Importance of Loose Smut [*Ustilago nuda* (Jensen) Rostrup] of Barley (*Hordeum vulgare* L.) in Western Amhara Region, Ethiopia

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Abstract: Barley occupies 10.72, 7.23 and 5.78% of the total land allocated for cereal crops in South Gondar, West Gojam and Awi Zones, respectively. This contributes to 9.19, 5.29 and 3.58% of the total cereal crop production in each zone, respectively. However, barley production has been constrained by different biotic and abiotic factors, of which loose smut [*Ustilago nuda* (Jensen) Rostrup] is the major biotic factor in the study areas. This piece of work was carried out to assess the level of loose smut incidence at field and on farmers' saved barley seeds. The field survey was done by randomly taking barley fields to determine the loose smut incidence level in the three zones of western Amhara Region (Awi, South Gondar and West Gojam) during the 2014 main cropping season. The seed health test on randomly taken farmers' saved barley seeds was conducted following the International Seed Testing Association (ISTA) 2014 rules. It was found that a minimum incidence of 1.17 to 2.00% and maximum incidence ranging from 4.04 to 10.64% occurred at field level, whereas seeds collected from these zones for embryo test in the laboratory showed the minimum seed infection of 8.35% and maximum infection of 25.65%. It can be concluded that loose smut infection rates in the surveyed areas were high. Hence, selecting disease-free barley seeds and screening resistant varieties together with seed treatments with selected fungicides need to be promoted to tackle loose smut and to sustain barley production.

Keywords: Barley; *Hordeum vulgare*; Loose Smut; Seed Treatment; *Ustilago nuda*

1. Introduction

In Ethiopia, the long history of cultivation and the diverse agro-ecological and cultural practices have resulted in a wide range of barley diversity (Eticha *et al*., 2010). Vavilov (1951) declared that nowhere else in nature he had observed such a diversity of barley forms and genes. Therefore, he proposed Abyssinia (the former Ethiopian Empire) as a center of origin for the cultivated barley (Eticha *et al*., 2010). Barley grows well at altitudes of 1500–3500 meters above sea level and is predominantly grown at 2000–3000 meters above sea level in Ethiopia (MoA, 1998).

Barley is the major staple crop and is deeply-rooted in the socio-cultural lifestyle of the Ethiopian communities (Eticha *et al*., 2010). It is used for the preparation of different foodstuffs in the country, such as flattened pancake (*injera*), porridge, *Kolo* (roasted grains); and local drinks, such as *tela*, *borde*, and *arekie* as well as malt production. Its straw is a good source of feed and the stem stubbles and straw can be used for roof thatching and bedding materials (Kuma *et al*., 2011). Barley is cultivated in some regions in two distinct seasons: *Belg,* which relies on the short rainfall period occurring from March to April, and *Meher,* which relies on the main long rainy season that takes place from June to September (Lakew *et al*., 1997; Berhanu *et al*., 2005).

Barley is the fifth most important cereal crop after tef, wheat, maize and sorghum in total production in the Amhara region. The total area coverage of barley in this region is about 370,000 ha with total production of 390,000 tons and productivity of 1.1 tons per hectare (CSA, 2013), which is below the national average of 1.2 tons per hectare. Its productivity in the country is also very low compared to that of most other countries. This is due to low level of cultural practices and other abiotic and biotic factors. Barley crop diseases in Ethiopia incur yield and quality losses. Research efforts have been underway for a long time to mitigate these problems and various high yielding and disease resistant varieties have been released and appropriate agronomic practices have been recommended (Kuma *et al*., 2010).

Earlier research reviews on cereal diseases, pathogens involved, their distribution and importance and their management in Ethiopia showed that 28 plant pathogens were reported to affect barley, at various agro-ecologies and in different farming systems in the country. The great majority of the pathogens identified were of fungal origin (Eshetu, 1986).

Ustilago nuda (Jensen) Rostrup, the causal agent of loose smut of barley (*Hordeum vulgare* L.), is a common world-wide seed-transmitted pathogen. The mycelium localized in the embryo spreads systemically and asymptomatically in the developing plant and during flowering the inflorescence is largely replaced by sori containing teliospores of the fungus (Punithalingam and Waterston, 1970; Vánky, 1994). This fungus is an important pathogen of cereal crops, reducing yield and quality of harvested grain (Thomas, 2011a). Loose smut is an internally seed-borne disease where the pathogen is localized within the embryo of the seed; hence,

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contaminated machinery and soil will not be the transmission mechanisms for this disease (Thomas, 2011b).

Seeds infected by loose smut produce normal and healthy-looking tillers up until the time of ear emergence. Thus, the affected plants can compete for light, water and nutrients alongside healthy plants. Affected plants cannot produce any grain so there is a clear relationship between percentage of seed infection and yield loss. A 2% seed infection will give a corresponding 2% loss in yield. An infection of 0.1% in the field (one in a thousand ears affected) can appear dramatic, even though the yield loss would be negligible (Paveley *et al.*, 1996).

Grain losses attributed due to loose smut of barley are generally less than 1% in modern times; but losses of 15 to 25% can occur if proper management practices are not used (Punithalingam and Waterston, 1970; Menzies *et al.*, 1997).

Loose smut can effectively be managed through the use of certified seed, smut-free seed, as determined using an embryo infection test (Rennie, 1982), resistant host varieties, or by applying systemic seed treatment fungicides (Bailey *et al.*, 2003). Seeds known to carry high levels of loose smut fungus should not be sown (Thomas *et al.,* 2011b). Rouging infected plants as early as possible has been recommended (Zemede, 1989), particularly in small isolated seed plots. Killing of the mycelium without damaging the embryo achieved by hot water treatment is taken as physical management of the disease; anaerobic seed treatment using air-tight storages were also used as physical management (Bilgrami and Dube, 2001).

