

Evaluation of Gray Leaf Spot Tolerant Genotypes from CIMMYT in the Highland Maize Production Eco-systems of Bhutan

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Abstract: Bhutan is a small landlocked country located in the eastern Himalayas. Over 69% of the population is engaged in agriculture. Rice, maize, wheat, barley, buckwheat and millets are the major cereal crops cultivated. Rice is the most preferred food crop of the Bhutanese. Maize is a primary food crop after rice and it ranks first among food crops in production. The cultivation ranges from less than 300 m asl (metres above sea level) nearly up to 2,800 m asl. In 2007, a new, extremely serious problem of GLS (gray leaf spot) in maize that was previously never reported in Bhutan was confirmed. This disease spread rapidly in the highland maize growing areas causing production losses of over 50% to 70%. All the maize varieties cultivated in the country were found to be highly susceptible to the disease. In order to contain this devastating disease, the national maize program drew short and long term strategies with the help of a CIMMYT Expert. As an immediate short term action to contain GLS, systemic fungicide Tilt 25 EC (active ingredient propiconazole) was supplied free of cost to the farmers. A longer term strategy pursued was the introduction, evaluation and selection of GLS tolerant genotypes for the highland ecosystem. Over 100 GLS tolerant genotypes were introduced from CIMMYT Colombia, Mexico, Zimbabwe and Nepal. These materials were initially evaluated in a disease hotspot sites and then further tested in multi-location trials in GLS affected areas across the country. Farmers were engaged for Participatory Variety Selection by organizing farmer's field days at the trial sites. Finally, in 2011 considering the need of GLS tolerant varieties for farmers, two GLS tolerant genotypes ICAV305 and S03TLYQAB05 were provisionally released. In the 2011 season, these two provisionally released genotypes were put under large scale demonstration in the GLS affected areas in nine districts across the country. In 2012, the two genotypes were formally released by the Technology Release Committee of the Ministry of Agriculture and Forest. Rapid seed increase of the new varieties was initiated through farmers from Community Based Seed Production groups and so far 75% seed replacement of GLS affected farmers has been accomplished.

Key words: Gray leaf spot, hotspot, yield loss, participatory variety selection, community based seed production and seed replacement.

1. Introduction

Bhutan is a small landlocked mountainous country located in the southern slopes of eastern Himalayas. It is sandwiched between the two great Asian civilizations, China to the north and India in the east, west, and south. The country lies between latitudes 26°45'N and 28°10'N, and longitudes 88°45'E and

92°10'E. The country has a total geographical area of 38,394 km² with a population of 745,600 people [1]. The forest (tree) cover of the country is about 70.46%, arable land 2.93%, meadow land 4.10%, shrub land 10.43%, snow cover land 7.44% and bare areas 3.20% of the total geographic area [2]. Agriculture is the mainstay of the people with an estimated 69% of the population engaged in farming. Rice, maize, wheat, barley, buckwheat and millets are the major cereal crops cultivated in Bhutan and rice is by far the most

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important and preferred food crop of the Bhutanese.

Maize (*Zea mays* L.) is a major food crop cultivated by 69% of the rural households for subsistence. The total area under maize in 2010 was 61,476 acres; the total production was 57,666 Mt with a national average yield of 2.38 t·ha⁻¹ [3]. Maize ranks first in the extent of area cultivated amongst the food crops. Maize cultivation in the country ranges from less than 300 m to nearly up to 2,800 m asl owing to its versatile capacity to adapt to different environments. It plays a critical role in ensuring the household food security. It is estimated that 80% of the total production is consumed at the household level by the farmers which is valued at Nu. 353 Million (1 USD = 54 Ngultrum (Nu.)) annually [4]. About 6% of the total production is sold which is an important source of household income. The rest of the production is used as seed, processed into different products and fed to the livestock. The maize production environment in the country is broadly categorized into three zones mainly based on the altitude. The three production zones are: sub-tropical maize production zone I (< 1,200 m asl) or low altitudes; sub-tropical maize production zone II (1,200-1,800 m asl) or mid altitudes; and the highland maize production zone (> 1,800 m asl) [5]. These different production zones vary widely in their production potentials and constraints.

