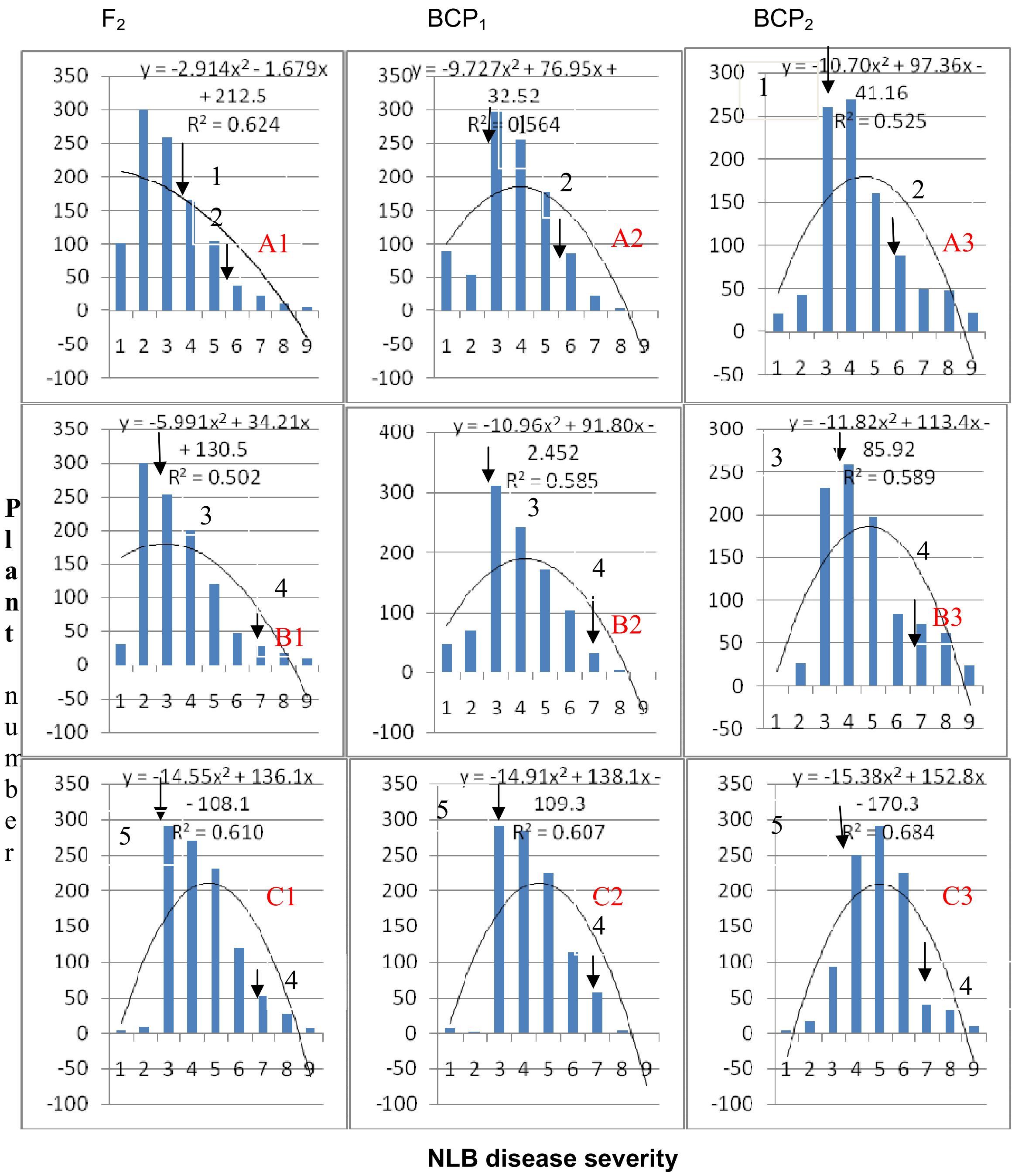

Figure 6.1: Frequency disrtibutions of six populations evaluated for NLB disease resistance by generation mean analysis in Tanzania for 2010 and 2011 growing season

Where, A1- A3 = Set a, A1 = F2 (P145-95 X CML 395), A2= BCP1 (P145-95) and A3 = BCP2 (CML 395). B1- B3 = Set b, B1 = F2 (K530-106-1 X P86-95-1), B2= BCP1 (K530-106-1) and B3 = BCP2 (P86-95-1). C1- C3 = Set c, C1 = F2 (E29 X P86-95-1), C2= BCP1 (E29) and C3 = BCP2 (P86-95-1). 1 = P145-95, 2 = CML 395, 3 = K530-106-1, 4 = P86-95-1 and 5 = E29 parents.

Set C revealed continuous population distribution with majority of group members of F_2 genotypes occupying the scale of 3 - 7 (Figure 6.1). Few plants were found to be highly resistant (scale 1-2). The backcross to resistant parent (E29) recorded majority of plants at the scale of 3 – 7 with trace plants attached to highly susceptible scale. When backcross to susceptible parent P86-95-1 was performed, majority of plants were found to concentrate on the scale 3 -8 with some plants being highly susceptible reaching the scale of 9. Generally, transgressive segregants for both resistance and susceptibility were observed in F_2 populations for all sets. In this case, offspring were more resistant or susceptible than their parents.

6.3.4 Analysis of generation means per site

In total, 1293 plants were individually evaluated for NLB disease resistance per set in each site. Each set had 105, 104, 102, 335, 327 and 320 plants for P_1, P_2, F_1, F_2, BCP_1 and BCP_2 populations respectively. In all three sets tested and six populations per set, the resistant parent one (P_1) differed significantly ($P<0.05$) from the susceptible parent two (P_2). At set A (Table 6.3a), the rank order for generation means was $P_2>F_2>BCP_2>F_1>P_1>BCP^1$ in all sites except at Selian where P_1 exchanged order with F_1. At set B (Table 6.3b), the ranking order was $P_2>F_2>BCP_2>F_1>P_1>BCP_1$ except at Uyole site where BCP_1 exchanged ranking order withP_1 and at Selian site where P_1 exchanged ranking order with F_1. At set C (Table 6.3c), the ranking order was $P_2>BCP_2>F_1>F_2>BCP_1>P_1$ for all three site.

Table 6.3a: Set A site generation means analysis for NLB disease resistance evaluated in Tanzania for 2010/2011 growing season

	Set A P145-95 X CML 395				
Tumbi	Mean	Uyole		Selian	
Generations	Ranked mean♣	Generations	Ranked mean	Generations	Ranked mean
P_2	4.76a	P_2	4.75a	P_2	4.23a
F_2	4.62a	F_2	4.38b	F_2	3.55b
BCP_2	3.93b	BCP_2	3.78c	BCP_2	3.18c
F_1	3.58c	F_1	3.29d	P_1	2.93c
P_1	2.59d	P_1	2.69e	F_1	2.53d
BCP_1	2.52d	BCP_1	2.52e	BCP_1	2.46d

♣Transformed data of severity (%), means with the same letter are not significant different at 0.05.

Table 6.3b: Set B site generation means analysis for NLB disease resistance evaluated in Tanzania for 2010/2011 growing season

	Set B K530-106-1 X P86-95-1				
Tumbi	Mean	Uyole		Selian	
Generations	Ranked mean♣	Generations	Ranked mean	Generations	Ranked mean
P_2	4.44a	P_2	4.39a	P_2	5.09a
F_2	4.01ba	F_2	3.91b	F_2	4.26b
BCP_2	3.95ba	BCP_2	3.89b	BCP_2	4.21b
F_1	3.92b	F_1	3.82b	P_1	3.91b
P_1	2.43c	BCP_1	2.38c	F_1	2.71c
BCP_1	2.42c	P_1	2.28c	BCP_1	2.55c

♣Transformed data of severity (%), means with the same letter are not significant different at 0.05.

Table 6.3c: Set C site generation means analysis for NLB disease resistance

	Set C E29 X P86-95-1				
Tumbi	Mean	Uyole		Selian	
Generations	Ranked mean♣	Generations	Ranked mean	Generations	Ranked mean
P_2	5.50a	P_2	6.22a	P_2	5.95a
BCP_2	3.41b	BCP_2	3.52b	BCP_2	3.49b
F_1	3.23b	F_1	3.28b	F_1	3.35b
F_2	3.06b	F_2	3.15b	F_2	3.09b
BCP_1	2.02c	BCP_1	2.01c	BCP_1	2.10c
P_1	1.70c	P_1	1.84c	P_1	1.59c

♣Transformed data of severity (%), means with the same letter are not significant different at 0.05.

6.3.5 Estimation of average degree of dominance

The average degree of dominance varied across sites and sets. It ranged from -0.52 observed at Tumbi site in set B to 0.88 recorded at Uyole in set A (Table 6.4).

Table 6.4: Average degree of dominance for NLB disease across sets and sites

	Set A			Set B			Set C			
	P145-95 X CML 395			K530-106-1 X P86-95-1			E29 X P86-95-1			
	Tumbi	Uyole	Selian	Tumbi	Uyole	Selian	Tumbi	Uyole	Selian	Mean
ADD	0.74	0.88	0.82	-0.52	-0.44	-0.37	0.34	0.17	0.42	0.23

Where ADD= average degree of dominance

6.3.6 Narrow sense heritability and mid parent heterosis

There were variations for NLB disease severity across sites and sets on narrow sense heritability. However in many cases, it was higher than 65%. The highest narrow sense heritability was recorded at Tumbi site (84.76%) in set C, while the lowest was 54.24% which was recorded at Uyole in set B. Variations existed on midparent heterosis among sets and sites. Set A and C had positive mid parent heterosis for NLB disease severity (Table 6.5). The mid parent heterosis ranged from -18.98 as recorded at Uyole site in set A to 1.11% as observed at Uyole in set B.