In the last ten years, the incidence of loose smut of barley had been assessed long before decades in western Amhara region, as reviewed previously (Yitbarek *et al.*, 1996). Incidence of covered and loose smuts was 3.3 and 8.5%, respectively, whereas Bekele *et al.* (1994) reported an incidence of 28% for loose smut in the area. However, the result did not clearly indicate the real status of the disease at field and farmers' saved barley seeds at the moment. Therefore, this work was carried out to assess the current loose smut incidence as well as importance of the disease caused by the fungus [*Ustilago nuda* (Jensen) Rostrup] in the field and in samples of farmers' saved barley seeds in western Amhara Region.

2. Materials and Methods

A field survey was conducted in some districts and Farmers' Associations' (FA) selected based on accessibility and extent of barley production in western Amhara region from mid-July to mid-August, 2014. Barley fields were randomly assessed in each FA, giving a total of seventy-two assessed fields in five surveyed districts, namely Farta, Guagusa Shikudad (Tilili), Lay Gayint, Sekela and Yilmana Deinsa in three zones, viz. Awi, South Gondar and West Gojam zones (Figure 1).

2.1. Description of the Surveyed Areas

The geographic location, average temperature and rainfall of each surveyed district were recorded using the primary and secondary data. The locations of the surveyed areas lied between longitude 37^{o}03' and 38^{o}78' E, and latitudes ranging from 10^{o}76' to 11^{o}88' N. The altitudes of these areas ranged from 2174 to 3185 meters above sea level.

South Gondar Zone is located in western Amhara Region. According to CSA (2013), barley occupies over 10.72% of the total land allocated for cereal crops and it contributes to 9.19% of the total cereal crop production in the Zone. Two districts, namely Lay Gayint and Farta were selected for this study. Lay Gayint district has altitude that ranges from 2652 to 3185 meters above sea level located between 11^{o}73' and 11^{o}79' N latitude and ranges from 38^{o}27' to 38^{o}78'E longitude (NAE, 1984). It receives an annual rainfall of 921.3 mm and the temperature ranges from 8.0 to 18.4 ^{o}C. Farta district is located southwest of Lay Gayint and has annual rainfall of 1500.9 mm, the temperature ranges from 6.1 to 25.5 ^{o}C.

West Gojam Zone is located in the western part of Amhara Region. According to CSA (2013), barley occupies over 7.23% of the total land allocated for cereal crops and it contributes to 5.29% of the total cereal crop production in the Zone. Yilmana Densa and Sekela districts were selected for this study. Yilmana Densa has altitudinal ranges from 2174 to 2865 meters above sea level, located 11^{o}13' to 11^{o}32'N latitude and 37^{o}42' to 37^{o}56'E longitude (NAE, 1984). It receives an annual rainfall of 921.3 mm and the temperature ranges from 7.3 to 31.3 ^{o}C. Sekela District is located south of Yilmana Densa and has temperature that ranges from 8.2 to 23.4 ^{o}C. The district receives an annual rainfall of 1804.9 mm.

Awi Zone is located in the southwest of West Gojam Zone in western Amhara Region. According to CSA (2013), barley occupies over 5.78% of the total land allocated for cereal crops and it contributes to 3.58% of the total cereal crop production in the Zone. In this Zone, only one district, *i.e.* Guagusa Shikudad (Tillili) sows barley in May. The rest barley producing districts in this Zone sow barley in late August; hence, only this district was included in the survey. It has an altitudinal ranges from 2562 to 2718 meters above sea level and receives annual rainfall of 2036.8 mm.

2.2. Data Collection

Estimation of loose smut incidence

Barley was inspected in a total of 72 fields at flowering stage for loose smut incidence in five districts within three zones (Figure 1) from July 12 to August 16, 2014. Randomly identified barley fields were assessed at intervals of 5-10 km along the road. A 0.5 m x 0.5 m quadrate was placed five times on 5 to 10 meters apart in 'X' manner at two diagonals across the inspected fields. In each quadrate, total headed plants were observed and recorded as diseased or healthy. Disease incidence was computed as a percentage of diseased plants to the total number of plants observed using the following formula.

$$\text{Disease Incidence (\%)} = \left(\frac{\text{No. of diseased plants}}{\text{Total no. of plants observed}}\right) * 100$$

The global positioning system (GPS-Garmin) and digital photo-camera were used to measure the geographic locations.