Bhutanese maize farmers in the Highland and Sub-tropical Zone II are facing a new, extremely serious problem of GLS (Gray Leaf Spot), fungal disease which was previously never reported in the country [6]. According to [7], the incidences of GLS has increased during past two decades and today it is one of the greatest threats to global maize production [8]. In Bhutan, GLS and TLB (Turcicum leaf blight) have become economically important diseases especially in areas above 1,200 m asl. Serious outbreaks of these diseases were reported from 12 major maize growing districts in 2006. GLS spread rapidly in the highland maize growing areas and

attained epidemic scale in 2007. It affected 4,193 maize growing households. The total area affected was 4,821.89 acres and the total production loss due to the disease was 6,504.12 Mt [3]. The estimated production loss of the affected farmers ranged from 50% to 70% [3]. In Zambia, when similar GLS epidemic occurred in the mid 1990s, the average yielded losses ranged from 28% to 54% with an average of 33.5% [9]. The impact of this disease, farmers crop husbandry practices, disease management strategies and the disease reaction on the GLS resistant genotypes introduced from CIMMYT Colombia are discussed in this paper.

2. Materials and Methods

An initial report of premature drying of maize was received from 12 maize growing districts in 2006. Such reports mostly came from maize growing areas above 1,500 m asl. Accordingly, field visits and rapid surveys were conducted in the affected areas by the researchers from the NPPC (National Plant Protection Centre) and the RNR RDCs (Renewable Natural Resources Research and Development Centers). The crop was in the late maturity stage and most of the lower leaves had completely dried. Some lesions of TLB were observed in the few upper leaves which were partially green. TLB was suspected to have caused the damage. To assist the maize program in further confirming this problem, technical assistance of CIMMYT (International Maize and Wheat Center), South Asia Regional Office, Kathmandu was sought. The CIMMYT office immediately recommended the input of Dr. Carlos De Leon, a former plant pathologist of CIMMYT for two weeks. The technical expert made extensive field visits in the major disease affected areas to determine the level of disease incidences and identify the disease. Informal discussions were held with the affected farmers to understand their field and crop management practices. Infected leaf samples were collected for laboratory investigation. The collected samples were incubated

and observed under the microscope and finally GLS and TLB were confirmed in 2007. GLS was observed to be more serious and damaging as compared to TLB. Immediately after the confirmation of the disease, the expert recommended an immediate and a long term strategy for the management of the disease considering the serious impact of the disease on the household food security of the maize dependent farmers. All the traditional maize varieties including the most popular improved variety Yangtsipa (Suwan 1) were found to be highly susceptible to GLS.

The immediate and short term strategy adopted was the spray of systemic fungicide Tilt 25 EC (active ingredient propiconazole). The longer term and a more sustainable strategy was the introduction, evaluation and selection of GLS tolerant maize genotypes as the majority of Bhutanese farmers cannot afford expensive fungicides. Moreover, repeated use of fungicides are not feasible both economically and environmentally [10] besides physical constraints due to steep terrain slopes more than 50%. The use of genetic resistance has been noted as the most sustainable means to prevent maize production losses from GLS in Africa, especially for subsistence farmers who cannot afford to purchase expensive fungicides [11].

To initiate the development and selection of GLS tolerant genotypes for the country, GLS tolerant germplasm were requested from CIMMYT International. Accordingly in 2007, 45 GLS tolerant genotypes from CIMMYT Colombia, 23 from Mexico, eight from Zimbabwe and six from the National Maize Program of Nepal were introduced to the country. As the quantity of seeds was small, the first step was the off season increase of the seed at Lingmethang research farm (640 m asl) through controlled pollination. The first batch of materials to arrive in the country was 45 entries from CIMMYT Colombia. From the 45 entries, 39 entries were planted at Lingmethang Research Sub Centre (640 m asl) in October 2007 as winter nurseries for seed increase through controlled pollination. From the 45 entries,

four entries had enough seed and one which was a hybrid was dropped from seed increase. To screen the introduced materials under disease pressure, a disease hotspot site at Chaskar (1,960 m asl), in Mongar district where the disease occurs naturally and in abundance due to continuous cropping of maize was identified. Verma [9] reported that new materials were screened for tolerance to GLS in the Zambian Seed Company's farm at Lusaka that had turned into a good "hotspot" for GLS due to continuous cropping of GLS susceptible maize varieties. The screening and evaluation for tolerance to GLS in Bhutan started from March 2008. To facilitate the screening of the materials, two acres of land was leased at Chaskar from a farmer by the maize research program. After initial evaluation, promising materials were promoted to the nationally coordinated trials for evaluation in multi-location trials across the country through the Regional Research Centers. At the trial sites, farmer's field days were organized to engage farmers for PVS (Participatory Variety Selection). As GLS was new to the country, on the job training were provided to the researchers and extension staff in monitoring the disease. In the trial sites, GLS was constantly monitored by the researchers. At the disease hotspot trial site in Chaskar, the introduced materials were planted in the third week of March and harvested in late September. GLS was scored based on the scale of 1-5 where 1 = no lesions visible, 2 = few lesion seen on two lower leaves, 3 = lesions visible on most leaves below the ear, 4 = many lesions visible on leaves above the ear and 5 = all leaves dead. All other agronomic parameters were recorded at the time of harvest. Existing improved and released variety Yangtsipa and a local variety from Chaskar were used as the check varieties in the trial site.