Table 6.5: Narrow sense heritability and mid parent heterosis of six maize generations evaluated for NLB disease resistance

	Set A			Set B			Set C			
	P145-95 X CML 395			K530-106-1 X P86-95-1			E29 X P86-95-1			
	Tumbi	Uyole	Selian	Tumbi	Uyole	Selian	Tumbi	Uyole	Selian	Mean
h^2	68.45	72.38	67.18	60.76	54.24	64.95	84.76	77.27	62.31	68.67
MPH	-12,11	-18.98	-7.27	2.36	1.11	1.18	-6.34	-3.56	-5.87	-0.01

Where, MPH = mid parent heterosis (%), h^2 (%) = narrow sense heritability,

6.4 Discussion

6.4.1 Environment and population genetic effects

All parameters mean sum of squares for environment, replication with the nested environment, populations, population x environment interactions were highly significant ($P<0.001$) in all sets. The high significant environmental effects and its interaction with populations justify testing genotypes under varying environments and disease pressure to test population stability. Breeding implication exhibited by high significant environment effects calls for breeding for specific environments according to genotype adaptations (Beyene et al., 2011; Eberhart and

Russell, 1966). At the same time, highly significant effects of population means justify the use of generation mean analysis so as to partition additive, dominance and non-allelic gene interactions effects.

6.4.2 Gene actions for NLB disease resistance for the studied populations

There were additive, dominance and non-allelic gene actions involved in the control of NLB disease resistance with different level of magnitude which are in accordance to Lingam et al. (1989). In all sets and sites, additive and dominance gene action effects were highly significant and negative significant ($P<0.001$). This implies that both gene action were involved in the NLB disease inheritance control in all three sets (Dingerdissen et al., 1996; Ogliari et al., 2005; Vieira et al., 2009). However, for non-allelic gene interaction, some differences on the magnitude of effects were noted. For example, additive x additive, additive x dominance were highly negative significant ($P<0.001$) in set A and C and additive x dominance had significant contribution ($P<0.05$) in set B. The observed differences could be attributed to resistance or susceptible gene frequencies and proportion of dominance and recessive genes possessed by parents (Viana et al., 1999). Another explanation could be gene linkages and maternal effects which probably operative in these parents. Parent selection was based on the assumption that, there was no linkage and loci had equal allelic effects (Wright, 1968). For dominance x dominance non-allelic gene interactions, all sets showed highly positive ($P<0.001$) effects to indicate the effect being skewness to susceptibility direction. In all sets, dominance had (-) and dominance x dominance had (+) sign which is the indication of duplicate types of epistatic gene interactions. These types of gene interactions make the choice of parents with contrasting traits complicated and demanding (Lyimo et al., 2011).

There was an indication of NLB disease resistance inheritance being mainly controlled additively. Although there were variations for additive gene actions contribution among sites and sets, the evidence showed that, relative genetic contribution of additive effects was high in all sets and sites compared to dominance and non-allelic gene interactions with the range of 56.80% to 89.12 %. On the other hand dominance and non-allelic gene interactions contributed 0.02 – 24.38% to sum of squares variations. Other investigators have also revealed the high contribution of additive compared to dominance and non-allelic gene actions in NLB disease studies (Carson, 1995; Lingam et al., 1989; Opio et al., 2010; Sigulas et al., 1988; Welz and Geiger, 2000).

6.4.3 Frequency distributions

Generally, all crossing sets P145-95 X CML 395, K530-106-1 X P86-95-1 and E29 X P86-95-1 involved crosses of resistant (first) and susceptible (second). The NLB disease severity graphic illustration showed the continuous distribution in all sets for F_2 populations which is the indication of quantitative nature of inheritance and additive gene effects. Other researchers reported the quantitative inheritance nature of NLB disease (Freymark et al., 1993; Sigulas et al., 1988). Continuous population distribution was further emphasized by coefficient of determination which was higher than 50% in all sets. The continuous population distribution implies the involvement of more than one gene in the control of NLB disease resistance. Furthermore, continuous distribution support observations of polygenic inheritance and thus, one can speculate the quantitative traits loci (QTLs) perhaps there is a major QTL that biases distribution on the left (resistant) in all sets. The study also found the distribution being skewed left towards resistance direction to speculate additive resistant QTL effects operating in these populations. Population distribution obtained from backcross to resistant parent (BCP_1) showed distribution skewed to resistance direction to indicate that, several genes could be involved in NLB disease inheritance. When backcross to the susceptible parent (BCP_2) was performed, population distribution skewed left to the susceptible direction. Highly susceptible individuals to the scale of 7-9 differed across sets in BCP_2 populations in the order of decreasing magnitude set B > set A > set C which could be attributed to differences in gene frequency for resistance and susceptibility of parents.

In general, backcrossing to susceptibility increases frequency of alleles for susceptibility hence skew in favour of 6 – 9 scale on the susceptible direction. Where backcrossing to resistant parent increases alleles for resistance hence skews towards left (resistant) direction. However, the presence of transgressive segregants that perform better or worse than parents exists. In this study, transgressive sergeants were also observed in F_2 population which resulted in more resistant plants or more susceptible plants than their parents.

6.4.4 Generation means of studied populations

This study individually analysed 1293 plants per set which were satisfactory to study the genetic variation using generation mean analysis. For stable germplasm like P_1, P_2 and F_1, relatively few plants were used compared to segregating populations like F_2, BCP_1 and BCP_2. In all three sets and six populations tested per site, the resistant parent one (P_1) differed significantly ($P<0.05$) from the susceptible parent two (P_2). This result satisfied the condition that, in generation mean analysis, parents have to possess contrasting levels for the traits of interest. Although there was changing rank order of means in some sets and site which mainly involved P_1 and BCP_1, the consistence generation performance was observed. The changed rank order of some few cases in population is the indication of G x E interactions which frequently occur in experimental sites.

6.4.5 Average degree of dominance

Findings revealed high variation in terms of average degree of dominance. The high range of genetic factors was caused by differences in populations means across sites and sets. The average degree of dominance in all three sets and sites ranged from -0.52 to 0.88. According to Edwards and Lamkey (2001), a range of 1 to -1 average degree of dominance denotes complete dominance. With reference to findings of this study, these populations could be probably under partial dominance gene action which is in line with additive gene effects. The degree of dominance of populations under study were below 1 and -1 to indicate the partial dominance in all three sets which confirm the predominance of genes with additive effects for the inheritance of NLB disease resistance.

Generally, findings further suggest that, inheritance of NLB disease severity is mainly controlled additively and quantitatively. The presence of highly significant additive gene action, high heritability and negative heterosis which leaned towards resistance gives the possibility of employing breeding for durable NLB disease resistance in maize (Sharma and Payak, 1990). Findings also suggest selection strategies like recurrent and pedigree selection for maize population improvements. However, presence of highly significant dominance and epistatic gene actions are likely to slow the progress (Sofi et al., 2007). But, they were quite minimal in these populations to cause complications.

6.4.6 Narrow sense heritability and heterosis traits

Across sites and sets analysis on narrow sense heritability revealed variations. However in many cases, it was high than 65%. The range of narrow sense heritability was 54.24% - 84.76%. Stanfield (1991) classified heritability values as lower (<20%), medium (20–50%) and high (>50%). By adapting this type of classification, heritability obtained in the study was higher because it exceeded 50% in all sites and sets. High heritability is the indication of additive gene action involvement in controlling particular traits (Jawaharlal et al., 2011). Heritability information assists plant breeders in designing appropriate breeding strategies. Therefore, selection strategies like recurrent selection could be used to improve maize populations under study as stated by Ceballos et al. (1991).

Negative heterosis is desired for disease resistance in maize populations. From the study, mid-parent heterosis ranged from -18.98 – 1.11%. In this study, set A (P145-95 X CML 395) and B (E29 X P86-95-1) had negative mid parent heterosis in all sites and sets while set C (K530-106-1 X P86-95-1) had positive mid parent heterosis across sets and sites. This implies that resistant parents in crosses P145-95 X CML 395 and E29 X P86-95-1 contributes to NLB disease resistance while K530-106-1 X P86-95-1 inclined and leaned to susceptibility class. However, the same susceptible parent P86-95-1 was used for both set B and C crossing. It seems that, the resistant parent K530-106-1 which was used in set B probably had susceptible genes that contributed to susceptibility to the K530-106-1 X P86-95-1 cross in set B.

6.5 Conclusions

The following can be drawn from this study:
The following were the research highlights from the study:

- The additive gene effects were predominant ranging between 57% to 89%, while dominance and epistasis were generally negligible. This was further confirmed by large heritability (54-85%). At the same time, the NLB disease severity showed continuous distributions in all sets for F_2, BCP_1 and BCP_2 populations indicating that inheritance of resistance was quantitative in nature.
- The average degree of dominance ranged between -0.52 to 0.88 supporting observations of partial dominance.
- On the other hand the mid parent heterosis ranged from -19 to 1% indicating NLB disease resistance in the populations.
- Therefore resistance to NLB disease could be improved through selection procedures like recurrent selection. Epistatic gene effects would cause minor complications because they were negligible (<25%).

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Chapter 7

7 Client oriented breeding for maize northern leaf blight disease resistance in western Tanzania

Abstract

In Tanzania, the majority of farmers grow unimproved and NLB disease susceptible maize varieties despite their low production potential probably due to community preferred traits they possess. Modern varieties were developed without the community involvement. Therefore, the objective of this study was to perform farmers and stockists assessment on 110 F_1 experimental maize hybrids and compare them with breeders' selection criteria. Breeding materials were planted in two replications at Agricultural Research Institute-Tumbi. Statistical analysis ranked 10 top high yielding experimental hybrids. Farmers developed 14 while stockists developed 13 hybrids selection criteria. The most preferred hybrids by farmers were VL 05616 x CML 159, CML 159 x KS03-0B15-47 and EB04-0A01-304 x CML 442 while stockists preferred VL 05616 x CML 395, EB04-0A01-304 x CML 442 and VL 05616 x CML 159. Two F_1 experimental hybrids EB04-0A01-304 x CML 442 and CML 159 x CML 442 appeared in all five top most ranked hybrids of breeders, farmers and stockists. Generally, findings showed that, farmers, stockists and breeders can generate useful information which can supplement each other.