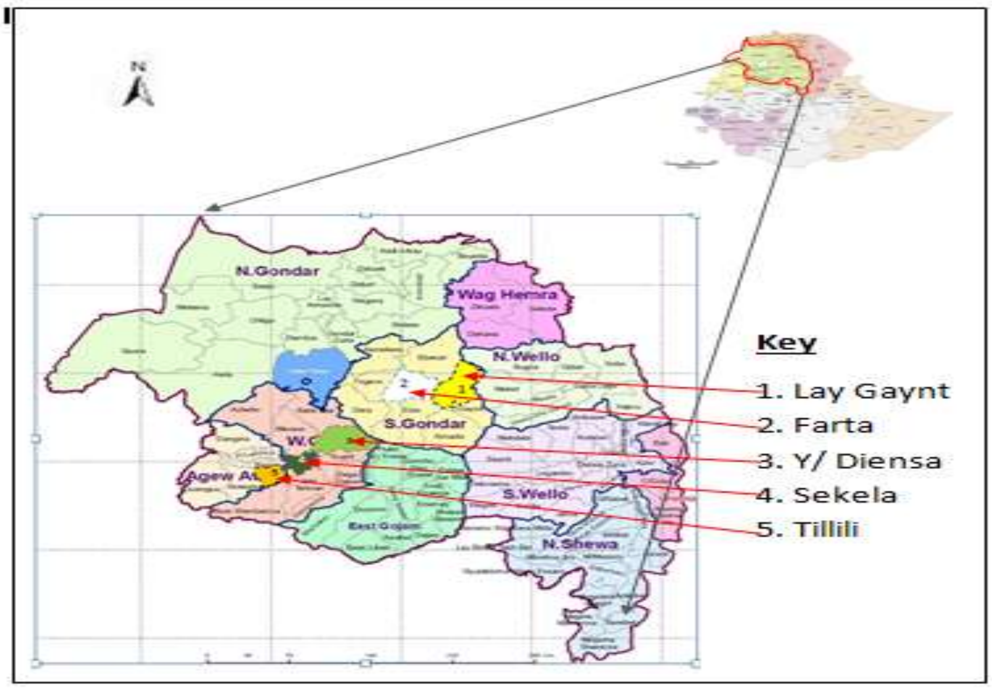

Figure 1. Map of the Amhara Regional State showing the loose smut surveyed districts.

2.3. Agronomic and Other Relevant Data

During the survey, additional data on field history, field size, crop variety, cropping systems, altitude, latitude and longitudes, planting date, and soil types were collected to determine their relationships with barley loose smut.

2.4. Seed Health Tests (Embryo Test)

To relate the incidence of the disease on field survey results with seeds used at sowing, the samples of barley seeds that remained from sowing during the cropping season were collected and used for seed health tests. Farmers' saved barley seed samples were collected from different altitude ranges of western Amhara Region in 2014. The locations ranged from 10°76' to 11°88' N latitude and 37°03' to 38°78' E longitude; the altitude of the study area ranged from 2474 to 3185 meters above sea level. The mean temperature ranged from 6.1 to 31 °C.

2.5. Seed Sampling

Fifteen Farmers' Associations (FAs') were selected based on the accessibility and the extent of barley production. One farmer from each of 15 FAs was randomly considered for sample collection. All barley cultivars in the study area were distinctly named and grown for food and local drinks. The six-rowed barley, named '*Kinkina*', or *Tikurdirib gebis* and two-rowed barley, named '*Tsebel gebis*', were the dominantly grown cultivars in the Lay Gayint and Farta districts of South Gondar Zone. *'Semerieta', 'Saldimi', 'Temeji'* and *'Wonteka'* (two-rowed, mostly used as roasted grain, locally called *qolo*) were predominantly grown cultivars in West Gojam and Awi Zones. Thus, four seed samples of *Awura gebis*, three seed samples from *Tsebel gebis,* two seed samples from *Wonteka,* five seed samples of *Semereta* and one seed sample of *Tikurdirib gebis* were collected from the 15 farmers taken randomly.

A 1 kg seed sample was taken from a seed lot used for planting based on the procedures suggested by the International Seed Testing Association (ISTA, 2014). Thus, a total of 15 kg seed samples were obtained. Seed sampling was made from different parts (store depths and points) of each seed container. The test was carried out on a working sample as described in the rules of International Seed Testing Association (ISTA, 2014), where the method requires 2000–4000 seeds, but for this test 2000 seeds were sampled by counting and 1000 embryos were examined and this was replicated twice.

For embryo extraction, seeds were placed in 1 L of a freshly prepared 5% aqueous solution of sodium hydroxide (NaOH) and maintained at 20 °C for 24 hrs. After soaking, the entire sample was transferred to a suitable plastic container and washed in warm water to separate the embryos, which appeared through the softened pericarps. Embryos were collected using sieves of 1 and 2 mm meshes. The embryos were transferred to a mixture of equal quantities of glycerol and water in which further separation of the embryos and chaff could be made. The embryos were also transferred to fresh glycerol for examination. The scutellum became more transparent when embryos were left in glycerol for 1–2 hr, making the embryo examination easier.

2.6. Embryo Examination

The extracted embryos were examined by stereomicroscope (Model No.ZS2500) at 4×20 magnification with adequate sub-stage illumination for the characteristic golden brown mycelium of *Ustilago nuda* following ISTA rules (ISTA, 2014).

2.7. Data Analysis

All data on disease incidence and seed health tests were analyzed using SAS 9.1 version software. Disease incidence was associated with the independent variables, using the logistic regression model by forming binomial data.

3. Results and Discussion

3.1. Incidence of Loose Smut of Barley on Farmers' Fields

3.1.1. South Gondar Zone

In South Gondar Zone, two districts, namely Lay Gayint and Farta were surveyed. In Lay Gayint district, the altitude of the surveyed area ranged from 2652.8 – 3185.2 meters above sea level. (Table 1). The local barley cultivar *Manie gebis*, *Tikurdirib gebis*, *Tsebel gebis, Kinkina gebis* and *Wasiera* were widely cultivated. Barley loose smut appeared at different levels of incidence in all these local barley cultivars. The maximum (4.45%) and minimum (1.78%) disease incidence was recorded in this district (Table 3), while the overall mean incidence was 2.91% (Table 1).

In Farta district, the altitudes of the surveyed areas ranged from 2579.3 – 3020 meters above sea level (Table 1). The local cultivars *Manie gebis*, *Tikurdirib gebis* and *Tsebel gebis* were widely cultivated and the malt barley variety Holker was also cultivated in few fields. In this district, incidence of loose smut was higher than in Lay Gayint district in the Zone. The maximum (7.79%) disease incidence and the minimum (1.89%) incidence was recorded (Table 3). The overall mean incidence of loose smut in this district was 4.42% (Table 1), which was higher than that of the incidence in Lay Gayint district of South Gondar Zone, covered by the present survey.