3. Results and Discussions

3.1 Disease Confirmation and Conditions That Favoured Disease Development

The observation of symptoms in the field and the

subsequent observation of spores under a simple microscope confirmed the disease to be GLS (Fig. 1) caused by the fungus *Cercospora zea-maydis* (Tehon and Daniels 1925). Another fungal disease TLB caused by *Exserohilium turcicum* (Synonymous; *Helminthosporium turcicum*) locally known as *Songsongma* was also confirmed. TLB was present in the country, but serious infection was noticed only after 2006. GLS was previously never reported in the country and was confirmed for the first time. Severe incidences of GLS were observed at elevations nearly and above 1,500 m asl. At these elevations, GLS was found to be causing the most damaging effects with some farmers losing almost 100% of their crop [6]. At the elevations 1,500-1,800 m asl, both GLS and TLB were prevalent. Below 1,700 m asl, TLB was more prevalent but with incidences of lesser economic significance. GLS symptoms were also seen at 600 m asl, however, the impact was much lesser as compared to areas above 1,500 m asl.

The cause for outbreak of these two diseases in Bhutan is attributable to the farming practices which are congenial for the development of the diseases. The predominant farming practices adopted by the maize growers in the country are: (1) mono-cropping of maize with occasional rotation with potato; (2) use of maize stalks and crop residues as livestock bedding, and subsequently as FYM (farm yard manure)—a major source of nutrients for the crop; (3) reduced or minimum tillage due to steep terrain.

Apparently, the main source of inoculum for GLS was the infected crop residues especially the leaves and the leaf sheath left on the soil surface. Bhutanese farmers in the highlands practice the continuous cultivation of maize, use maize stover for feeding the cattle as animal bedding and also collect and heap the stover in the field to be spread in the field at the time of planting. The leftover residues from the feeding stalls go into the compost yard which is later spread in the maize field. These practices seemed to greatly favor the development of the disease.

Majority of Bhutanese farmers use locally made ploughs drawn by the bullocks which do not penetrate very deep in the soil. The plough does not adequately incorporate the crop residues deep in the soil. Farmers in the highlands (> 1,800 m asl) normally practice continuous cultivation of maize without crop rotation due to the limited land holdings, short growing season and lack of other suitable optional crops. As a result of the disease, the maize production has substantially declined from 2005 (Fig. 2).

All these cropping practices like continuous mono-cropping of maize [12-14] and use of minimum or conservation tillage practices [12, 15, 16] have been found to increase the incidence of the disease. In



Fig. 1 GLS conidia as observed under simple microscope, 2007.

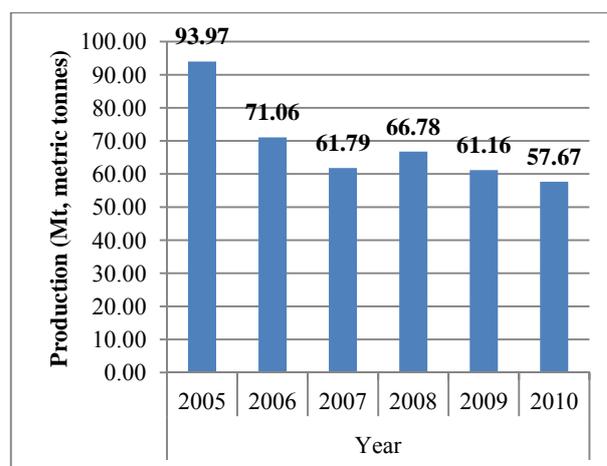


Fig. 2 Maize production trend in Bhutan [3].