7.1 Introduction

Maize (*Zea mays* L.) is one of the five most important cereal crops in the world (FAO, 2008). It can be utilized in a number of ways ranging from traditional food preparations to ethanol extraction (Sticklen, 2006). In America maize is one of the most important ingredients of animal feed formulation (Yamka et al., 2004). Studies show that, maize plays an important role by providing calories and proteins for the majority of people in Africa (FAO, 1992). With regards to East Africa, it is estimated that, every year there is an increase of 3% of maize demand for home consumption and export (CIMMYT, 2002). In Tanzania, maize is primarily used as food and cash crop for the majority of people living in rural and urban areas (Ramadhani et al., 2002).

Despite the great role played by maize for enhanced food security and income, maize yield per unit area is still very low in sub-Saharan Africa. For example in Tanzania, Bisanda and Mwangi (1996) mention that, the national average yield of maize production is less than 1.5 t ha^{-1}. The principal contributors of lower yields are the use of germplasm with poor yield potential and highly susceptible to diseases like NLB.

Although, there are success stories of plant breeding around the world such as introduction of maize hybrids in the 1900's in the USA (Kutka, 2011) which increased yield significantly to more than 10 t ha^{-1} and the Green Revolution that happened in the 1960's in Asia, both operated under different socio-economics, farming systems and production environments which are quite different to Tanzania situation. A breeding system that suites Tanzania environment is needed.

Currently, variety breeding in Tanzania starts with problem identification which is mainly done by breeders. Parent selection follows and application of appropriate mating designs is done to get progenies for further testing. Before a variety reaches farmers, it has to go through on station and multi-location trials. Breeders use selection indices for breeding materials advance. Selection indices weighting depends on objectives. However selection indices are highly influenced by distinctness, uniformity, and stability (DUS) and value for cultivation and use (VCU) tests. The last stage is the application for release which is done by the variety releasing committees. Variety releasing committees must be satisfied with DUS and VCU tests. All above procedures do not involve any farmers and stockists opinions and preferences. Few farmers are only involved through participatory variety selection and not participatory plant breeding after a variety has been released. Procedures for releasing varieties takes long time and chances of being rejected are high despite wasting time, human and finance resources (Setimela et al., 2009). This situation has led to to low adoption rate and subsequent rejection of developed varieties. Mafuru et al. (1999) argue that, adoption of modern cultivars in the western zone of Tanzania ranges from 6-12% only.

The low rate of adoption of high yielding cultivars is one of the reasons of low yield in the country. The majority of farmers still prefer composite and landraces despite of low yield potential they portray. The ability to be recycled and possession of desired traits are some of reasons farmers revert to them. On the other hand, modern cultivars were developed at breeding centres in absence of community involvement and hence have undesired qualities to respective communities.

In the process of introduction of new hybrids, socio-economic factors should not be underrated and ignored. They determine hybrids' adoption and utilization in varying farming systems. Worrying reports of rejection and abandonment of promising cultivars exists in many countries. In beans, Assefa et al. (2005) reports the reversed order of ranking between farmers and breeders' statistical evaluation approaches in Ethiopia. In Tanzania, Limbu (1999) reports on the rejection of early maturing and bird attack resistant sorghum variety called Serena due to its red colour and poor local food preparation qualities. In maize, high yielding maize varieties H6302 and H614 were rejected and abandoned by farmers of Tanzania due to their susceptibility to insect-pests and diseases (Kaliba et al., 1998).

For a variety to be accepted in the community, it needs involvement of stakeholders from planning stage, implementation and evaluation (Gyawali et al., 2007; Magigi and Majani, 2006; Mugo et al., 2005; Obeng and Ugboro, 2008). A mechanism is needed by which breeders and communities collaborate in searching for desired cultivars. Involvement of communities in the breeding process is the typical characteristic of client oriented research which is a community demand driven approach (Assefa et al., 2005; Mulatu and Zelleke, 2002). However, most of participatory breeding approaches ignored the presence of stockists who play a great role in supplying needed cultivars. They are the first breeding clients to sell (adopt) or not sell (rejection or abandon) a developed cultivar. Although farmers and stockist variety selection might differ, there must reach a point by which they match. This is because, both farmers and stockists deals with crops in same societies. Stokists aims at profit maximization and farmers thrive for high yield and household food security. Breeders are obliged to combine high yielding and profit maximization (marketing) traits during variety breeding processes which is the essence of the current scenario termed as value chain approach.

Generally, the past maize breeding in Tanzania ignored farmers and did not consider stockists as important partners in breeding. Failure to acknowledge farmers and stockists widened the gap between them and agricultural research centres. As a result has faced variety rejection and abandonments which resulted to the low rate of variety adoption. The overall effect was the inefficiency utilization of time, human and finance resources. Subsequently, these factors contributed to the low maize yield in the country.

This study was conducted to contribute to the increased rate of variety adoption and maize yield. It involved breeders, farmers and stockist assessment of 110 F_1 maize hybrids for resistance to NLB disease. The study started by utilizing PRA information generated from farmers and stockists in 2008/2009 season. The specific objectives were to perform farmers and stockists assessment on 110 F_1 maize hybrids and compare them with normal breeders ranking based on statistical analyses.

7.2 Materials and methods

7.2.1 Western zone location and description

The study was conducted in the western zone of Tanzania which covers two regions Tabora and Kigoma. Western zone extends from the western plateau to Lake Tanganyika shores to share boundaries with Burundi, Democratic Republic of Congo in the west and Zambia in the south. It is situated between 31 – 34^0 (Longitude) east and 4 – 7^0 south (latitude) with unimodal type of rainfall which normally occurs in November to May. The zone covers total area of 113,160 km^2 with about 2,991,486 populations (URT, 2011). The main food crop grown in the zone is maize which can be used as cash crop in the case of surplus and bumper harvests.

7.2.2 Experiment site description

The experiment was conducted at Agricultural research institute (ARI)-Tumbi, Tabora region in the western zone of Tanzania. Table 7.1 depicts site description of the experiment site.

7.2.2.1 Soils

A composite soil analysis from the experiment site (0 - 20 cm) revealed that, Acidic soils, sandy loam, low total nitrogen and low Na^+ dominated the trial site which calls for high rate of fertilizer application, addition of organic matter and liming materials.

Table 7.1: Site descriptions of NLB disease test site for evaluation of 110 F_1 maize hybrids at ARI-Tumbi for 2010/2011 growing season

Attributes/test site	ARI-Tumbi
Altitude (masl)	1124
Latitude (South)	$05^0$06.485
Longitude (East)	$032^0$66.630
Agro-ecological zone	Low to medium
Research zone	Western
Rainfall (mm)	912.90
Temperature (^{0}C)	22.92
Soil pH (Water)	5.9
Soil pH (KCl)	4.8
Clay%	14
Silt%	10
Sand%	76
TN%	0.13
OC%	1.24
C:N	9
Zn (mg/kg)	
P (mg/kg)	18
CEC(Cmol (+) /kg)	7.6
Exchangeable bases (Cmol ($^+$) /kg)	
Ca^{+2}	4.83
Mg^+	0.85
Na^+	0.4

7.2.2.2 Rainfall, temperature and relative humidity

The site received 793.1 mm rainfall amount for 2010/2011 growing season. The relative humidity reached 70% with temperature of about 25^{0}C (Figures 7.1 and 7.2) during the growing period (November-April) which was ideal for seasons to expose the site to be vulnerable to disease infections like NLB and other fungal diseases.

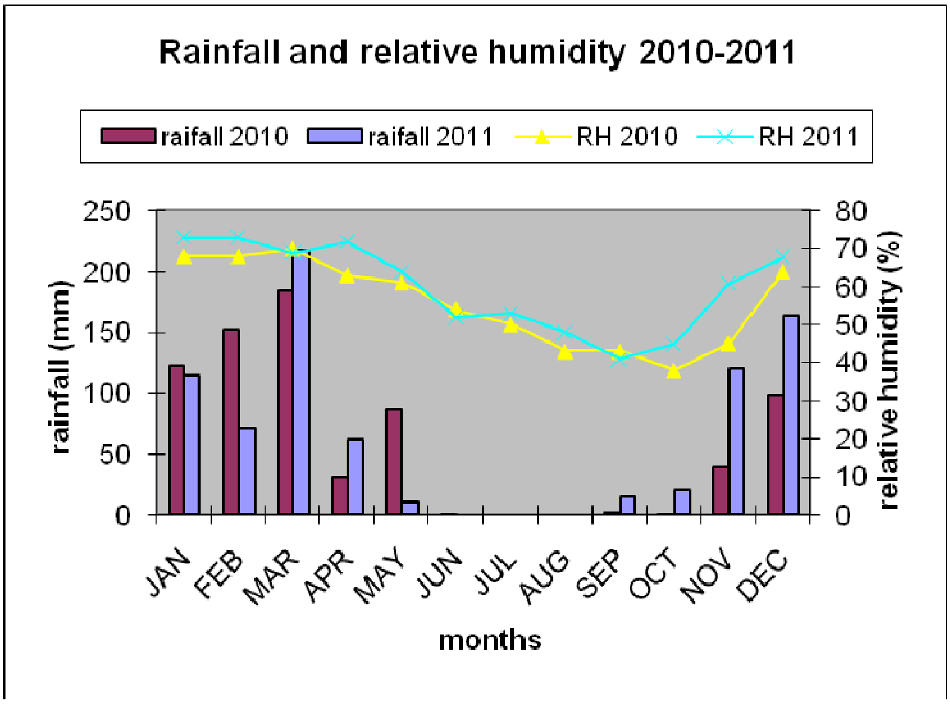

Figure 7.1 Rainfall (mm) and relative humidity (%) distribution at ARI-Tumbi for 2010/2011 growing season