In these two districts, viz. Farta and Lay Gayint that got high incidence of loose smut on some fields, farmers also indicated that the disease was widespread in their fields and was mentioned to be among the major problems of barley production. The wide cultivation of susceptible barley cultivars and the use of own-saved seed for next planting season year after year might be responsible for the higher occurrence of loose smut in both districts.

3.1.2. West Gojjam Zone

In West Gojjam Zone also two districts (Yilmana Densa and Sekela) were surveyed. In Yilmana Densa district, the altitude of the surveyed area ranged from 2174.1 to 2865.2 meters above sea level (Table 1). The maximum (10.64%) and minimum (1.17%) loose smut incidence was recorded (Table 3). An overall mean incidence of the disease recorded was 4.52% (Table 1). In this district, the local barley cultivar (*Semereta*) was dominantly grown. This cultivar showed maximum loose smut incidence; most probably due to high distribution of infected seeds or due to year-after-year use of the seed of this cultivar without seed treatment.

In Sekela district, the altitude of surveyed areas ranged from 2562.1 to 2717.9 meters above sea level (Table 1). Loose smut was found in fields with mean incidence level of 3.94% (Table 1). The maximum (5.55%) and the minimum (1.95%) disease incidence was recorded in this district (Table 3). Farmers asserted that loose smut disease, locally called *Yewofare*, occurred more in some years and less in other years and they told that they did not practice seed treatment before (Personal communication).

3.1.3. Awi Zone

In Awi Zone, only one district, namely Guagusa Shikudad, was selected to assess the incidence of loose smut. In this district, the altitude of the surveyed areas ranged from 2425.3 to 2566 meters above sea level (Table 1). Maximum (4.04%) and minimum (2.00%) incidence of loose smut was recorded in this district (Table 3). All the surveyed fields were planted with the barley cultivar *Semerieta* and it appears that this variety might have some degree of susceptibility to the disease; the mean disease incidence was 3.13% (Table 1). It was also recognized that some farmers developed a practice of rouging out infected plants from the field, but keeping the critical time for rouging was under debate (Personal communication with farmers).

3.2. Association of Independent Variables with Disease Incidence

The data on the mean disease incidence were categorized into the variable classes that were compared (Table 2). The result of logistic regression and the likelihood ratio test on the deviance ratio showed that mean disease incidences were not significantly different ($Pr>\chi^2$) except from the altitude parameters which was highly significant ($p\leq0.01$) (Table 4).

This may be due to temperature differences that might have favored the disease to express itself in the respective location. To support this result, the ten years' mean monthly temperature data that barley crop stayed in the field were taken at each surveyed district except Guagusa Shikudad and were compared with loose smut incidence of the current year. The result of simple linear regression showed that for each increase in temperature there was an increase of loose smut incidence (Figure 2).

This significant variation is consistent with the finding of Dean (1969) who reported that maximum loose smut disease development occurs at 23 °C. It is also known that atmospheric temperatures during the seedling to spike emergence stage usually influence the number of smutted ears. However, if the temperature prevails at 29 °C, especially during the period between the beginning of internode elongation and boot emergence, spore formation in the smut-infected plants is completely prevented and there would be no loose smut incidence.

Another study done by Agrawal *et al.* (1984) indicated that the optimum temperature for seed infection is 22-25 °C. If at the time of flowering these meteorological conditions prevail and the inoculum is present in the locality, heavy infection of healthy flowers can be expected.

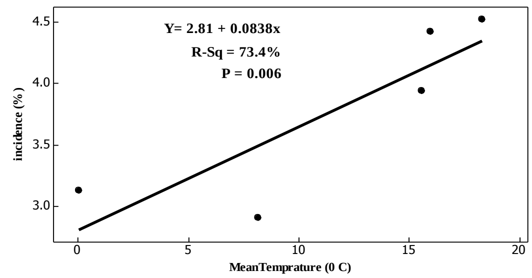

Figure 2. Linear regression between incidence of loose smut and temperature.

Table 1. Mean incidence of loose smut of barley in western Amhara Region in 2014 cropping season.

Zone	District	Altitude (m)	Incidence range (%)	Mean Incidence (%)
South Gondar	Lay Gayint	2651.8 - 3185.2	1.78- 4.45	2.91
	Farta	2579.3 – 3020	1.89 - 7.80	4.42
West Gojam	Yilmana Densa	2174.1 – 2865.2	1.17 - 10.64	4.52
	Sekela	2562.1 – 2717.9	1.95 - 5.55	3.94
Awi	Guagusa Shikudad	2425.3 – 2566	2.00 - 4.04	3.13

Table 2. Categorization of variables used for logistic regression analysis of loose smut in barley fields (n=72) in western Amhara Region, Ethiopia, during the 2014 main cropping season.

Independent variable	Variable classes	Number of fields with loose smut			
		Incidence (%)		Prevalence within the field (%)	
		≤ 4	> 4	≤ 80	> 80
Districts	Lay Gayint	7	2	7	2
	Farta	7	1	15	3
	Yilmana Densa	12	3	20	5
	Sekela	5	6	10	1
	Guagusa Shikudad	8	1	7	2
Variety	Local	37	3	57	13
	Improved	1	1	2	0
Sowing date	Early May	14	4	24	3
	Mid May	14	8	17	6
	Late May	10	2	18	4
Previous crop	Potato	11	4	21	4
	Wheat/Triticale	10	7	14	2
	Faba bean/Fieldpea	5	3	5	3
	Tef	11	5	12	4
	Maize	1	4	5	0
	Barley	1	1	2	0
Soil	Light sandy	30	5	45	10
	Heavy black	9	8	14	3
Altitude	≤ 2300	14	7	15	6
	> 2300	24	27	44	7

Table 3. Minimum, maximum and mean incidence of loose smut for different independent variables in western Amhara Region in 2014 main cropping season.