Uganda, Africa where maize is one of the main crops, leaving the disease infected stover in the soil surface and planting of susceptible variety were identified as the key factors responsible for the perpetuating of GLS [17]. Further, in most maize growing areas of the country, cloudy weather with high humidity and extended period of wetness prevails with the onset of monsoon from late May till September, which favours the growth and development of the pathogens [18].

3.2 Immediate Management of the Disease

Since the diseases had a serious threat on the household food security of the farmers, spraying of systemic fungicide Tilt (active ingredient propiconazole) was recommended as an immediate measure. Tilt 25 EC (active ingredient propiconazole) was imported from India and was recommended to be sprayed at 2 mL per litre of water with minimum of one spray, two weeks before flowering. The DoA (Department of Agriculture) with fund support from the EU-ASSP (EU-supported Agriculture Sector Support Project) supplied 1,650 L of fungicide Tilt (propaconazole) worth Nu. 1.65 Million the affected farmers free of cost. However, spraying was cumbersome and ineffective due to steep terrain where water had to be fetched from downhill, lack of appropriate spraying machines and accessibility of far flung maize fields.

3.3 Introduction, Evaluation and Selection of Disease Tolerant Varieties

The use of genetic resistance has been noted as the most sustainable means to prevent maize production losses from GLS, especially for subsistence farmers who cannot afford to purchase expensive fungicides [11]. Currently, there are no GLS tolerant varieties released for the highland maize growing areas in the country and therefore, the introduction, evaluation and selection of GLS tolerant maize genotypes were given the highest priority. The 45 GLS tolerant genotypes from CIMMYT Colombia, 23 from Mexico, eight

from Zimbabwe and six from the National Maize Program of Nepal were the primary source for adapting and releasing GLS tolerant genotypes. In 2008 season, most of the genotypes appeared to be promising with reasonably good level of field tolerance to GLS and TLB. In the first season, the main selection parameter was the tolerance to GLS and TLB and not too much emphasis was given to other agronomic traits. Among all the genotypes, the genotypes from CIMMYT Colombia were most stable and exhibited better tolerance to GLS while genotypes from CIMMYT Mexico appeared to be inbred lines with poor plant type. All the materials from CIMMYT Zimbabwe were white maize which is less preferred by farmers and the ones from Nepal were all susceptible to GLS. From the 45 genotypes from CIMMYT Colombia, 15 genotypes which received an average GLS and TLB score of less than 2.5 [19] were selected for further evaluation (Table 1).

In 2009 maize season, the 15 selected entries were evaluated in five different locations above 1,500 m asl where GLS occurs in abundance. The results indicated significant difference on GLS and TBL incidences and yield among the different genotypes evaluated (Table 2). The results also showed that there was no large difference on disease tolerance and yield among the different genotypes as compared to the check varieties. This is mainly because the introduced genotypes are new to the highland maize ecosystem and are still undergoing adaptation whereas the check varieties are well adapted to the highland ecosystem.

To engage farmers and extension as active research partners in evaluating and selecting suitable GLS tolerant genotypes, farmer's field was organized for the PVS (participatory variety selection) at the disease hotspot trial site in Chaskar. A total of 34 farmers and extension staff who participated in the PVS ranked Cap. Miranda 99Bact1F-1 (Entry No. 1) as their first choice, S03TLYQAB05 (Entry No. 35) as second, GLS1Y01/SPMAT (Entry No. 6) as third and Villavicencio 03Asp1C (LET-EARLY) (Entry No. 21)

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Table 1 The 15 promising GLS tolerant genotypes selected from CIMMYT Colombia materials, 2008.

Si No.	Entries No.	Pedigree	GLS score*	TLB score*	Yield t/ha
1	1	Cap. Miranda 99Bact1F-1	1.8	2.2	7.91
2	13	Menegua 01 Phaeo	2.2	1.5	5.13
3	2	Granada 01Phaeo1AS2	2.3	2.3	4.85
4	23	Villavicencio 03Phaeo1A(SA4)	2.3	1.7	4.58
5	35	S03TLYQ AB05	2.3	2.0	8.58
6	6	GLSIY01/SPMAT	2.3	1.4	5.07
7	38	ICA V305	2.4	2.3	7.58
8	21	Villavicencio 03Asp1C (LET-EARLY)	2.5	1.3	7.58
9	33	Cimcali 03HCG1A	2.5	1.8	6.10
10	3	Turipana 01DMR 1D(1)	2.5	2.5	6.01
11	5	GLSIY01HG"A"	2.5	1.6	4.53
12	15	Granada 03Poly1A(SA4)	2.5	1.7	4.43
13	17	Villavicencio 03Asp1C(QPM)	2.5	1.8	3.88
14	25	Villavicencio 03Phaeo1A(Elites)	2.5	1.6	4.16
15	9	ACROSS S9624-1	2.0	2.0	4.05
16		Yangtsipa (Improved Check)	3.1	4.0	4.74