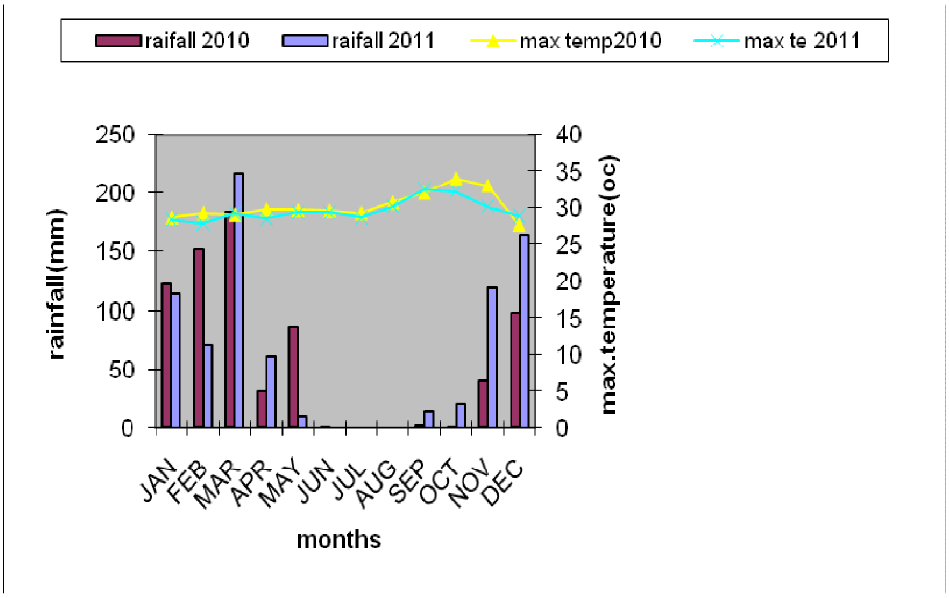

Figure 7.2 Rainfall (mm), maximum and minimum temperature (^{0}C) distribution at ARI-Tumbi for 2010/2011 growing season

7.2.3 Farmers and stockist selection

Farmers and stockists participants were selected by the extension department in each district. Farmers selection was based on district representation, farmer field school and farmer research group membership and non-membership and gender. In total 60 farmers and 12 stockists (Table 7.2) were selected to participate in evaluation of 110 F_1 maize hybrids.

Table 7.2: Number and descriptions of farmers and stockists participated in evaluation of 110 F1 maize hybrids resistant to NLB disease

	Farmers		Age range		FRG member		FSS member		Stockists	
Districts	M	F	M	F	M	F	M	F	M	F
Sikonge	11	9	25-58	32-51	5	7	6	4	3	1
Urambo	13	7	30-56	28-57	7	2	7	2	3	0
Nzega	10	10	29-60	25-42	2	3	3	5	3	2
Total	34	26			14	12	16	11	9	3
Total farmers	60									
Total FRG	26									
Total FSS	27									
Total stockists	12									

Where, M = male, F= female, FRG = farmers research groups, and FSS = famers field schools.

The 110 F_1 hybrids in this study were obtained from a 11 x 11 full diallel cross conducted in 2009/2010 after a PRA study which was carried out in the community during the 2008/2009 growing season.

7.2.4 Breeding materials

Table 7.3: Maize (F_1 hybrid) assessed by breeders, farmers and stockists during 2010/2011 growing season in western Tanzania

F_1 hybrid	F1 hybrid	F_1 hybrid	F_1 hybrid	F_1 hybrid	F_1 hybrid	F_1 hybrid	F_1 hybrid
HxG	GxA	IxC	ExJ	KxG	IxH	CxI	IxG
BxJ	FxJ	FxI	ExB	FxB	KxJ	KxA	FxG
CxJ	DxH	BxF	HxA	BxE	DxK	DxE	ExH
CxG	JxB	HxK	BxG	IxE	FxE	ExA	BxA
BxD	HxI	GxK	GxH	FxH	DxF	FxA	ExG
IxA	KxB	BxI	DxA	DxI	ExK	GxB	FxD
CxE	JxG	HxF	GxE	IxB	GxJ	AxJ	AxK
FxC	AxH	JxA	AxE	CxD	JxK	DxB	AxI
HxJ	ExI	CxF	CxA	HxC	JxH	AxC	IxF
JxD	KxH	IxJ	JxC	HxB	KxC	AxF	BxK
GxC	CxH	KxI	KxE	ExC	ExF	AxD	JxI
BxC	CxB	FxK	DxJ	HxE	CxK	IxK	JxE
DxG	HxD	AxB	KxD	DxC	JxF	GxF	
BxH	AxG	ExD	KxF	GxI	IxD	GxD	

Where, A = KS03-0B15-126, B = EB04-0A01-304, C= CML 159, D = KS03-0B15-2, E = KS03-0B15-45, F = VL 05616, G = KS03-0B15-47, H = CML 395, I = KS03-0B15-12, J= CML 442 and K = CKL 05007-B-B

7.2.5 Field management

The 110 F_1 hybrids were planted in 13 x 10 alpha lattice design in two replications One F_1 hybrid CML 159 X VL 05616 was doubled per replication to fulfil the condition of filler material. Maize was planted at a spacing of 0.75 m x 0.30 m, which gives a plant population of 44,444 maize plants per hectare. Fertilizer, NPK basal fertilizer was applied at a rate of 40 Kg P ha^{-1}. Murate of Potash at the rate of 40 kgK_20 was also applied. Top dressing was done by using UREA (46%) to achieve the recommended rate of 100 Kg N ha^{-1}. A single row plot, 3m long were employed to conduct the experiment. The experiment was kept free from weeds by hand weeding. To ensure uniform disease infestation, artificial inoculation was conducted according to procedures described by Reid (2005) as follows: A sample grinder machine [Laboratory mill model-4, Thomas –Wiley, Thomas scientific (TM), U.S.A] was used to grind infected leaves into powder form. Bazooka equipment was used to apply powder in the whorl of plants. One dose of powder from Bazooka application amounts to 0.1 g of leaves powder. Two applications, one at 6

– 8 leaf stage and the second at 11-12 leaf stage were conducted. Furthermore, two rows of a spreader local variety (Situka-1) which is highly susceptible to NLB disease was planted around the field and after every 10 rows of the test materials. The uses of spreaders have been used successfully in screening germplasm for other disease studies (Singh et al., 2004).

In addition to NLB disease resistance, maize genotypes were assessed on other agronomic characteristics. Other collected agronomic data included: Days to 50% anthesis, days to 50% silking, plant height, ear height, husk cover, kernel type, grain texture and grain yield kgha^{-1}. Data were recorded according to IBPGR (1991) maize descriptors list. Yield and disease resistance data were validated and analyzed in Genstat statistical computer software (Payne et al., 2007).

7.2.6 Data collection from farmers and stockists

Factors that enhance or hinder adoption of new cultivars in western zone were solicited from farmers during registration process. Each farmer was given a form to fill during registration. Contents of the form included farmer field school membership, food security, food quality, age of the farmer, education of the farmer, sex of the farmer, farmer research group membership, potential maize yield, disease and drought resistance prevalence, availability of extension services, attending agricultural shows and income status. In total sixty farmers were used to assess 110 F_1 hybrids planted at ARI-Tumbi field in 2010/2011 growing season. Assessment was done twice. The first assessment was at milk and the second at harvest stage. Farmers and stockist selection criteria were provided by themselves. Each respondent was given a piece of paper to put down five most criteria she/he uses for variety selection. One representative from farmers and one from stockists collected pieces of papers and pinned on the board. Similar selections were merged and resulted into 14 for farmers and 13 for stockists variety selection criteria. During field assessment, a scale of 1-5 was used with the following definitions: 1 = almost rejected, 2= preferred with some bad characteristics, 3= moderately preferred, 4= preferred with good characteristics and 5 = highly preferred with excellent characteristics. Cards were designed and assigned rating number from 1-5. Each farmer was given many cards and asked to put on only 10 F_1 hybrids. The number of cards with their associated scores was summed to get totals. The top ten were subjected to group assessment which involved all 60 farmers. All (12) stockists in three districts where PRA study were invited to perform stockists field assessment on the 110 developed F_1 hybrids. The exercise was performed in separate sessions with farmers to avoid communication and inbreeding of ideas between the two groups. Assessment methodology was the same as that used during farmers' assessment. A wrap up session was conducted which involved breeders, farmers and stockists which resulted to the proposed model of breeding procedures to be adopted in the zone. The proposed model was developed by farmers and stockists in collaboration with breeders.

7.2.7 Data analysis

Statistical analysis was performed on the gathered data by normal ranking and analysis of variance using Genstat computer software to test the existence of significant differences on measured maize traits (Payne et al., 2007). The following model was used

$Y_{ij} = \mu + \propto_i + \beta_j + + \varepsilon_{ijk}$,

Where Y_{ij} is the observed trait

μ is the overall mean

$\propto$i is the treatment effect

βj is the block effect

eij is the random error.

Logit regression analysis was performed by using Wald test in SPSS (2006) computer software programme to assess factors influencing adoption rate of cultivars in the area. In the model ten variables were used (Table 7.4). Furthermore, income distribution among farmers which is considered to affect the adoption rate of cultivars in the area was estimated by using Lorenz curves procedures. Gini coefficients were determined by assessment of income proportion with their corresponding respondents' proportions in the area (Hina and Kanwal, 2011). The asymmetry coefficient was estimated In Genstat computer programme by using the equation developed by Payne et al. (2007) by which, asymmetry = Fmu + Lmu.

Where Fmu = (m + d) / n, Lmu = (CDsort_m + d × Dsort_{m+1}) / $\mathrm{CDsort}_{n,\, m}$ = the index of the largest number less than mean (data), CDsort = cumulative (Dsort) and d = (mean (data) - Dsort_m) / (Dsort_{m+1} - Dsort_m).

7.3 Results

7.3.1 Income distribution among farmers

Figure 7.3 show the Lorenz curve of income distribution among farmers. Analysed revealed that, the Gini coefficient was 0.6405 and the Lorenz curve leaned right below the line of equality.

The coefficient of asymmetry was 0.6927 to further explain the nature of income distribution among farmers (Beach and Russell, 1983).