Independent variable	No. fields	Incidence (%)			
		Min	Max	Mean	SD
District					
Lay Gayint	9	1.78	4.45	2.91	0.93
Farta	18	1.89	7.80	4.42	1.78
Yilmana Densa	25	1.17	10.64	4.52	1.92
Sekela	11	1.95	5.55	3.94	1.22
Guagusa Shikudad	9	2.00	4.04	3.13	0.77
Variety					
Local	70	1.17	10.64	3.98	1.65
Improved	2	1.89	2.16	2.03	0.33
Sowing date					
Early May	28	1.89	6.37	3.85	1.27
Mid May	23	1.17	10.64	4.17	1.98
Late May	21	1.78	7.80	4.13	1.81
Previous crop					
Potato	24	1.89	10.64	4.17	1.99
Wheat/Triticale	17	1.78	7.83	3.95	1.57
Maize	5	1.17	6.37	4.25	1.95
Faba bean/Fieldpea	8	2.07	6.22	3.74	1.55
Barley	2	2.89	5.29	4.09	1.69
Tef	16	2.44	7.80	3.98	1.38
Soil					
Light sand	57	1.89	10.64	4.13	1.78
Heavy black	15	1.17	5.23	3.67	1.11
Altitude					
≤ 2300	21	1.17	5.23	3.58	0.99
> 2300	51	1.78	10.64	4.27	1.83

Where: SD = Standard deviations.

The insignificant results of these variables showed that loose smut was not affected by these parameters, implying that it is a completely seed-borne disease (Table 4). In general, it appears that the disease was more prevalent in fields assessed in Yilmana Densa and Farta districts. This might be attributed to the wide distribution of the local cultivars *Semereta* and *Tsebel gebis* in Yilmana Densa and Farta districts, respectively. Use of farmer-saved untreated seed and suitable environmental conditions might be responsible for high levels of this internally seed-borne disease.

In Lay Gayint and Guagusa Shikudad districts, the mean incidence of barley loose smut was 2.91 and 3.13%, respectively, when compared to Farta and Yilmana Densa that had a 4.42 and 4.52% loose smut incidence, respectively. This might not be due to resistance but it might be due to the fact that farmers mostly grow the locally diverse cultivars, planting crop mixtures and temperature difference.

Table 4. Independent variables used in logistic regression of loose smut incidence and likelihood ratio test (LRT) for six variables as single predictor of disease outcome.

Independent variable	df	Loose smut incidence LRT > 4%		Variable class	Natural logarithms of odds ratio		
		DR	Pr > χ^2		Estimate $Log_e^{(OR)}$	Odds ratio	Prob.< 4%
District	4	47.86	0.1143				
Altitude	1	31.95	0.0036	≤2300	-0.4323	0.65	0.61
				>2300	0.00	1.00	
Sowing date	2	31.94	0.4979				
Previous crop	5	31.10	0.9745				
Soil	1	30.93	0.6752				
Variety	1	28.33	0.1071				

Where: LRT = likelihood ratio test, DR = deviance ratio.

Only the altitude variable showed significant difference and hence the probability of reducing loose smut incidence below 4% was 61% higher of the variable classes than its reference group (100%). As the odds ratios indicate, there were 0.65 times less probabilities that loose smut incidence exceed 4% at the variable than its reference group variable (Table 4).

When the overall incidence of loose smut was considered in all surveyed areas, the highest (4.56%) mean disease incidence was recorded in Yilmana Densa, followed by Farta (4.42%) and Sekela (3.94%) (Table 1). The mean incidence of the disease in the surveyed areas ranged from 2.91 to 4.52%, signifying a yield loss range of 2.91 to 4.52% due to the loose smut. It was stated that barley yields are reduced in proportion to the percent smutted heads; since most infected heads produce no seed (Gothewel *et al.*, 1972). Disease surveys in northwestern Ethiopia during 1988–1992 were reviewed previously (Yitbarek *et al.*, 1996), indicating incidences of loose smuts ranging between 3.3 and 8.5% at that moment, whereas Bekele *et al.* (1994) reported an incidence of 28% for barley loose smut.

The local cultivars *Semereta* and *Tsebel gebis* were more affected by the disease as indicated by higher incidence observed on these two cultivars. Less (1.89%) loose smut incidence occurred on the variety Holker; most farmers growing this variety were supplied with this seed by Dashen Beer Factory through Amhara Region Seed Enterprise. Thus use of certified seed might have contributed to lessen loose smut incidence. Cultural measures, such as use of disease-free seeds for planting and removal of smutted-heads before the spores are disseminated to healthy plants may help in minimizing the occurrence of the smut (Yilma and Abebe, 1987).

The cultivation of the diverse local cultivars (*Mesino, Semereta, Saldimi, Wonteka* and *Temeji*) and mixed cropping system (e.g. the locally practiced '*Wasera*', *i.e.* cropping of barley and wheat in mixture on one field simultaneously) in Lay Gayint district might have contributed to the lower level of loose smut incidence. The two local varieties, i.e. *Wonteka* and *Semereta* predominantly grown in Sekela and Guagusa Shikudad, respectively, were highly infected by loose smut.