*: disease score based on scale of 1-5, where: 1 = No lesions are visible; 2 = Few lesion seen on two lower leaves; 3 = Lesions visible on most leaves below the ear; 4 = Many Lesions visible on leaves above the ear; 5 = All Leaves dead.

Table 2 GLS, TLB and yield of 15 selected genotypes from CIMMYT Colombia at five different locations, 2009.

Entry	Pedigree	Mean of 5 locations		
		GLS*	TLB*	Yield (t·ha ⁻¹)
1	Cap. Miranda 99Bact1F-1	1.9	1.9	5.94
2	Granada 01Phaeo1AS2	1.7	1.7	5.31
3	Turipana 01DMR 1D(1)	1.9	1.7	5.4
5	GLSIY01HG"A"	2.0	1.6	4.16
6	GLSIY01/SPMAT	1.9	1.7	4.76
9	ACROSS S9624-1	1.8	1.7	4.35
13	Menegua 01 Phaeo	1.7	1.6	4.03
15	Granada 03Poly1A(SA4)	1.8	1.8	3.57
17	Villavicencio 03Asp1C(QPM)	2.2	1.7	4.05
21	Villavicencio 03Asp1C(LET-EARLY)	1.9	1.8	4.51
23	Villavicencio 03Phaeo1A(SA4)	1.9	1.7	5.28
25	Villavicencio 03Phaeo1A(Elites)	2.2	1.7	3.90
33	Cimcali 03HCG1A	1.9	1.7	5.26
35	S03TLYQ AB05	1.9	1.8	4.15
38	ICA V305	1.9	1.9	5.35
	Yangtsipa	2.0	1.7	5.57
	Local	2.2	1.8	5.45
Location (L)		**	**	**
Entry (E)		**	ns	**
L × E		**	ns	ns
S.E.D		0.2435	0.3122	1.339
CV		13	18	28

** $P > 0.01$.

as fourth choice. Farmer's selection criteria included plant height, husk cover, tolerance to GLS, yellow

flint gains and size of the cobs. Based on the results and farmer's preferences, three genotypes namely

Entry No. 1, 35 and 21 were selected for further evaluation. The genotypes Entry No. 6 which was preferred by the farmers was dropped due to less quantity of seed. One additional genotypes, ICA V305 (Entry No. 38) that was not selected by the farmers during the PVS, was included for further evaluation based on the advice of the CIMMYT experts. This genotype ICA V305 is a stable variety released in Colombia and one of the parents is Suwan 1 (Yangtsipa) which is a popular variety in Bhutan. All the selected genotypes showed higher tolerance to GLS than the check varieties (Table 2).

In 2009 season, a very close monitoring of GLS was done for the four selected genotypes and the two check varieties. The GLS incidence starts by the last week of July and reaches the peak in the last week of August. All the four new genotypes including the check varieties were infected by GLS, however, the incidences of GLS was much higher in the two check varieties right from the initial stage and continued to increase until all the leaves were dead by end August (Fig. 3).

This strongly indicates that in the event of an early incidence of GLS, the new genotypes can tolerate

much better as compared to the check varieties. The results from multi-location trial of four selected genotypes in four locations in 2010 revealed that new genotypes showed much higher tolerance to GLS as compared to the local check variety (Table 3). There was significant difference in yield and the highest yield of 5.33 t·ha⁻¹ was recorded for Yangtsipa, the improved check variety. Among the new genotypes, the highest yield of 5.27 t·ha⁻¹ was recorded for Cap. Miranda 99Bact1F-1 (Entry No. 1) (Table 3). This genotype has semi dent grains and higher percentage of open husk which is less preferred by the farmers. The mean data of three years indicated that this genotypes had high incidence of Turcicum Leaf blight and yield was comparable to that of genotypes S03TLYQ AB05 (Entry No. 35) and ICAV305 (Entry No. 38) and therefore was not considered for immediate release (Tables 4 and 5). Although the yield of ICA V305 (Entry No. 38) is lower than that of the check varieties, it had better tolerance to GLS and good husk cover. Due to the urgency for GLS tolerant varieties for maize growing areas above 1,500 m asl, the two genotypes S03TLYQ AB05 (Entry No. 35) and ICAV305 (Entry No. 38) were selected for large