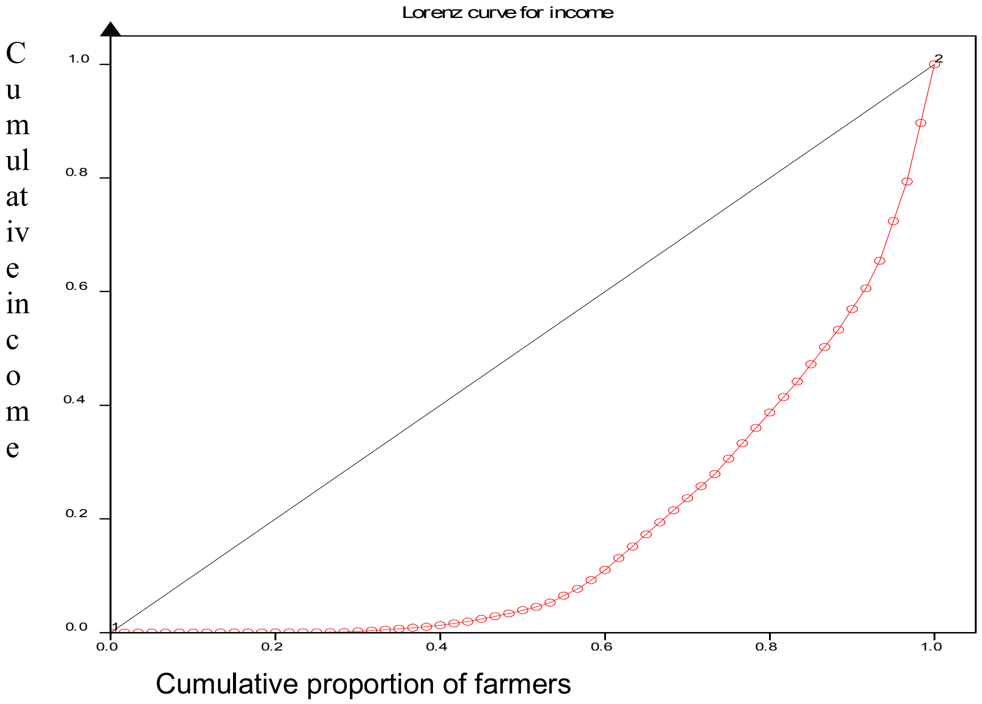

Figure 7.3: Lorenz curve for farmers income distribution in western zone of Tanzania, 2010/2011 growing season

7.3.2 Logit regression analysis

Table 7.4: Description of variables used in the regression model for farmers' maize varieties adoption in western zone, Tanzania for 2010/2011 growing season

Variables (X_j)	Description of variables included in the regression model
FFSmember	Farmer field school member
Foodsecure	Food security
Foodqua	Food quality
Age	Age of the farmer
Education	Education of the farmer
Sex	Sex of the farmer
FRG	Farmer research group member
Yield	Potential cultivar yield
Diseasedrought	Disease and drought resistance of the cultivar
Extension	Availability of extension services

Wald test which indicates the relative contribution of individual variable to probability of adoption of cultivars showed three variables highly loading the model. These variables were food security (5.93), cultivar yield potential (4.7) which determines farmers' income and cultivar disease and drought resistance (4.53). These were the three most important factors determining adoption rate of maize cultivars in the area (Table 7.5).

Table 7.5: Logit regression results for factors influencing maize cultivar adoption in western Tanzania for 2010/2011 growing season

	B	S.E.	Wald	DF	Sig.	Exp(B)
Age	2.02	1.49	1.85	1	0.17	7.55
Yield	2.44	1.12	4.77	1	0.03*	11.43
Education	0.00	0.00	0.19	1	0.67	1.00
Sex	0.00	0.00	1.36	1	0.24	1.00
FRG	0.00	0.00	2.33	1	0.13	1.00
Foodqua	1.02	0.60	2.90	1	0.09	2.76
Foodsecure	-3.22	1.32	5.93	1	0.02*	0.40
Diseasedrought	-2.20	1.03	4.53	1	0.03*	0.11
Extension	0.48	0.90	0.28	1	0.59	1.62
FFSmember	0.82	0.92	0.80	1	0.37	2.27
Constant	0.97	2.89	0.11	1	0.74	2.64

Prediction rate = 86.40%, $X^2 = 0.45$, DF = 11 and -2 Log likelihood = 41.83

7.3.3 Statistical analysis of F_1 hybrids

There were highly significant difference ($P<0.001$) in yield performance among hybrids with cross CML 395 x KS03-0B15-47 leading (8.52 t ha^{-1}) followed by EB04-0A01-304 x CML 442 with 8.34 t ha^{-}1 (Table 7.6). Hybrids also showed differences on percentage means and yield advantages. The range was 48.95 – 145.52 and 0.92 – 15.6% for percentage mean and yield advantage respectively. This implies that, by growing the studied hybrids, there is a possibility of gaining 15.6% yield margin as compared with local checks. On severity, a high significant difference ($P<0.001$) was observed among F_1 hybrids.

Table 7.6: Performance analysis of 110 F_1 experimental hybrids in western Tanzania

Top ten sites	**(Yield)**	**across**						
F1 hybrid	Yield tha^{-1}	% mean	Yield advantage (%)	Severity (%)	Height (cm)	Ear ht (cm)	Anthesis days	Silking days
HG	8.52	145.52	15.06	36.25	201.83	101.67	70.17	71.83
BJ	8.34	142.34	14.59	8.02	193.67	87.50	70.67	72.50
CJ	8.09	138.13	13.98	5.50	182.67	83.33	70.67	72.33
CG	7.91	135.05	13.53	8.17	189.17	77.50	70.83	72.67
BD	7.89	134.66	13.47	14.92	198.50	93.00	70.17	72.00
IA	7.71	131.69	13.03	33.08	186.83	88.83	72.33	74.00
CE	7.70	131.47	13.00	8.67	199.67	96.67	70.83	72.67
FC	7.62	130.10	12.80	9.72	188.17	79.17	71.33	73.00
HJ	7.61	129.93	12.78	31.42	189.33	98.17	72.50	74.17
JD	7.40	126.40	12.26	5.50	174.50	74.17	71.83	73.50
Mean	**5.86**			**17.46**	**176.73**	**80.18**	**70.56**	**72.42**
Lsd	**2.3933**			**10.497**	**21.393**	**16,203**	**4.295**	**2.5652**
Cv	**36.00**			**52.90**	**10.70**	**17.80**	**5.40**	**3.10**
Prob	**<.001**			**<.001**	**<.001**	**<.001**	**<.001**	**0.001**

Where, A = KS03-0B15-126, B = EB04-0A01-304, C= CML 159, D = KS03-0B15-2, E = KS03-0B15-45, F = VL 05616, G = KS03-0B15-47, H = CML 395, I = KS03-0B15-12 and J= CML 442, Height= plant height, Ear ht= ear height

7.3.4 Farmers and stockists F_1 hybrid assessment

Farmers developed 14 criteria to assess the developed F_1 hybrids performance (Table 7.7). The highly preferred maize traits in decreasing order of importance were endosperm colour, yield, grain texture, NLB disease and drought resistance. By applying matrix tool of analysis on ten farmers selected hybrids, Farmers ranked VL 05616 x CML 159, CML 159 x KS03-0B15-47, EB04-0A01-304 x CML 442, CML 395 x CKL 05007-B-B and CML 159 x CML 442 in decreasing order of preferences. The most preferred hybrids were VL 05616 x CML 159, CML 159 x KS03-0B15-47 and EB04-0A01-304 x CML 442 which possess the most preferred traits like white endosperm colour, high yield and flint or flint/dent grain texture.

Table 7.7: Ten best farmers F_1 hybrids assessment at ARI-Tumbi, Tanzania for 2010/2011 growing season

Char./genotype	CJ	HK	FC	BJ	CG	EK	DF	FE	AK	GD	Total	Rank
Height	1	1	3	2	2	1	1	1	3	2	17	9
Cob size	1	1	2	3	4	2	2	3	1	1	20	6
Maturity days	2	3	4	2	2	1	2	1	1	1	19	7
Lodging	3	1	2	2	2	2	2	1	1	2	18	8
Streak resistance	2	2	3	3	3	2	1	1	1	1	19	7
Striga resistance	2	2	2	1	3	1	1	1	1	1	15	10
Stalk rot resistance	3	2	2	2	2	2	2	1	2	1	19	7
Drought resistance	2	3	4	3	1	3	1	2	1	2	22	5
Cob rot	3	4	3	2	2	1	2	1	1	1	20	6
Leaf rust	1	2	2	1	3	2	1	1	1	1	15	10
NLB resistance	4	1	4	3	3	3	1	2	1	3	25	4
Yield	2	4	5	4	3	1	1	3	2	1	26	3
Texture	2	4	3	3	4	3	2	3	2	1	27	2
Endosperm colour	2	4	5	4	4	2	1	1	2	3	28	1
Total	30	34	44	35	38	26	20	22	20	21	290	
Rank	5	4	1	3	2	6	9	7	9	8		

Where, A = KS03-0B15-126, B = EB04-0A01-304, C= CML 159, D = KS03-0B15-2, E = KS03-0B15-45, F = VL 05616, G = KS03-0B15-47, H = CML 395, J= CML 442 and K = CKL 05007-B-B

Table 7.8 shows 13 traits developed and used by stockists to assess maize hybrids. The first ranked traits in order of decreasing importance were grain hardness, grain size, endosperm hardness, endosperm colour, twin cobs and cob size. Stockists identified ten most preferred crosses and subjected them to matrix ranking to reveal VL 05616 x CML 395, EB04-0A01-304 x

CML 442, VL 05616 x CML 159, CML 159 x CML 442 and EB04-0A01-304 x KS03-0B15-126 as best materials in order of decreasing preferences.