The recycling of infected seed year after year might have contributed to the higher level of the incidence in all the surveyed districts as also reported by Goel *et al.* (2001). Under favorable weather conditions, the barley produced from a field with only one percent head infection can have seed with 10% or more infection by loose smut in the next growing season (IPM, 1990). The present survey clearly indicated that loose smut caused varying levels of losses in the surveyed areas. In this respect, Farta and Yilmana Densa districts need more attention in view of the higher incidence and field prevalence of the disease at the current subsistence barley farming.

3.3. Seed Embryo Test

A highly significant ($p \leq 0.01$) difference in percent seed infection was obtained among the locations and local cultivars collected for seed health testing purpose (Table 5). The maximum (25.65%) seed infection by loose smut was observed on barley seed samples of the local cultivar '*Aura gebis*', collected from Kimir Dengia Farmers' Association in Farta district. This site is found at 2989 meters above sea level, followed by 21.40% seed infection on samples from the local cultivar *Semereta* collected from Debremawi Farmers' Association of Yilmana Densa district, followed by *Wonteka* and *Tsebel gebis* collected from Ambisi Farmers' Association in Sekela district and Ata Sifatira Farmers' Association in Farta district with 20.96 and 20.10% seed infection by loose smut, respectively (Table 5).

The minimum (8.35%) seed infection was recorded on seed samples of the cultivar *Semereta* collected from Addisalem Farmers' Association in Guagusa Shikudad district, followed by seed samples of *Tsebel* and *Tikurdirib gebis* cultivars collected from Tsegur Farmers' Association in Farta district and at GovGov Farmers' Association in Lay Gayint district with 11.00 and 11.95% infection, respectively.

In general, the minimum (8.35%) barley loose smut infection and the maximum (25.65%) infection with the mean (17%) seed infection were recorded across the whole seed samples collected for embryo examination. In this respect, *Awura gebis* and *Semereta* were more infected than the other local cultivars. However, when this result was compared with the field survey results, it was found to be higher than the field survey results.

This might be due to the pathogen (the mycelium within the embryo) might have not been favored by the temperature and moisture during the growth period of the plant (Figure 3). Different authors have also indicated that favoring environmental factors (mainly temperature and rainfall) are most decisive elements for loose smut pathogen development; if the temperature at sowing and, especially between beginning of internodes' elongation and root emergence, reaches 29 ^{o}C, smut formation is prevented (Dean, 1969). At 24 ^{o}C, loose smut incidence is moderate and at 19 ^{o}C, smut incidence is very heavy. According to Agrawal *et al.* (1984), the mean optimum temperature for seed infection by *Ustilago nuda* is within the range of 22 - 25 ^{o}C at the time of flowering.

Verma *et al.* (1984) also reported that occurrence of light and frequent rains (about 7 - 21 mm) for 4 – 5 days, with a temperature range of 6.4 - 23.3 ^{o}C during flowering favors high loose smut infection. Excessive heat or dry air lowers spore germination and germ-tube growth, delays the penetration of the ovary and precludes the entry of the fungus and environment can also cause florets to stay open for a shorter period, which also eventually reduces the chance of entry of the fungus into the seed (Atkins *et al.*, 1963).

With this context, the minimum (6.1 - 8.2 ^{o}C) and maximum (18.4 - 31.3 ^{o}C) temperatures as well as rainfall ranges from 921 to 3036 mm indicated that the pathogen may be affected within the temperature and rainfall extremes. Cockerell and Rennie (1995) generally reported farm-saved seeds were more frequently infected with *U. nuda* and at higher levels than certified seed.

Table 5. Welch's variance-weighted ANOVA for embryo test on barley seed infection with loose smut.

District	Location/ Peasant Association	Cultivar	Mean infection	Standard deviation
Farta	Kimirdengia	*Awura gebis*	25.65 (1.44)	0.019
Lay Gayint	Nefasmewucha	*Tsebel*	14.40 (1.21)	0.025
Farta	Ata sifatira	*Tsebel*	20.10 (1.33)	0.007
Lay Gayint	Titramikael	*Semerieta*	12.00 (1.14)	0.039
Lay Gayint	Chechoho	*Awura gebis*	13.00 (1.18)	0.016
Lay Gayint	Genboche	*Awura gebis*	13.00 (1.18)	0.036
Lay Gayint	Govgov	*Tikurdirib gebis*	11.95 (1.10)	0.013
Lay Gayint	Sali	*Awura gebis*	16.55 (1.29)	0.064
Sekela	Ambisi	*Wonteka*	20.96 (1.34)	0.006
Sekela	Gindatemem	*Semerieta*	9.90 (0.97)	0.098
Sekela	Ateta	*Wontaka*	15.05 (1.25)	0.063
Yilmana Densa	Aybar	*Semerieta*	11.25 (1.11)	0.028
Yilmana Densa	Debremawi	*Semerieta*	21.40 (1.36)	0.008
Farta	Tsegur	*Tsebel*	11.00 (1.10)	0.032
Guagusa Shikudad	Addisalem	*Semerieta*	8.35 (1.00)	0.044
Mean			14.97	
CV (%)			3.47	
SE			0.025	
R^2			0.95^{***}	
P-value			<0.0002	

Where: CV = Coefficient of variation, SE = standard error, values in parenthesis are log transformed.