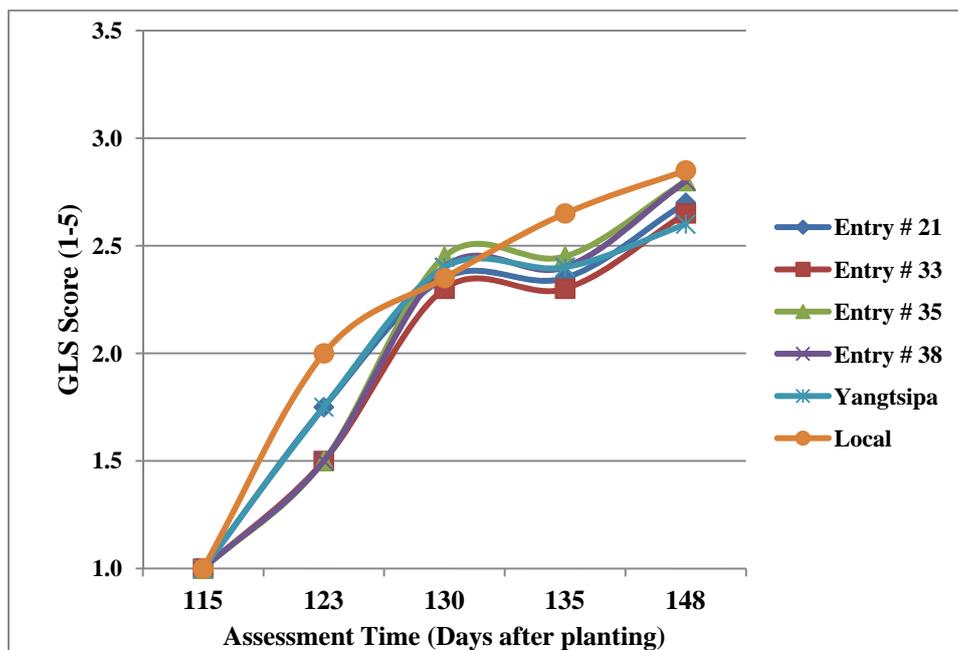


Fig. 3 GLS progression at Chaskhar, 2009.

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Table 3 Mean GLS, TLB score and yield t/ha for 2010 season (mean of four locations).

Entry No.	Pedigree	GLS, score*	TLB, score	Yield (t·ha ⁻¹)
1	Cap. Miranda 99Bact1F-1	2.1	2.2	5.27
21	Villavicencio 03Asp1C(LET-EARLY)	1.9	2.1	2.48
35	S03TLYQ AB05	2.1	2.1	4.78
38	ICA V305	2.1	2.2	3.83
	Yangtsipa	2.0	2.2	5.33
	Local	2.5	2.4	3.96
	Fprob			***
	LSD			1.606
	CV (%)			25

*** $P > 0.001$.

Table 4 Other agronomic traits of selected varieties, 2010 season.

Entry No.	Pedigree	Average plant height (cm)	Average ear height (cm)	Husk cover** (1-5)	Ear aspect *** (1-5)
1	Cap. Miranda 99Bact1F-1	217.4	93.8	2.5	2.6
21	Villavicencio 03Asp1C(LET-EARLY)	222.8	108.6	2.8	2.9
35	S03TLYQ AB05	226.6	94.5	2.3	2.2
38	ICA V305	242.6	116.7	2.4	2.7
	Yangtsipa	257.7	124.9	2.4	2.4
	Local	281.7	145.7	1.6	1.6

** Husk cover based on scale of 1-5 where 1 = best (fully covered) and 5 = open husk; *** Ear aspect based on scale of 1-5 where 1 = best quality with uniform ear size and good grain filling 5 = poor quality.

Table 5 Mean GLS, TLB and yield of four selected genotypes.