Table 7.8: Ten best stockists F_1 hybrids assessment at ARI-Tumbi, Tanzania for 2010/2011 growing season

Char./Genotype	FC	CJ	BA	BJ	FH	GF	IK	AD	ED	JH	Total	Rank
Cob size	4	1	3	3	4	1	2	1	2	2	23	5
Grain size	4	4	3	3	4	2	3	2	2	2	29	2
Yield	2	3	2	4	2	1	1	1	1	2	19	7
Twin cobs	3	3	2	4	3	3	1	1	3	2	25	4
Height	2	2	2	3	4	1	2	2	1	3	22	6
Maturity	2	2	2	3	4	2	1	1	1	1	19	7
Disease resistance	3	2	2	2	4	3	1	1	2	2	22	6
Drought resistance	2	3	2	3	3	1	1	1	1	2	19	7
Grain heaviness	4	4	5	3	4	1	3	3	2	2	31	1
Grain texture	2	3	2	3	3	1	1	1	1	1	18	8
Endosperm hardness	3	3	4	4	3	1	2	3	2	2	27	3
Endosperm colour	3	3	2	3	3	2	1	2	3	3	25	4
Grain rot	4	3	2	3	3	1	2	2	1	2	23	5
Total	38	36	33	41	44	20	21	21	22	26	302	
Rank	3	4	5	2	1	9	8	8	7	6		

Where, A = KS03-0B15-126, B = EB04-0A01-304, C= CML 159, D = KS03-0B15-2, E = KS03-0B15-45, F = VL 05616, G = KS03-0B15-47, H = CML 395, I = KS03-0B15-12, J= CML 442 and K = CKL 05007-B-B

7.3.5 Combined analysis

Statistical analyses, farmers and stockists combined analysis showed that, two F_1 hybrids EB04-0A01-304 x CML 442 and CML 159 x CML 442 were highly top ranked by farmers, stockist and breeders separately. These three hybrids are high yielding and have traits preferred by both farmers and stockists (Figures 7.4 and 7.5). At the same time, VL 05616 x CML 159, CML was ranked by both farmers and stockists among the best three hybrids. However, farmers and stockists differed for selection and ranking of the remaining varieties during assessments.

Figure 7.4: EB04-0A01-304 x CML 442 cobs (A) and (shelled B)

Figure 7.5: CML 159 x CML 442 cobs (C) and shelled (D)

7.4 Discussion

7.4.1 Factors influencing adoption rate of maize cultivars

Income in the community has an influence on the type of technology to be adopted. The community studied exhibited inequality in income distribution. This was shown by the Lorenz curve of income distribution among farmers which leaned right below the line of equality. The

Gini coefficient was 0.6405 which approached one to further signify income distribution inequality. At the same time, the coefficient of asymmetry was 0.6927. The coefficient of asymmetry less than one indicates income inequality distribution in the society. Income improvement is in this community can be done through the introduction of high yield and disease resistant varieties.

There are other factors which influence the rate of adoption of cultivars in the community. These factors can enhance or hinder the adoption rate of an intended cultivar transfer and dissemination. Logit regression by using Wald test was performed to assess ten factors affecting adoption of new cultivars in the area. Food security, cultivar yield potential and cultivar disease and drought resistance were found to be the major factors determining adoption rate of maize cultivars in the western zone of Tanzania. Other researchers report the importance of food security and other farmer desired traits in influencing cultivar adoption rate in societies (Cavane, 2011; Kaliba et al., 2000). Breeding for NLB disease resistance and high yielding F_1 hybrids could possibly contribute towards minimizing the effects of these factors. Scaling up and wide impact and adoption of F_1 hybrids could be realized if food security, disease and drought resistance which is coupled with high yield potential are instituted and introduced in the studied area (Doss et al., 2003; Mugisha and Diiro, 2010).

7.4 .2 Farmers and stockists F_1 hybrid assessment

Farmers and stockists develop criteria for germplasm assessment which largely depend on objectives and environmental circumstances of recommendation domain (Abebe et al., 2005; Bucheyeki et al., 2011). While farmers select a variety to meet multiple objectives like house hold food security, food preferences and weather condition, stockists require cultivars which maximize profit. In this study, farmers developed 14 criteria to assess 110 developed F_1 hybrids. Fourteen criteria developed by farmers seem manageable as other researchers report that more than 14 criteria for cultivar selection. For example, Assefa et al. (2005) reports 40 criteria developed by farmers to assess bean cultivars in Ethiopia.

In this study, farmers preferred cultivars with white endosperm colour, high yield potential and flint texture. These traits are linked to food security assurance as well as food processing qualities. In Tanzania, the major food dish preparation from maize flower is `Ugali`, the stiff porridge. According to farmers, ugali must be white and not otherwise (Katinila et al., 1998).

White ugali is a clear indication of wealth, no hunger and suffering. This inherited idea was brought by yellow corn supply through food aid during hunger time, the idea persisted and difficult to change despite the high levels of nutrients which can be supplied by yellow corn for example vitamin A rich varieties which are yellow. Based on farmers selection, VL 05616 x CML 159, CML 159 x KS03-0B15-47 and EB04-0A01-304 x CML 442 were highly ranked and possess preferred traits to meet their objectives. These cultivars are likely to receive high rate of adoption if released to farmers.

For increased income and profit maximization, stockists seek cultivars with traits that meet farmers demand and assurance of clients attraction. Based on these objectives, stockists developed 13 criteria for assessment of 110 developed hybrids. Stockists preferred cultivars with hard endosperm, large grain size and white endosperm. While, hard endosperm is linked to long storage period and resistance to storage pests to ensure high germination percentage, grain size has an implication on grain weight and high income because maize seeds are sold on weight bases. At the same time, white endosperm aims at customers' attraction as they prefer white flour for 'ugali' preparation. According to stockists ranking, Hybrids like VL 05616 x CML 395, EB04-0A01-304 x CML 442 and VL 05616 x CML 159 are likely to attract more stockists in maize seed industry. The increased number of stockists is expected to raise seed supply and probably reduce seed prices to attract farmers in adopting hybrid cultivars.

7.4.3 Farmer, stockist and breeders germplasm combined analysis implications

Selection criteria of breeders, farmers and stockists sometimes differ due to differences in intended uses. While breeders select parents to deal with traits like disease resistance, farmers and stockists might consider it as a secondary objective and preference. The created knowledge gap on variety selection criteria can be bridged through proper planning with the community concerned. In this study, statistical analysis ranked ten top most high yielding F_1 hybrids while farmers and stockists ranked five each. Although there were differences in ranking orders, the complementarily was also obvious. Abebe et al. (2005) found similar result while assessing maize cultivars in Ethiopia where farmers and community selections coincided but differed in other occasions. Two F_1 hybrids EB04-0A01-304 x CML 442 and CML 159 x CML 442 were highly ranked by farmers, stockist and breeders separately. These hybrids are high yielding and have traits preferred by both farmers and stockists. For increased maize yield production and rate of adoption, these hybrids are recommended for consideration for release.

This study demonstrated the significant importance of grassroots breeding which starts by breeders, farmers and stockists problem focus identification and implementation.

7.4.4 Proposed breeding and community linkage model

The held discussions with farmers and stockists after F_1 hybrid assessment in the field proposed a linkage model which aims at bridging the gap among practitioners (Figure 7.6).

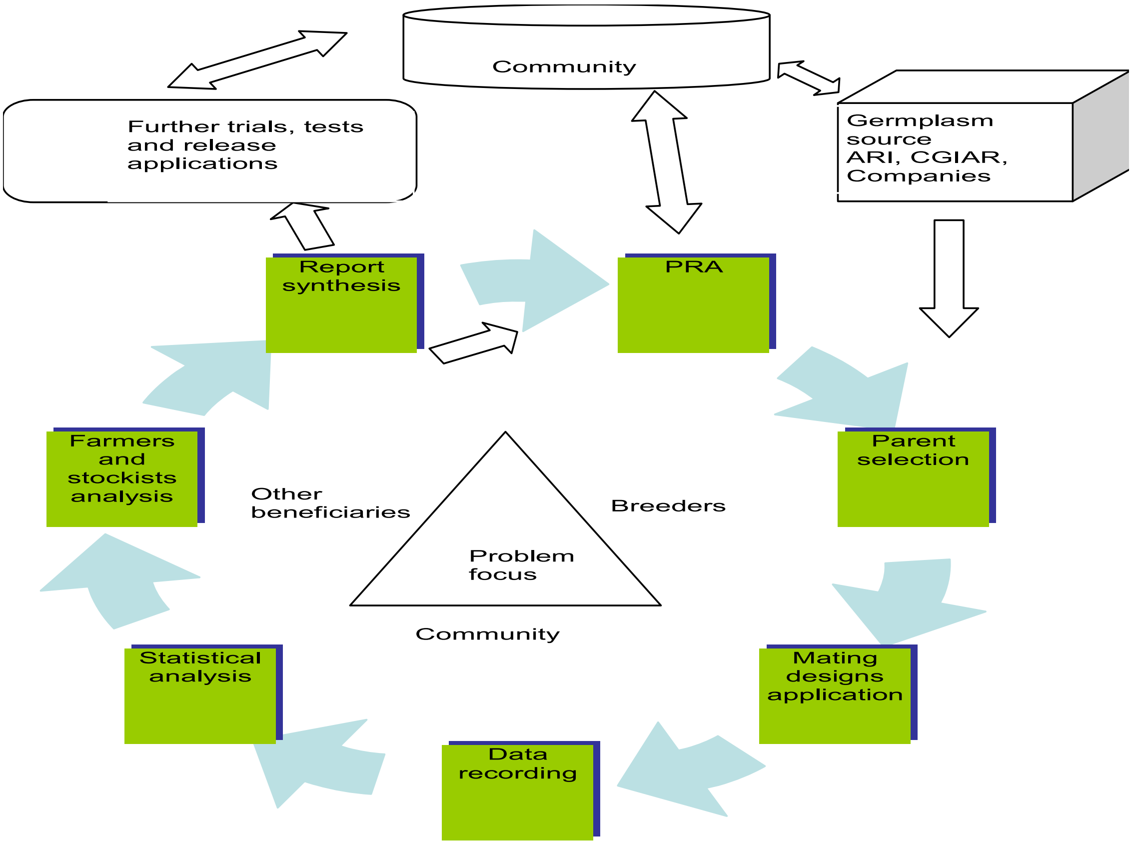

Figure 7.6: Proposed breeding and community linkage model for client oriented maize hybrid breeding development

Farmers and stockists in the western zone developed a functional linkage model for smooth cultivar development, transfer and increased rate of adoption. They proposed that, there must be a problem focus like low yield or disease related problem to tackle in the community. Breeders, community members and other beneficiaries plan to solve together. The problem solving starts with the participatory rural appraisal (PRA) so as to dissect the society and find the probable root cause of the problem. Best parent selection by breeders then follows which can be sourced from Agricultural research centres; CGIAR and the community itself (like provision of well adapted landraces). Parents are planted and crossed using appropriate mating designs depending on the nature of inheritance, number and nature of the problem to be solved. Data is recorded by breeders and community members to assess vegetative growth of breeding materials before harvesting.