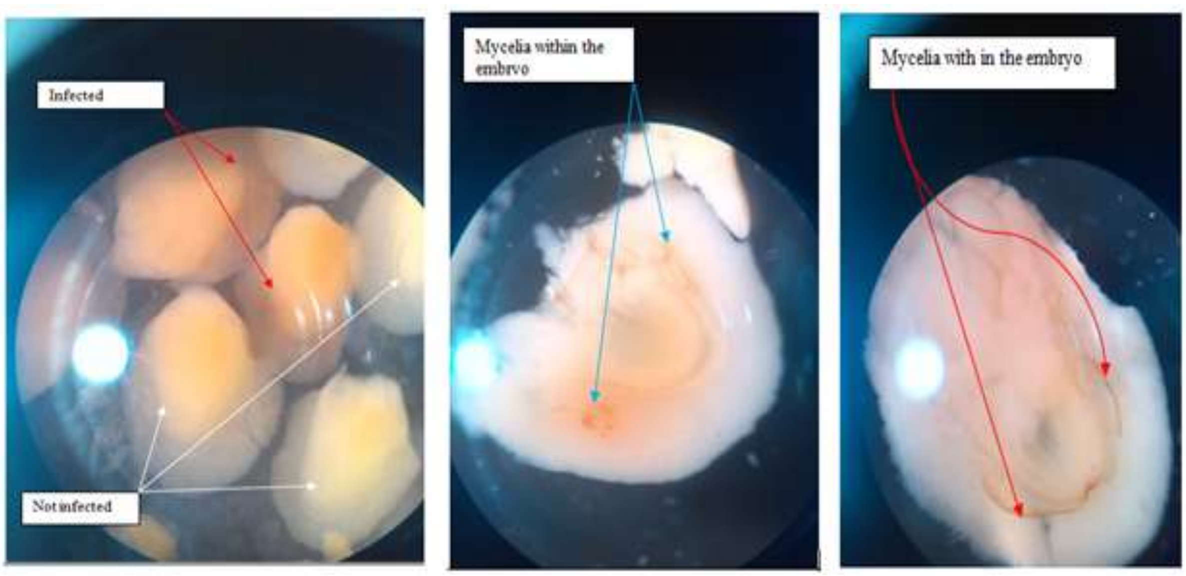

Figure 3. Extracted embryos under microscopic field showing infected and uninfected barley embryos by *Ustilago nuda* in seed samples from western Amhara Region in 2014 main cropping season.

4. Conclusions

Loose smut of barley, caused by *Ustilago nuda* (Jensen) Rostrup, is one of the commonly found diseases of barley in western Amhara Region. Survey of this disease was conducted in five districts (namely Farta, Guagusa Shikudad, Lay Gayint, Sekela and Yilmana Densa) of western Amhara Region, Ethiopia, to assess the incidence of the disease. Samples of barley seeds saved by farmers for planting purposes were also collected for laboratory seed health through embryo test technique.

It was found that loose smut was common in all the districts surveyed during the 2014 main cropping season. In South Gondar Zone, the mean incidences of barley loose smut were 2.91 and 4.42% in Lay Gayint and Farta districts, respectively. The barley loose smut incidence in West Gojam Zone was found to be relatively higher than in South Gondar Zone. Mean disease incidences of 4.52 and 3.94% were recorded in Yilmana Densa and Sekela districts, respectively. The mean incidence of loose smut in Awi Zone was found to be 3.13%, recorded in Guagusa Shikudad district.

In general, the mean incidence of loose smut of barley was higher (4.52%) in Yilmana Densa district than in the other four districts, whereas the lowest (2.91%) loose smut incidence occurred in Lay Gayint district. From these survey results, the incidence of loose smut can be related with yield losses ranging from 2.91 to 4.52% in parts of western Amhara Region covered by the study. The maximum (25.65%) seed infection by loose smut was observed on barley seed samples of the local cultivar '*Aura gebis*' collected from Farta district.

In conclusion, the present study revealed that barley loose smut occurred in the three zones of western Amhara Region, and it was widely spread in some districts, like Yilmana Densa and Farta. Hence there is a need for monitoring the smut development and coming with recommendation with fungicide seed treatments in the affected areas. Barley germplasms need to be screened against loose smut and varieties resistant to loose smut should be developed and farmers' should be advised to use certified seed.

As far as future directives are concerned for loose smut management, further surveys need to be conducted to assess the significance of the disease throughout the region since the current survey was conducted only in some districts of western Amhara Region. It is also desirable to search for loose smut resistance by testing many more barley germplasms and to test the efficacies of several seed treatment fungicides for the purpose of development of integrated loose smut management options for sustainable barley production.

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6. References

Agrawal, V. K., H. S. Verma, M. Agrawal and R. K. Gupta. 1984. Studies on loose smut of wheat. I. A simplified procedure for the detection of seedborne infection. *Seed Research* 9: 72 - 78.

Atkin, I. N., O. G. Markle, K. B. Proter, K. A. Lahr and D. E. Weibel. 1963. The influence of environment on loose smut percentage, re-infection and grain yields on winter wheat at four locations of Texas. *Plant Disease Reporter* 47: 92 - 96.

Bailey, K. L., B. D. G., R. K. Gugel and R. A. A. Morrall. 2003. Diseases of field crops in Canada. *The Canadian Phytopathological Society*, 37 - 38.

Bekele Hundie, Hailu Tefera, Melkamu Ayalew, Mesele Alemu and Y. S. Paul. 1994. Management of loose smut and leaf stripe of barley using seed dressing fungicides. *pp*. 14 - 15. *In*: *Crop Protection Society of Ethiopia,* Addis Ababa.

Berhanu Bekele, Fekadu Alemayehu and Berhane Lakew. 2005. Food barley in Ethiopia. *pp*. 53 - 82. *In*: Grando, S., Gomez Macpherson H (eds). Food barley: importance, uses, and local knowledge. Proceedings of the International Workshop on Food Barley Improvement, 14 - 17 January 2002, Hammamet, Tunisia. ICARDA, Aleppo.

Bilgrami, K. S. and H. C. D. 2001. A textbook of modern plant pathology. *pp*. 226 - 243. *Vikas Publication New Delhi*.