Entry No.	Pedigree	Mean of three years		
		GLS	TLB	Yield (t·ha ⁻¹)
1	Cap. Miranda 99Bact1F-1	2.1	2.4	4.96
21	Villavicencio 03Asp1C(LET-EARLY)	2.2	2.1	3.80
35	S03TLYQ AB05	2.3	2.3	4.75
38	ICA V305	2.3	2.3	4.81
	Yangtsipa	2.4	2.3	5.57
	Local	2.6	2.5	4.41

scale demonstration and provisional release in 2011 season considering the mean performances of three years (Table 4). Although the yield of ICA V305 (Entry No. 38) is lower than that of the check varieties, it had better tolerance to GLS and good husk cover (Tables 4 and 5).

In 2011, the TRC (Technology Release Committee) of the MOAF (Ministry of Agriculture and Forest) in its 15th meeting endorsed the provisional release of the two GLS tolerant genotypes S03TLYQ AB05 (Entry No. 35) and ICAV305 (Entry No. 38). The urgency of GLS tolerant variety was one important consideration for the release.

Further in the 2011 season, the two provisionally

released genotypes were put under large scale demonstration in the GLS affected areas in nine districts across the country. The mean yield reported from the large scale demonstrations for the two genotypes S03TLYQ AB05 (Entry No. 35) and ICAV305 (Entry No. 38) were 3.73 t·ha⁻¹ and 4.43 t·ha⁻¹, respectively. In order to facilitate the rapid seed replacement of the GLS affected farmers, seed production was done using the concept of CBSP (Community Based Seed Producers) group [20] and nine CBSP groups have been formed in five districts for seed multiplication of the two new GLS tolerant genotypes. Finally, in 2012 based on the overall performance in 2011 season and general acceptability

of the farmers, the two provisionally released genotypes were proposed for final release to the TRC of the MoAF. The TRC finally endorsed the release of the two genotypes in April 2012. After the release the two genotypes were given local names as *Chaskarpa* for genotype ICAV305 (Entry No. 38) and *Shafangma Ashom* for genotype S03TLYQ AB05 (Entry No. 35).

The main justification for the release were that GLS epidemics has gained the status of a national emergency as all the available maize varieties were found to be highly susceptible to the disease. The two new genotypes have shown much higher field tolerance to GLS as compared to the existing varieties. Further, in the event of early outbreak of GLS, the two new genotypes will perform much better in terms of disease tolerance and production. Both are open pollinated varieties for which seed production is easy and the two genotypes have a higher yield potential and have shown comparable yields with the check varieties despite the fact that they are relatively new to the highland ecosystem and are undergoing adaptation. Both have yellow flint grains, and *Shafangma Ashom* S03TLYQ AB05 (Entry No. 35) is a QPM (Quality Protein Maize) which is more nutritious than the traditional maize varieties. It is the first QPM maize variety released in the country and will immensely contribute to the nutritional requirement of the maize farmers particularly that of the children [21].

The two new varieties are recommended for the maize production zone II (1,200-1,800 m asl) and Highland maize production Zone (> 1,800 m asl) where the two disease GLS and TLB have severely affected maize production. Both the varieties also perform well in Sub-tropical maize production zone I (< 1,200 m asl) or low altitudes. By 2013 season, the maize program has accomplished around 75% seed replacement of the GLS affected farmers with the two new varieties mostly with the supports of DRDP-WB, DoA MoAF (World Bank through the Decentralized Rural Development Project).

4. Conclusion

The high altitude farmers in Bhutan are facing the serious problem of GLS and TLB. Although, use of fungicide Tilt 25 EC seems to be quite effective, yet the use of chemicals will not be sustainable. The small and subsistence Bhutanese farmers cannot afford expensive fungicides, besides the difficulty to spray manually in steep slopes where most of the maize is grown and the detrimental impact of continuous use of fungicides on environment does not make the use of fungicide a suitable disease management option. The release of two GLS and TLB tolerant maize varieties has come as a big respite for the Bhutanese maize farmers particularly those above 1,500 m asl. With rapid seed increase through the CBSP groups, 75% seed replacement of the affected farmers with the two GLS tolerant varieties has been accomplished by 2013 planting season. The seed increase and replacement of the affected farmers is rigorously being pursued in collaboration with the National Seed Center and the district extension services.

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contributed in improving the seed production system and technical capacity building of researchers, extension and farmers. We also acknowledge the financial support of CIMMYT International and the SLMP (Sustainable Land Management Project)-NSSC (National Soil Services Center) for supporting the participation of maize researchers in the 11th Asian Maize Conference at Nanning, China, where an abstract of this paper was presented as a poster on GLS management.