At harvest, data is again recorded with the involvement of community members. Data recording is done independently and separately to avoid breeders influence and biasness. Community members involvement during harvest is crucial because, farmers and other community members assess parameters related to maturity, yield and prediction of post harvest qualities of breeding materials. Data analysis is also done separately. Breeders analyse some important traits and community members also analyse cultivars according to their criteria. After analysis, breeders and community sit, interpret and digest information together. The outcome of the meeting can be advancement to next breeding procedures like further testing in multi- location trials for variety release. There is a possibility of early adjustments before taking number of several years in cultivar development and ended up with the community rejected variety. The proposed model by communities in Tanzania seems to fit maize breeding programme as is cost effect, serves time, finance and human resources. However, some strengths and weaknesses have to be put into considerations.

7.4.5 Proposed model strengths

- The model ideas came from the community so it represents opinions of farmers and stockists who are chief clients of variety dissemination and adoption.
- It is more efficient in time, human and finance resources serving.
- It allows the community active participation in the breeding process.
- The cultivar developed by using the proposed model is likely to achieve high adoption rate in the community.
- The model is flexible and allows adjustments during breeding cycles.

7.4.6 Proposed model weaknesses

- The model is likely to be influenced by socio-economic, environment and weather frequent changes. For example, when drought occurs, farmers could demand early and drought variety. At the same time, farmers could demand late maturity variety during heavy rainfall seasons. This complicates breeding programme planning.

- Sometimes, the community might think the immediate solution to a researchable problem and thus communities seeks for early outcomes of the technology. As time elapses, farmers might think that they are wasting time and hence discouraged in active participation. This result to drop out of some community members in the variety breeding process.
- Breeders prefer small plot sizes at on-station, medium during multi-location and big plot sizes during on-farm trials. On the other hand, farmers prefer big plot sizes right from the beginning of the trial (on-station). The plot size preference differences are due to yield measurements units used by farmers and breeders. Breeders yield measurements units are in metric systems such as grams and kilograms while farmers uses packages like sacks (1 sack = 90 kg) to bring confounding ideas.
- Community members operate under complex social, political and environment circumstances and are obliged to them. This limits their frequent participation in the variety breeding which time is demanding.

7.5 Conclusions

The following conclusions can be drawn from this study

1. Farmers developed 14 criteria for maize varieties selection. The five highly ranked criteria included endosperm colour, yield, grain texture, NLB disease and drought resistance**.** By using those criteria, farmers preferred VL 05616 x CML 159, CML 159 x KS03-0B15-47 and EB04-0A01-304 x CML 44 experimental F_1 hybrids.
2. Stockists developed 13 criteria which were related to community variety preferences for maize variety selection. These included grain hardness, grain size, endosperm hardness, endosperm colour, twin cobs and cob size. Stockists preferred VL 05616 x CML 395, EB04-0A01-304 x CML 442 and VL 05616 x CML 159.
3. By using statistical and normal ranking from the highest to lowest grain yield and resistant to NLB disease, breeders ranked ten F1 hybrids.
4. Comparison between breeders, farmers and stockists findings which were done independently of each other revealed EB04-0A01-304 x CML 442 and CML 159 x CML 442 F_1 experimental hybrids being ranked in the top five in each group.

5. Two F_1 hybrids EB04-0A01-304 x CML 442 and CML 159 x CML 442 emerged and appeared in all five top rankings of breeders, farmers and stockists.
6. Generally, this study observed that, although breeders, farmers and stockists might have different variety selection criteria but sometimes coincide.

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Chapter 8

8 General overview and the way forward

8.1 Introduction

The prevalence and severity of NLB disease in farmers' field coupled with susceptible cultivars grown in Tanzania formed the base of this study. The primary focal point was to identify locally adapted and resistant maize germplasm to be used in breeding for NLB disease resistance. The work started by acquiring maize inbreds, screening for NLB disease resistance and other suitable traits. In addition, maize landraces were also collected and morphologically characterized based on NLB disease reactions. Participatory plant breeding was applied through PRA and field evaluation of experimental F_1 hybrids by which both farmers and stockists were involved. The second step was to understand the NLB disease inheritance in inbreds which are locally adapted to Tanzania conditions. The NLB disease problem focus was used to generate the study hypothesis and objectives to be tested and accomplished by the study. Therefore, this chapter deals with the hypothesis tested and provides an overview of study major findings, implications and the way forwards.

The tested hypotheses are shown here under:

1. Factors limiting maize production in western Tanzania are known by farmers and can be taped for documentation. The increased understanding of these constraints by both farmers and stockists can be used to improve the maize programme agenda in Tanzania.
2. Farmers and stockists in western Tanzania have specific preferences and variety selection criteria to be documented. These criteria can be included in the breeding programme to enhance the selection index with a possible impact on variety release and adoption.
3. There is prevalence of NLB disease in farmers' fields of western Tanzania which compromise grain yield.
4. There are genetic variations among maize landraces found in Tanzania which can be exploited in a breeding programme to enhance variety release throughput.
5. There is high combining ability for NLB disease resistance among eleven maize inbred lines adapted to Tanzania condition selected parents. These lines can therefore be combined to develop productive hybrids that perform under NLB infestation.
6. There is no existence of epistatic gene action among maize inbred lines found in maize inbred lines adapted to Tanzania conditions to affect the effectiveness of additive and

dominance effects estimation. Therefore new inbreds can be developed through selection to enhance the level of resistance to the NLB disease.

8.2 Summary of the major findings

8.2.1 Maize production constraints, NLB disease status, stockists and farmers' opinions on varieties selection preferences in western Tanzania

The PRA study conducted in Sikonge, Urambo and Nzega districts of Tabora in Tanzania observed low maize yield of about 1.1 t ha^{-1} caused by biotic and abiotic factors. The research highlights were:

- Thirteen major maize production constraints were recorded. Five top ranked problems included Low-N, diseases incidences, lack of farm inputs, lack of improved varieties and drought prevalence. In addition 13 insect pests and vermins and were identified. The highly ranked were Stalk borer (*Busseola* spp), army worms (*Spodoptera* spp), large grain borer (Prostephanus spp), Striga *asiatica* and *Striga hermonthica.* The study also recorded 11 diseases which included Maize streak virus (*Gemini* spp), Northern leaf blight (*Helminthosporium turcicum),* Fusarium stalk rot (*Fusarium moniliforme),* Fusarium ear rot *(Fusarium moniliforme)* and Leaf rust (*Puccinia maydis*).
- Farmers preferred traits included resistance to abiotic and biotic stresses, early maturity, preferred milling qualities, high storage qualities and high yielding potentials.
- Stockists identified 12 preferred maize variety traits for selection for the business. The top ranked five included high yielding, disease and insect pest resistance, heavy grain, high yield, large cob size and large grain sizes.
- It was reported that, the NLB disease has invaded all farmers' fields and attacked the current cultivated cultivars in the field.
- Similarity between farmers and stockist variety preference ranking were found to exist. Although Farmers put more emphasis on food security and processing qualities, while stockists emphasized on traits linked to high prices and profit maximization. Farmers and stockist both ranked high milling qualities and high yield traits.

8.2.2. Occurrence and distribution of northern leaf blight disease in western Tanzania

The study for NLB disease prevalence in western Tanzania was conducted in seven districts for two seasons. The following were the study finding highlights:

- The disease incidence in season two (2009/2010) significantly increased from season one (2008/2009) t= -3.25 (348), P= 0.001.
- Altitude and NLB disease severity were highly positively correlated (0.117**).
- The NLB disease has changed its distribution pattern affecting all districts of the western zone of Tanzania. Both means of northern leaf blight disease incidence and severity approached 30% across districts.
- Out of 16 varieties grown by farmers only three cultivars were resistant to NLB disease. The resistant cultivars included Gembe landrace, TMV-1 (Composite) and one unknown was among the three observed resistant varieties. The susceptible cultivars included both landraces and modern varieties.

8.2.3. Characterization and screening of maize landrace for northern leaf blight disease resistance in the western zone of Tanzania

Characterization study based on NLB disease resistance on 71 landraces and 19 commercial varieties were planted at Agricultural Research Institute Tumbi in Tabora. The following were research highlights of the study:

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- Variation existed among maize landraces in terms of morphological and NLB disease reactions. Landraces showed high variations in lesion width with the mean of 7.84 mm and incidence (11.67%). Five principal components contributed 71.98 % of total variations in maize landraces. Leaves /plant, infested leaves/plant, lesion number, lesion length, lesion width and NLB incidence traits highly contributed to variations and grouping of landraces. Landraces were further clustered in five distinct groups of maize landraces to denote their breeding potentials in the area.
- Landrace TZA 3075 was identified as NLB disease resistant. This was recorded in principal component 3 which had the lowest NLB incidence of 11.25%.
- The important agronomic traits found in maize landraces included yield potential, dent grain texture, white endosperm and husk cover

8.2.4 Combining ability analysis for northern leaf blight disease resistance on Tanzania adapted inbred maize lines

A 11 x 11 full diallel analysis for northern leaf blight resistance was carried out at three sites of Tanzania. The following were the research highlights from the study:

- The mean sum of squares for GCA was highly significant ($P < 0.001$) on disease severity indicating additive gene action.
- The GCA contribution was high for disease severity (91%) and lesion number (85%) to further denote additive gene action on the disease expression.
- The majority of parents had negative GCA to imply contribution to disease resistance on their progenies.
- The mid parent heterosis for NLB disease severity ranged from -93.46 to 361.99%.
- Maternal effects had non significant ($P>0.05$) effects on the inheritance of the NLB disease severity.

8.2.5 Generation mean analysis of northern leaf blight disease resistance in five tropical maize inbred lines

Generation mean analysis was conducted using five inbred lines in a six parameter model comprising P_1, P_2, F_1, F_2, BCP_1 and BCP_2 generation progenies. The following were the result highlights of the study:

- The full model of additive, dominance, additive x additive, additive x dominance epistatic effects and dominance x dominance epistatic effects was highly significant ($P<0.001$).
- The additive gene effects were predominant ranging between 57% - 89% which was confirmed by large heritability (54-85%).
- The average degree of dominance ranged between -0.52 to 0.88 supporting observations of partial dominance.
- The NLB disease severity showed the continuous distribution in all sets for F_2, BCP_1 and BCP_2 populations indicating that inheritance of resistance was quantitative.
- The mid parent heterosis ranged from -19 to 1% indicating NLB disease resistance in the populations.