Cockerell, V. and W. J. Rennie. 1995. Survey of seed-borne pathogens in certified and farm saved seed in Britain between 1992 and 1994. Home-Grown Cereals Authority Project Report No. 124. London: Home-Grown Cereals Authority.

CSA (Central Statistical Agency). 2013. Agricultural Sample Survey. Volume III. Report on Area and Production *(Private Peasant Holdings, Meher Season)*. Addis Ababa.

Dean, W. M. 1969. The effect of temperature on loose smut of wheat (*Ustilago nuda*).*Annals of Applied Biology* 64: 75 - 83.

Eshetu Bekele. 1986. A review of research on disease of barley, teff and wheat in Ethiopia. pp 79 - 108, *In:* Tsedeke Abate (ed.). Proceedings of the Review of Crop Protection Research in Ethiopia. Institute of Agricultural Research, 4 - 7 February 1985, Addis Ababa, Ethiopia.

Eticha, F., W. Sinebo and H. Grausgruber. 2010. On-farm diversity and characterization of barley (*Hordeum vulgare* L.) landraces in the highlands of West Shewa, Ethiopia. *Ethnobotany Research and Applications* 8: 25 - 34.

Goel, L. B., D. P. Singh, V. C. Sinha, S. Amerika, K. P. Singh, A. N. Tewari, M. S. Beniwal, S. S. Karwasra, S. S. Aujla and A. S. Grawal. 2001. Efficacy of Raxil (tebuconazole) for controlling the loose smut of wheat caused by *Ustilago segetum* var. *tritici*. *Indian Phytopathology* 54 (2): 270 - 271.

Gothewel, B. D. 1972. Effect of loose smut on the growth and morphology of spring wheats. *Indian Journal of Mycology and Plant Pathology* 2: 171.

IPM (Integrated Pest Management). 1990. Loose smut of wheat. Reports on Plant Disease. RPD No. 112. University of Illinois at Urbana Champaign.

ISTA (International Seed Testing Association). 2014. International Rules for Seed Testing. Annex to Chapter 7 Seed Health Testing. Seed Health

Testing Methods. International Seed Testing Association, Bassersdorf, Switzerland.

Kuma Berhanu, Shenkut Ayele, Tilaye Teklewolde and Yalembirhan Molla. 2011. Achievements in barley technology transfer and extension in Ethiopia. Barley Research and Development in Ethiopia, 377.

Lakew Brehane, Yitbarek Semeane, Fekadu Alemayehu, Hailu Gebre, S. Grando, J. A. G. van Leur and S. Ceccarelli. 1997. Exploiting the diversity of barley landraces in Ethiopia. *Genetic Resources and Crop Evolution* 44: 109 - 116.

Menzies, J. G., G. H. and F. M.. 1997. Incidence and severity of cereal smuts in Manitoba and Saskatchewan, 1996. *Canadian Plant Disease Survey* 77: 58.

MoA (Ministry of Agriculture). 1998. Agricultural productivity report. Addis Ababa, Ethiopia.

NAE (National Atlas of Ethiopia), 1984. Ethiopian mapping agency. Addis Ababa., Ethiopia.

Paveley, N., W. Rennie, J. Reeves, M. Wray, D. Slawson, W. Clark, V. Cockerell and A. Mitchell, 1996. Cereal seed health and seed treatment strategies. Home Grown Cereals Authority.

Punithalingam, E. and J. M. Waterston. 1970. *Ustilago nuda*. CMI Description of Pathogenic Fungi and Bacteria, 2pp.

Rennie, W. J. 1982. ISTA Handbook on Seed Health Testing. Barley loose smut. Working Sheet. ISTA Secretariat, Zürich, Switzerland, pp.6.

SAS (Statistical Analysis Systems). 2002. SAS/STAT User's guide, Version 9.1. SAS Institute Inc., Cary, NC.

Thomas, P. L. 2011a. The occurrence of loose smut of barley on commercially-grown cultivars possessing genes for resistance from Jet. *Canadian Journal of Plant Science* 54: 453 - 456.

Thomas, P. L. 2011b. Barley smuts in Manitoba and eastern Saskatchewan, 1972-74. *Canadian Plant Disease Survey.* 54: 124 - 128.

Vánky, K. 1994. *European Smut Fungi*. Gustav Fischer Verlag, Stuttgart, Germany, 570 pp.

Vavilov, N. I. 1951. The Origin, Variation, Immunity and Breeding of Cultivated Plants. *Chronological Botany* 13: 1 - 366.

Verma, H. S., A. Singh, and V. K. Agrawal. 1984. Application of seedling crown test for screening of systemic fungicides against loose smut of wheat. *Seed Research* 12: 56 - 60.

Yilma Kebede and Abebe Menkir. 1987. Current activities and recommendations and future strategies of the sorghum improvement program in Ethiopia. *In*: Proceedings of the 19th National Crop Improvement Conference, IAR., Addis Ababa, Ethiopia.

Yitbarek Semeane, Bekele Hunde, Getaneh Woldeab and Dereje Tadesse. 1996. Disease surveys and loss assessment studies on barley. *pp.* 105-115. *In:* Hailu Gebre and J. A. G. van Leur (eds.). Barley Research in Ethiopia: Past Work and Future Prospects. Proceedings of the 1st Barley Research Review Workshop, 16-19 October 1993, Addis Ababa. IAR/ICARDA, Addis Ababa, Ethiopia.

Zemede Asfaw. 1989. Relationships between spike morphology, *Hordeins* and altitude within Ethiopian barley. *Hereditas*, 110, 203 - 209.