References

- [1] MOIC, Bhutan Portal [Online], available: http://www.bhutan.gov.bt/government/abt_geography.php (accessed Jan. 15, 2013).
- [2] LCAR, Land cover assessment report, in: Agriculture, The National Soil Service Center and the Policy and Planning Division, Thimphu, Bhutan, 2010.
- [3] DOA, Agriculture Statistics, in: Agriculture, Thimphu, Bhutan, 2010.
- [4] T.B. Katwal, P. Dem, G.B. Chhetri, L. Bockel, M. Punjabi, Maize commodity chain analysis, A working document, Department of Agriculture, Ministry of Agriculture, Thimphu, Bhutan, 2006.
- [5] S. Shrestha, T.B. Katwal, B.B. Ghalley, Adoption and Impact Assessment of Improved Maize Technologies in Bhutan, Council of RNR Research of Bhutan, RNR RDC Wengkhar, 2006.
- [6] C. de Leon, Report of activities and recommendations on a consultancy to Bhutan, August 12-26th, 2007, RNR Research Centre, Bajo, Wangdiphodrang, Council of RNR Research of Bhutan, Bhutan, 2007.
- [7] J. Wang, M. Levy, L.D. Dunkle, Sibling species of *Cercospora* associated with gray leaf spot of maize, *Phytopathology* 88 (1998) 1269-1275.
- [8] J.M.J. Ward, E.L. Stromberg, D.C. Nowell, F.W. Nutter, Gray leaf spot - A disease of global importance in maize production, *Plant Disease* 83 (1999) 884-895.
- [9] B.N. Verma, Gray leaf spot disease of maize - loss assessment, genetic studies and breeding for resistance in Zambia, in: 7th Eastern and Southern Africa Regional Maize Conference, 2001.
- [10] F.M. Latterell, A.E. Rossi, Gray leaf spot of corn: A disease on the move, *Plant Disease* 67 (1983) 842-847.
- [11] A. Menkir, M. Ayodele, Genetic analysis of resistance to gray leaf spot of midaltitude maize inbred lines, *Crop Sci.* 45 (2005) 163-170.
- [12] P.M. Beckman, G.A. Payne, External growth, penetration, and development of *Cercospora zeaе-maydis* in corn leaves, *Phytopathology* 72 (1982) 810-815.
- [13] J.C. Rupe, M.R. Siegel, J.R. Hartman, Influence of environment and plant maturity on gray leaf spot of corn caused by *Cercospora zeaе-maydis*, *Phytopathology* 72 (1982) 1587-1591.
- [14] S.I. Harlapur, Epidemiology and management of Turcicum leaf blight of maize caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. Ph.D., University of Agricultural Sciences, Dharward, India, 2005.
- [15] C.W. Roane, Observations on gray leaf spot of maize in Virginia, *Plant Disease* 58 (1974) 456-459.
- [16] N.R.X. de Nazareno, P.E. Lipps, L.V. Madden, Survival of *Cercospora zeaе-maydis* in corn residues in Ohio, *Plant Disease*, 76, 1992.
- [17] G. Bigirwa, R.C. Pratt, P.E. Lipps, E. Adipala, Farming components for gary leaf spot disease severity in districts of contrasting incidence, in: Seventh Eastern and South Africa Regional Maize Conference, 2001.
- [18] P.A. Paul, G.P. Munkvold, Influence of temperature and relative humidity on sporulation of *Cercospora zeaе-maydis* and expansion of gray leaf spot lesions on maize leaves, *Plant Disease* 89 (2005) 624-630.
- [19] T.B. Katwal, D. Wangchuk, N.B. Adhikari, N. Wangdi, S. Wangdi, P.B. Biswa, First year report on maize breeding and selection for tolerance to gray leaf spot and turcicum leaf blight diseases: A working document, RNR RDC Wengkhar, Field Crops Sector, Mongar, 2009.
- [20] T.P. Tiwari, G. Ortiz-Ferrara, C. Urrea, R.B. Katuwal, K.B. Koirala, R.C. Prasad, et al., Rapid gains in yield and adoption of new maize varieties for complex hillside environments through farmer participation. II. Scaling-up the adoption through community-based seed production (CBSP), *Field Crops Research* 111 (2009) 144-151.
- [21] K. Mbuya, K.K. Nkongolo, A. Kalonji-Mbuyi, Nutritional analysis of quality protein maize varieties selected for agronomic characteristics in a breeding program, *International Journal of Plant Breeding & Genetics* 5 (2011) 317-327.