8.2.6 Client oriented breeding for maize northern leaf blight disease resistance in western Tanzania

Sixty farmers and 12 stockists participated in assessing 110 experimental hybrids in the western Tanzania. The following results were recorded:

- Farmers revealed 14 criteria for maize selection. The highly ranked criteria included endosperm colour, yield, grain texture, NLB disease and drought resistance. The most preferred hybrids by farmers were VL 05616 x CML 159, CML 159 x KS03-0B15-47 and EB04-0A01-304 x CML 44

- Stockists developed 13 criteria which are related to community variety preferences for maize variety selection. These included grain hardness, grain size, endosperm hardness, endosperm colour, twin cobs and cob size. Stockists preferred VL 05616 x CML 395, EB04-0A01-304 x CML 442 and VL 05616 x CML 159.
- By using statistical and normal ranking from the highest to lowest grain yield and resistant to NLB disease, breeders ranked to ten F1 hybrids.
- Comparison between breeders, farmers and stockists findings which were done independently to each other revealed EB04-0A01-304 x CML 442 and CML 159 x CML 442 F_1 experimental hybrids being ranked in top five in each group.

8.3 Implications for breeding NLB disease resistance and the way forward

The PRA study revealed maize yield was low caused by abiotic, biotic and social factors. In combination, they hinder production to the extent of 1.1 t ha^{-1}. Some of the problems can be solved by multidisciplinary team that involves breeders, pathologists, socio-economists, agronomist, soil scientists and anthropologist. There are reports of agricultural problem solving which resulted to increase rate of adoption and enhanced maize yield by multidisciplinary approaches (Efron et al., 1989; Pixley, 1994).

The identified problems like low rate of adoption of improved hybrids and the persistence of using locally available low yield and disease susceptible varieties are probably some of the chief causes of low yield and increased disease problem in the area. The study found that, 65% of farmers still grow landraces because they can be recycled, locally available and possess community preferred traits. Efforts should be therefore be concentrated to the breeding, introduction and campaigning for the use of high yielding and disease resistant hybrids.

The NLB disease prevalence and severity observed to increase in farmers field which calls for NLB disease resistance traits exploitation. However, Gembe landraces was recorded to be NLB disease resistant. Breeding procedures should be employed to study the mechanism and mode of Gembe landrace resistance. The use of molecular markers could be used to map the Gembe NLB disease resistance and then introgression studies to susceptible germplasm can be done.

Because Gembe landrace possesses the community preferred traits, it could be better if breeding procedures like recurrent backcrossing selection is employed to improve its yield potential (Zwonitzer et al., 2009). Improvement of landraces in the maize breeding programme is part of the participatory breeding which advocates community active participation in breeding procedure.

The research found that, community involvement in breeding programme was lacking. Few farmers were involved in participatory variety selection (PVS) only leaving behind stockists who played a great role in variety selection for sale and supply. Breeding for community preferred traits that involves farmers and stockists is desired in the area. Incorporation of farmers and stockists desired traits is expected to improve the rate of adoption of improved varieties and subsequently to the increased yield. Traits like resistance to abiotic and biotic stresses, early maturity, high milling qualities, white endosperm colour, dent grain texture and yield were preferred by both farmers and stockists. These traits should be incorporated in the maize breeding program. However, high yield in the area is likely not to be realized if the community mentioned agricultural production constraints are not solved and sorted out.

Problems like low-N, drought, disease incidences and low yield can be solved by the maize breeding programme. Breeding for low-N and drought tolerant in maize could assist in minimizing the problem. At the same time, breeding for disease resistance could help to solve maize susceptibility problem in the area. Other mentioned constraints like farm inputs availability and supply related can be solved by agricultural extension departments in the respective districts.

The NLB disease occurrence and distribution study revealed that, the disease is moving to lowland maize production affecting majority of maize cultivars growing in studied districts. Both means of blight incidence and severity approached 30%. This is an indication and prediction of NLB disease outbreak in the near future. The situation could be aggravated if farmers continue to grow susceptible varieties as recorded by this study. In addition, the observed NLB disease susceptible varieties possess a potential danger in the area. This is because; they act as sources of inoculum to the next season. This study established that both landraces and improved maize cultivars were invaded by the disease. Only three cultivars were observed to be resistant. These included landrace Gembe, TMV-1 which was released in 1987 and one unknown. Whether the observed resistance was due to genetic make up or disease escape needs further investigation. This can be done by applying on-station screening trials under standardized condition and environments for obtaining suitable genotypes in the area.

A study on characterization and screening of maize landrace based for northern leaf blight disease resistance revealed variations in maize landraces which are of importance to breeders. Number of infested leaves per plant, days to tassel emergence, plant height and NLB disease lesion characteristics were the principal traits that discriminate maize landraces into distinct groups. Breeders should utilize this information for selecting parents with different contrasting traits from different principle component groups and clusters recorded by this study. However, environmental factors could have probably affected the results as morphological markers can be affected by the prevailing environmental conditions. Although morphological markers provide useful information, a combination of morphological and molecular markers on genetic relationships provide more précised results (Bucheyeki et al., 2009; Doebley and Stec, 1991). Therefore, maize landrace clusters generated by this study can be confirmed by the molecular markers characterization which is less affected by environmental factors (Redfearn et al., 1999). This study also revealed TZA 3075 landrace as NLB disease resistant. The identified landrace can be utilized in maize breeding as a source of NLB disease resistance.

The combining ability analysis for northern leaf blight disease resistance in maize lines revealed high GCA contribution for disease severity (91%) and lesion number (85%) to denote additive gene action predominance on the disease expression. This study identified parent EB04-0A01-304 which had significant positive GCA effects on yield and highly negative effects on NLB disease resistance. This indicates that, parent EB04-0A01-304 contributes towards NLB disease resistance and high grain yield. Results also showed that, all the top ten F_1 experimental hybrids had negative heterosis to NLB disease severity to denote the enhanced resistance performance of progenies. The negative heterosis showed by F_1 experimental hybrids could be utilized in maize breeding for development of NLB disease resistant cultivars. This study also recorded several NLB disease resistant F_1 hybrids which can be advanced by testing in multi-location trials with the aim of releasing the best ones. Crosses like CKL 0500-B X KS03-OB15-12 showed resistances across sites which is the implication of wide adaptation to environmental conditions. Breeding materials which showed NLB disease resistance could be advanced to multi-location trials for further release application procedures.

The generation mean analysis of northern leaf blight disease resistance study revealed the additive gene effects being predominant over dominance and epistatic non-allelic interaction effects which was confirmed by large heritability. Therefore, resistance to NLB disease could be improved through selection by exploiting the additive gene effects. Crosses like P145-95 X

CML 395 and E29 X P86-95-1 showed high heritability and negative heterosis for NLB disease resistance. These crosses could be incorporated in maize breeding programme as potential additional genotypes resistant to NLB disease and a base population to extract new inbred lines within superior NLB resistance. Findings have established that, it is possible to improve resistance to NLB disease in maize through breeding strategies like recurrent selection as suggested by Ceballos et al. (1991) to concentrate the frequency of resistance alleles in the population.

The study on client oriented breeding for maize northern leaf blight disease established that, it is possible to plan breeding, implement and evaluate breeding materials with the surrounding community. Findings of the PRA study were incorporated in the maize breeding programme. Breeders, farmers and stockists separately identified two F_1 experimental hybrids EB04-0A01-304 x CML 442 and CML 159 x CML 442 as high yielding crosses with preferred community traits. Introduction of these breeding materials to farmers is likely to get high rate of adoption due to involvement of community right from the planning stage to variety release.

Generally, there is a high potential for increased and improved maize yield in the western Tanzania due to:
The presence and willingness of farmers and stockists to provide their opinion on maize variety and active participation in the research processes denoted the assistance they need and aspire for increased maize production. This was supported by the presence of landraces with contrasting traits. Landraces like Gembe and TZA 3075 were observed as NLB disease resistant but had low yield potential while Kabalagata another landrace was observed as early maturing variety but highly susceptible to NLB disease. Breeding procedures like recurrent backcrossing by which varieties with some desired traits but also possess some undesired traits can be improved by lowering the undesired traits. In this case Gembe landrace can be used as a potential source of NLB resistance alleles which can be transferred into the other high yielding but NLB susceptible varieties.

The presence of indigenous technical knowledge (ITK) like the ability of farmers and stockist to select the desired variety and application of crop husbandry practices such as managing NLB disease by burning crop debris during land preparation and grazing on maize stovers are some of the messages and opportunities to breeders. Another opportunity realized by this study included soil fertility reclamation by application of fertilizer trees like *Gliricidia sepium*. The above ITK could be improved and supplemented by breeders through introduction of improved fallows and rotation woodlots so as to increase soil fertility in farmers' field. Breeders would test and select new varieties under this fertility management systems.

Complex intercropping which involved more than five crops at space and time was also observed in the area. While many maize breeding programmes normally aims at maximizing yield or increasing resistance in monocropping system, farmers practice complex intercropping which is a challenge and an opportunity to breeders to select varieties that could perform under both monocropping and intercropping farming systems (Kasenge et al., 2001). This is because maize variety performs differently when grown as pure culture or intercropped. It seems this could be another reason of variety abandonment due to differences for selection in different farming systems. Breeders select varieties under pure culture and when these varieties are subjected to complex intercropping, they probably fail to show their expected production potential because they have low competitive ability.

The tested inbred lines showed the predominance of additive gene action which was shown by high GCA (91%) contribution for NLB disease severity in diallel cross, high heterosis in diallel cross (-93.46%) and in GMA (-19%) studies. The predominance of additive gene effects was also supported by high additive variation contribution (57 - 89%) which was confirmed by large heritability (54-85%) in GMA study. The continuous distribution of progeny frequency in the F_2 population in GMA study further denoted that, the NLB disease in maize is inherited polygenically. This makes the possibility of maize improvement through breeding procedures like recurrent selection. The presence of epistatic interaction effects noted in the study would bring less complication because they were negligible.

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